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Emerging techniques for manipulating endocrine function in animal production: A comprehensive review

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ABSTRACT

The endocrine system plays an important role in regulating various physiological processes in animals, encompassing growth, reproduction, metabolism, immune response, and overall homeostasis. This review delves into the fundamental definition and profound significance of endocrine function in animals, shedding light on its complex mechanisms and the vital role it plays in maintaining health and optimizing various aspects of animal production.

Keywords: endocrine glands, growth promoter, reproduction, hormones, endocrine disruptive chemicals.

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Introduction

The endocrine system, a complex network of glands and organs, is responsible for the secretion of hormones – vital molecules that govern a multitude of bodily functions. These functions encompass growth and development, metabolic processes, electrolyte balances, and reproduction (Nagy and Malcomson, 2022). This complex system comprises several key components, including the hypothalamus, pituitary gland, adrenal glands, gonads (testes and ovaries), thyroid gland, parathyroid glands, pancreas, and thymus (Skórka-Majewicz et al., 2020). Hormones, important to this system, are released into the bloodstream in response to specific triggers, reaching their target cells to convey critical messages (Nagy and Malcomson, 2022).

The endocrine system maintains a vigilant watch over the hormone levels in the blood, expertly regulating their release through complex biochemical mechanisms and the feedback loop (Hackney and Lane, 2015). Examples of these complex feedback loops are the hypothalamus-pituitary axis and the pituitary-adrenal axis, which finely tune hormone production and release (Paragliola et al., 2017). This complex system is instrumental in overseeing growth and development, tissue function, metabolism, and reproductive processes (Barzilai et al., 2012).

Undoubtedly, manipulating endocrine processes holds considerable potential in the realm of animal production. The significance of endocrines in the extents of nutrition, breeding, and overall production of farm animals cannot be overstated (Velazquez et al., 2008). The manipulation of endocrine processes promises enhanced animal growth, reproductive outcomes, and overall health. This can be achieved through a variety of strategies, including the administration of growth hormones as growth promoters, estrous synchronization, augmentation of milk production, the regulation of immune function to bolster health, and the reduction of stress by modulating cortisol levels. Such manipulation presents

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a nuanced approach to optimizing animal production, aligning with the sophisticated science of endocrine function.

Endocrine regulation of growth and reproduction

Overview of key endocrine glands and hormones involved in growth and reproduction

The endocrine system, often referred to as the hormone system, is a fundamental component found across the animal kingdom, encompassing mammals, birds, fish, and numerous other species. This complex system comprises an array of glands distributed throughout the body, each with its specific function. These glands produce hormones, which are subsequently released into the bloodstream or the fluid surrounding cells. These hormones act as messengers, and their actions are recognized and responded to by receptors in various organs and tissues (Jameson, 2015). In essence, hormones function as the body's communication network, transmitting vital messages from one part of the body to another (Pert, 1988).

The endocrine system plays a important role in regulating an extensive spectrum of biological processes, from the earliest stages of life, through adulthood, and into old age. Its influence extends to the development of the brain and nervous system, the growth and function of the reproductive system, and the complex control of metabolism and blood sugar levels (Jameson, 2015).

Among the key players in this system are: Pituitary Gland: Often termed the "master gland," the pituitary gland exerts control over various other endocrine glands. It produces a plethora of hormones, including growth hormone, which fosters the growth of bones and other bodily tissues. Furthermore, it plays a crucial role in nutrient and mineral utilization. Additionally, the pituitary gland produces luteinizing hormone and follicle-stimulating hormone, which regulate the production of sex hormones – estrogen in females and testosterone in males, and also govern the production of eggs in females and sperm in males (Bharati et al., 2023).

Thyroid gland: The thyroid gland is responsible for the production of thyroid hormones. These hormones wield influence over all cells within the body and hold sway over biological processes such as growth, reproduction, development, and metabolism (Jameson, 2015).

Adrenal glands: These remarkable glands synthesize several hormones, including aldosterone, which maintains salt and water balance and blood pressure regulation. Furthermore, cortisol, produced by the adrenal glands, governs metabolism, influencing growth, maturation, and immune function (Nagy and Malcomson, 2022).

Pancreas: Serving as an exocrine and endocrine gland, the pancreas primarily focuses on controlling blood sugar levels. It achieves this through the production of insulin and glucagon (Knight, 2021).

Ovaries: In female animals, the ovaries are the primary source of sex hormones, including estrogen and progesterone. These hormones play a important role in regulating the estrous cycle, and they are key actors in processes such as pregnancy and parturition (Persson, 2000).

Testes: In males, the testes serve as the primary source of sex hormones, particularly testosterone. This hormone is instrumental in the development of male sex organs and the emergence of secondary sex characteristics (Toppari et al., 1996).

In addition to these primary actors, a host of other hormones are involved in the complex web of growth and reproduction. This includes hormones like growth hormone-releasing hormone, thyrotropin-releasing hormone, corticotropin-releasing hormone, gonadotropin-releasing hormone, oxytocin, vasopressin, dopamine, and somatostatin (Nagy and Malcomson, 2022). Each of these hormones, glands, and their functions, contribute to the awe-inspiring symphony that is the endocrine system, underscoring its indelible impact on the biological processes that shape life in the animal kingdom.

Hormonal interactions and pathways regulating growth and reproduction

The endocrine system is complex and involves a network of hormones and pathways that work together to ensure proper growth and reproductive function. Hormonal interactions and pathways play a crucial role in regulating growth and reproduction in animals. Here are some key points about the hormonal regulation of growth and reproduction:

1. Hormones regulating growth*:* Growth Hormone (GH): Produced by the pituitary gland, GH promotes protein synthesis throughout the body, especially in cartilage, bone, and muscle (Isaksson et al., 1985)

Somatotropin: A hormone produced by the pituitary gland, somatotropin is responsible for promoting protein synthesis and growth (Isaksson et al., 1985) .

Steroid sex hormones: Testosterone and estrogen also have a role in regulating growth in humans. The hormone surge during adolescence produces a growth spurt in both male and female animals (Rogol et al., 2002)

2. Hormones regulating reproduction: Gonadotropin-Releasing Hormone (GnRH): Released by the

hypothalamus, GnRH stimulates the release of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) from the anterior pituitary, which are essential for reproductive processes (Yaron and Levavi-Sivan, 2011)

Follicle-Stimulating Hormone (FSH): Produced by the anterior pituitary, FSH stimulates the growth and development of ovarian follicles in females and sperm production in males (Chappel and Howles, 1991) Luteinizing Hormone (LH): Also produced by the anterior pituitary, LH triggers ovulation in females and stimulates testosterone production in males (Chappel and Howles, 1991)

Sex Steroid Hormones: Estrogen and progesterone in females, and testosterone in males, play crucial roles in regulating reproductive processes, including the development of secondary sex characteristics and the regulation of gonad function (McEwen, 1992).

3. Interactions and pathways

Growth hormone and reproduction: There is evidence to suggest that growth hormone (GH) also directly modulates reproduction, exerting both gonadotropin-dependent and gonadotropin-independent effects (Hull and Harvey, 2014).

GH-insulin-like growth factor (IGF)-1-gonadal axis: This axis plays a significant role in reproduction, with GH and IGF-1 influencing gonadal function and the production of sex steroid hormones (Dosouto et al., 2019).

Hormonal control of animal performance: Hormones, including growth hormone and cortisol, interact and impact the development and performance of animals, particularly during niche shifts and metamorphosis (McCormick and Romero, 2017).

Manipulation of growth hormones

Exogenous administration of growth-promoting hormones

The practice of administering exogenous growth-promoting hormones is a widespread strategy in animal production, aimed at augmenting growth rates and enhancing feed efficiency (Qaid and Abdoun, 2022). These hormones encompass androgens, estrogens, glucocorticoids, progestogens, and synthetic compounds like trenbolone acetate and zeranol (Yazdan et al., 2022). It's noteworthy that the Food and Drug Administration (FDA) in the United States has sanctioned the utilization of these hormones in animal production (Qaid and Abdoun, 2022).

Exogenous administration of growth hormone to animals has far-reaching consequences, impacting various physiological processes, including growth and lactation (Peel et al., 1983). The key hormonal substances employed for growth promotion encompass naturally occurring steroids such as estradiol-17β, progesterone, and testosterone, alongside synthetic compounds like zeranol, trenbolone acetate, and melengestrol acetate (MGA) (Jeong et al., 2013). Nevertheless, the application of growth-promoting hormones has stirred debate concerning the safety of livestock products for human consumption. Despite the ongoing controversy, several countries routinely employ these hormones, concurrently reducing greenhouse gas emissions, energy use, water consumption, and reactive nitrogen loss in beef production compared to hormone-free methods (Skoupá et al., 2022). In the context of an evolving demand for sustainability, growth-promoting hormones hold the potential to enhance production efficiency, thereby contributing significantly to the overarching goal of producing more food with fewer resources (Hume et al., 2011).

Effects of growth hormone manipulation on growth rates and body composition: Growth hormone manipulation wields substantial influence over the growth rates and body composition of animals. Notably, most insights into GH's mode of action have been gleaned from laboratory rather than farm animals (Berryman et al., 2008). Additionally, the precise mechanisms underpinning GH's effects on metabolism remain a subject of ongoing inquiry (Chaves et al., 2013).

1. Effects on growth rates: Pituitary growth hormone (GH) emerges as a potent anabolic agent in animal production, exemplified by swifter growth rates and reduced feed consumption per unit of body weight gain in treated animals, alongside diminished carcass fat compared to untreated counterparts (Williams et al., 1994). The consequential impact on body composition is a product of GH's dichotomous effects on lean and fat mass – promoting the former while curtailing the latter (Palmer et al., 2009).

2. Effects on body composition: GH exercises a profound lipolytic effect, resulting in the reduction of adipose tissue mass, all while preserving lean body mass (Berryman and List, 2017). GH's effects are mediated either directly or through the induction of IGF-1, which governs overall body growth (Oberbauer, 2015). This hormone also drives the growth of lean tissue while curbing the accumulation of adipose tissue (Berryman and List, 2017). It's imperative to acknowledge that the dietary conditions to which animals are exposed assume a important role when assessing the effects of hormonal manipulation on body weight and adiposity in whole-animal models (Ribaroff et al., 2017).

Ethical and safety considerations of using growth hormones in animal production

The utilization of growth hormones in animal production gives rise to ethical and safety concerns that warrant attention.

1. Ethical considerations: From an animal welfare perspective, the application of growth hormones can inflict discomfort and pain on animals, potentially leading to health problems like lameness and joint pain (Ormandy et al., 2011). The genetic modification of some growth hormones through genetic engineering further raises ethical concerns concerning animal welfare and potential risks to human health (Ormandy et al., 2011).

2. Safety considerations: In the context of human health, the use of hormonal substances in food-producing animals has been linked to potential health concerns, including an elevated risk of breast cancer and other health issues (Nachman and Smith, 2015). Moreover, the use of growth promoters, including antibiotics, can contribute to the emergence of resistance genes, rendering infections harder to treat in both animals and humans (Jeong et al., 2013). The presence of growth hormones in meat may pose potential health risks for consumers (Jeong et al., 2013).

Induction of superovulation and multiple pregnancies

The induction of superovulation and multiple pregnancies represents a vital technique in animal production, involving hormonal treatments to stimulate the recruitment and development of multiple follicles in animals, leading to the production of a larger number of embryos (González-Bulnes et al., 2004). This technique is frequently employed alongside embryo transfer to expedite the propagation of animals with desired genetic traits (Jainudeen et al., 2016). Follicle-stimulating hormone (FSH) stands as the most commonly used hormone for inducing superovulation, but the yield of embryos can be variable and influenced by factors such as breed, age, nutrition, and management practices (S. Khan et al., 2023). Quality variations in embryos produced through superovulation in cow have also been documented (S. U. Khan et al., 2022). Consequently, ongoing research endeavors aim to refine and simplify superovulation protocols. Selection of multiparous animals with high pregnancy experience and consideration of ovarian follicular development activity may enhance the outcomes of simplified superovulation (Khan et al, 2022).

Inducing multiple pregnancies is a sought-after outcome of superovulation in animal production, facilitating the generation of a larger number of offspring harboring desirable genetic traits (Moore and Thatcher, 2006). In cattle raised for beef production, the induction of twin pregnancies holds particular significance.

Thyroid hormone manipulation

Influence of thyroid hormones on metabolism and growth

Thyroid hormones have a critical role in animal metabolism, growth, and development. Altering thyroid hormone levels in animal production can significantly impact growth and metabolism, as these hormones are fundamental for normal growth and development. Thyroid hormone supplementation has been demonstrated to increase growth rates in animals (Todini, 2007). Conversely, a deficiency in thyroid hormones can lead to growth retardation and reduced productivity (Choksi et al., 2003). Additionally, thyroid hormones are important in regulating animal metabolism. They elevate the metabolic rate, resulting in increased energy expenditure and heat production (Bianco et al., 2005). Manipulating thyroid hormones can thus influence the energy balance of animals and, in turn, impact their productivity.

In terms of reproduction, thyroid hormones also play a role. Studies have shown that thyroid hormone deficiency can lead to reproductive issues in animals, including reduced fertility and litter size (Choksi et al., 2003). Supplementation of thyroid hormones can enhance reproductive performance in animals. Moreover, maternal thyroid hormone levels during pregnancy and lactation can influence offspring growth and development. Studies have indicated that maternal thyroid hormone supplementation can increase offspring growth rates and enhance their survival (Hsu et al., 2022).

Stress hormone regulation and animal welfare

Stress hormones can have profound effects on animal well-being and productivity. Chronic stress can lead to severe health problems, reduced productivity, and behavioral changes (Ghassemi Nejad et al., 2022). Stress can result in decreased growth, impaired reproductive success, and compromised cognitive abilities (Martínez-Miró et al., 2016). In livestock, stress can increase mortality and morbidity, decrease growth efficiency, and lead to less desirable end products (Edwards, 2010). To effectively manage the consequences of stress in animals, it is crucial to assess the level of stress, identify stressors, and consider various factors that affect the stress response (Ghassemi Nejad et al., 2022).

Hormones involved in stress responses can lead to physiological and behavioral changes in animals (Tilbrook and Ralph, 2018). Various biomarkers are employed to evaluate stress, categorized into four groups based on the physiological system or axis assessed: the sympathetic nervous system, the hypothalamic-pituitary-adrenal axis, the hypothalamic-pituitary-gonadal axis, and the immune system (Martínez-Miró et al., 2016).

Strategies to minimize stress-related hormonal responses in animal production

There are several strategies that can be implemented to minimize stress-related hormonal responses in animal production, enhancing both animal health and productivity:

Environmental Modifications: Providing animals with a comfortable environment, including proper ventilation, temperature control, and sufficient space, can reduce stress (Collier et al., 2006).

Nutritional Management: Ensuring animals receive a balanced diet with adequate nutrients can lower stress, as nutritional deficiencies can lead to increased stress and reduced productivity (West, 1999).

Handling and Management Practices: Proper handling and management practices that minimize stressors, reduce noise levels, and provide a calm and quiet environment can help alleviate stress in animals (Lloyd, 2017).

Genetic Selection: Selecting animals with calm temperaments that are less susceptible to stress can contribute to reduced stress-related hormonal responses (Chen et al., 2015).

Health Management: Implementing proper health management practices, such as regular vaccinations, parasite control, and timely treatment of sick animals, can minimize stress (Pertanika and Trop, 2018).

Behavioral Enrichment: Providing opportunities for natural behaviors through environmental enrichment, socialization, and access to toys can reduce stress in animals (Maria Dimova and Stirk, 2019).

Supplementation: The supplementation of specific nutrients, such as chromium, can help alleviate stress in animals (El-Kholy et al., 2017).

Heat Stress Management: In hot climates, providing shade, cooling systems, and access to cool water can help mitigate heat stress in livestock production systems (Dourmad et al., 2022).

Balancing productivity goals with animal welfare concerns

Balancing productivity goals with animal welfare concerns under hormone manipulation in animal production is a complex and multifaceted challenge. Although hormones are often used to enhance productivity, their use can have both positive and negative effects on animal welfare. It is essential to recognize that while animals require a minimum level of care to be productive, productivity does not automatically equate to good welfare (Lusk and Norwood, 2011). Animal welfare cannot be solely determined by productivity; it encompasses various aspects of an animal's overall well-being (Fraser, 1995).

Management practices play a critical role in enhancing both animal welfare and productivity. However, the impact of these practices on livestock performance can vary depending on factors such as species, environment, and specific management protocols (Morgado et al., 2023). It is also essential to consider the individual needs and requirements of animals to achieve a balanced approach.

Efforts to increase productivity while reducing greenhouse gas emissions per unit of product are in line with goals set by organizations like the World Bank (Laborde et al., 2021). This dual focus on welfare and productivity is particularly relevant in the dairy industry, where farmers aim to boost productivity using fewer resources (Oltenacu and Algers, 2005). Additionally, animal welfare in extensive production systems is an ongoing area of concern, with research primarily focusing on welfare issues commonly associated with intensive systems (Temple and Manteca, 2020).

Hormonal manipulation in aquaculture

Aquaculture, the cultivation of aquatic organisms like fish, crustaceans, mollusks, and aquatic plants, frequently employs hormonal manipulation techniques to regulate reproductive functions in captive fish. The use of hormones is vital for sustaining commercial aquaculture production. The fish reproductive cycle is separated into the growth (gametogenesis) and maturation phase (oocyte maturation and spermiation), both controlled by the reproductive hormones of the brain, pituitary, and gonad (Mylonas et al., 2010).Hormonal manipulations of reproductive function in cultured fishes have focused on the use of either exogenous luteinizing hormone (LH) preparations that act directly at the level of the gonad, or synthetic agonists of gonadotropin-releasing hormone (GnRHa) that act at the level of the pituitary to induce release of the endogenous LH stores (Mylonas et al., 2010). Hormones are used in fish farming to increase fish production when one sex of a species has the capacity to grow bigger and faster than the other sex (Hoga et al., 2018).

For successful aquaculture, determination of the reproductive condition of captive broodstock is important for administering hormonal therapies and inducing spawning (Mylonas et al., 2010). The first step for hormone-induced spawning is to determine the type of hormone suitable for the fish species of interest (Chatakondi et al., 2018). Efficacy of hormones is determined by the dose and timing of administration (Chatakondi et al., 2018). The use of hormones in aquaculture has been studied extensively, and analytical methods have been developed to determine their residues (Hoga et al., 2018). The first methods employed freshly ground pituitaries collected from reproductively mature fish, which contained gonadotropins (mainly LH) (Zohar and Mylonas, 2001). Hormonal manipulation is an important key factor for the sustainability of commercial aquaculture production of wild captive fish (Mylonas et al., 2010).

Hormonal control of sex differentiation

Sex differentiation in fish and other aquatic species is influenced by a combination of genetic, physiological, and environmental factors. Hormones, particularly gonadotropins from the pituitary gland, play a significant role in regulating gonadal development and differentiation in some fish species (Arcand-Hoy and Benson, 1998). Environmental factors, such as temperature, can interact with hormonal cues to influence sex determination (Budd et al., 2015).

Hormonal sex reversal treatments, utilizing exogenous hormones and other chemicals, have been applied to many fish species. These treatments enable changes in an individual's sex and are particularly useful in aquaculture (Piferrer, 2001). Aromatase and estrogens have been identified as key components in the process of sex differentiation in fish (Guiguen et al., 2010).

Hormone-assisted breeding techniques in aquaculture

Hormone-assisted breeding techniques are widely utilized in aquaculture to increase fish production and enhance the quality of offspring. These techniques involve administering hormones to fish for various purposes, including:

Inducing and sustaining vitellogenesis: To stimulate egg production, hormones like estrogens are employed.

Sex reversal: Hormones can be used to change the sex of fish to favor more productive sexes.

Chromosome Set Manipulation: Hormones can be used to manipulate the chromosome set in fish to generate desired characteristics or traits.

Hybridization: Hormones are used to facilitate the hybridization of different fish species to create new varieties or species (Mylonas et al., 2010).

For example, European eel aquaculture aims to close the life cycle in captivity, overcoming natural inhibitions to sexual maturation in both sexes by applying assisted reproduction protocols (Benini et al., 2022). Studies continually investigate the efficacy of various synthetic hormones in inducing breeding in different fish species, striving to improve the efficiency of hormone-assisted breeding techniques and ensure high-quality offspring (Nazir et al., 2023).

Environmental considerations and challenges in hormone use in aquaculture

The use of hormones in aquaculture comes with various environmental considerations and challenges:

Eutrophication: Aquaculture effluents with high biological oxygen demand (BOD) and suspended solids can contribute to eutrophication, characterized by excessive growth of algae and aquatic plants, which can lead to oxygen depletion and fish mortality (Hlordzi et al., 2020).

Accumulation of waste products: Aquaculture generates waste products, including uneaten food and feces, which can accumulate in the environment and affect water quality (Fraga-Corral et al., 2022).

Release of Chemicals: The widespread use of bioactive compounds, such as hormones, raises concerns about their release into the aquatic environment (Okeke et al., 2022).

Water quality concerns: Specific environmental steroids are a significant concern for water quality due to their potential adverse effects on aquatic organisms (Agrawal et al., 2010).

Biosafety concerns: The use of advanced biotechnological tools like CRISPR/Cas in fish aquaculture introduces biosafety concerns related to gene flow to wild populations and potential unintended effects on non-target organisms (Bohua et al., 2023).

Balancing the benefits of aquaculture with these environmental concerns requires careful management, regulation, and the development of sustainable and environmentally friendly practices in the industry.

Endocrine disruptors and unintended effects

Introduction to endocrine-disrupting compounds (EDCs)

Endocrine-disrupting compounds (EDCs) are natural or human-made chemicals that may mimic, block, or interfere with the body’s hormones, which are part of the endocrine system (Crawford et al., 2017). EDCs can be found in the environment, food sources, personal care products, and manufactured products (Archer et al., 2017). Exposure to EDCs can occur through diet,

air, skin, and water (Metcalfe et al., 2022). The endocrine system is a network of glands and organs that produce, store, and secrete hormones, which regulate the body's healthy development and function throughout life (Nagy and Malcomson, 2022).

EDCs can interfere with the normal function of the endocrine system by acting like "hormone mimics" and tricking the body into thinking that they are hormones, blocking natural hormones from doing their job, increasing or decreasing the levels of hormones in the blood, or changing how sensitive the body is to different hormones (Uthayanan and Sundareswaran, 2023). According to the Endocrine Society, there are nearly 85,000 human-made chemicals in the world, and 1,000 or more of those could be endocrine disruptors, based on their unique properties (Nagy and Malcomson, 2022). EDCs are associated with a wide array of health issues, including male reproductive problems, early female puberty, leukemia, brain cancer, and neurobehavioral disorders (Gore et al., 2014). Research is ongoing to better understand how EDCs work and define their role in health and disease. Research areas in progress include developing new models and tools to better understand how EDCs work, developing and applying high throughput assays to identify substances with endocrine disrupting activity, conducting animal and human health research to define linkages between exposure to EDCs and health effects, developing new assessments and biomarkers of exposure and toxicity, and identifying and developing new (Crews and Mclachlan, 2006).

The Endocrine Society and International POPs (Persistent Organic Pollutant) Elimination Network (IPEN) have joined together to develop an EDC Guide to raise global awareness about EDCs. The guide draws from each organization's strengths to present a more comprehensive picture of global EDC exposures and health risks than either could have done alone. Endocrine Society authors contributed the scientific and health-related content (Gore et al., 2014).

Potential risks of EDCs in animal production and the food chain

Endocrine Disruptive Chemicals (EDCs) are a group of chemicals that can interfere with the endocrine system, which is responsible for regulating hormones in the body. EDCs can have a significant impact on animal production and the food chain, as they can enter the food chain through various routes, including the living environment of food-producing organisms, direct use in food production, and release from food contact materials (Mantovani, 2016).

Here are some potential risks of EDCs in animal production and the food chain:

Reproductive and developmental effects: EDCs can interfere with the reproductive and developmental systems of animals, leading to reduced fertility, birth defects, and other reproductive problems (Mallozzi et al., 2016).

Hormonal imbalances: EDCs can disrupt the normal functioning of hormones in animals, leading to hormonal imbalances that can affect various body systems (Lee et al., 2013).

Cancer: Some EDCs have been linked to an increased risk of cancer in animals (Alsen et al., 2021).

Immune system effects: EDCs can weaken the immune system of animals, making them more susceptible to diseases and infections (Ansar Ahmed, 2000).

Environmental effects: EDCs can have a significant impact on the environment, as they can accumulate in soil, water, and air, and can affect wildlife and ecosystems (Kabir et al., 2015).

Human health effects: EDCs can also have an impact on human health, as they can enter the human body through the food chain and other routes of exposure. Some EDCs have been linked to an increased risk of various health problems, including cancer, reproductive problems, and developmental disorders (Yilmaz et al., 2020).

Strategies to mitigate the exposure and effects of endocrine disruptors

Endocrine disruptors are synthetic chemicals found in everyday products like plastics and fragrances that can mimic hormones and interfere with the delicate endocrine system. Exposure to these chemicals can cause irreversible changes in the body, especially during phases of accelerated development like in utero and throughout the developmental period (Mallozzi et al., 2016). To mitigate the risks of EDCs in animal production and the food chain, it is important to take steps to reduce exposure to these chemicals. This can include using alternative methods of pest control, reducing the use of plastics and other materials that contain EDCs, and improving waste management practices to prevent the release of EDCs into the environment (Campbell et al., 2006).

Emerging technologies in endocrine manipulation

Advances in genetic and biotechnological approaches for hormone manipulation

Advances in genetic and biotechnological approaches have been used to manipulate hormones in animal production. Here are some ways in which these approaches have been used:

1. Genetic engineering: Scientists have used genetic engineering to create transgenic animals that express

higher levels of growth hormone, which can increase productivity in farm animal species (Ormandy et al., 2011). For example, transgenic pigs and sheep have been genetically altered to express higher levels of growth hormone (Devlin et al., 2009).

2. Gene knock-out techniques: Gene knock-out techniques have been used to create designer companion animals. For example, some companies use genetic engineering techniques to remove the gene that codes for the major cat allergen Fel d1, creating hypoallergenic cats (Ormandy et al., 2011).

3. Modification of animal products: Modern biotechnological techniques have been used to alter the characteristics of animal products. For example, genetic manipulation in transgenic animals can alter the carcass to be lower in fat and cholesterol (Asaye et al., 2014).

4. Improved milk production traits: Genetic engineering and cloning have been used to improve milk production in livestock. Scientists hope to produce animals with altered traits such as milk composition (Moore and Thatcher, 2006). For example, a transgenic goat has a transgene that codes for a human protein under the control of a promoter region that targets expression specifically to the mammary gland. The human protein is secreted in the goat's milk but nowhere else in the animal (Pittius et al., 1988).

While these approaches have shown promise in improving animal productivity and food quality, there are also ethical and welfare concerns associated with genetic engineering of animals (Ormandy et al., 2011). The generation of a new genetically engineered line of animals often involves the sacrifice of some animals and surgical procedures on others (Ormandy et al., 2011). Therefore, it is important to consider the welfare of the animals involved in these processes.

Gene editing and its potential applications in altering endocrine functions

Gene editing is a powerful tool that allows for precise and efficient alteration of an animal's DNA (McFarlane et al., 2019). By using gene editing technology, targeted mutations can be made in specific genes, and novel lines of animals with valuable phenotypes can be produced (Li et al., 2020). Gene editing combined with animal production technologies provides the potential for accelerating the genetic improvement of livestock, including the alteration of production traits, enhancing resistance to disease, reducing the threat of zoonotic disease transmission, and improvement of livestock welfare (Perisse et al., 2021).

Endocrine functions play a crucial role in animal production, and gene editing can be used to alter these functions to improve livestock productivity. Here are some potential applications of gene editing in altering endocrine functions in animal production:

1. Improving growth and muscle development: The myostatin gene (MSTN) is a common target for research into increased growth and muscle development. Gene editing can be used to inactivate the MSTN gene, leading to increased muscle mass and improved meat quality (Zhao et al., 2022).

2. Enhancing milk production: Gene editing can be used to alter the genes responsible for milk production, leading to increased milk yield and improved milk quality (Kadarmideen et al., 2003).

3. Reducing stress and improving animal welfare: Gene editing can be used to alter the genes responsible for the stress response in animals, leading to reduced stress and improved animal welfare (Kramer and Meijboom, 2021).

4. Improving reproductive performance: Gene editing can be used to alter the genes responsible for reproductive performance in animals, leading to improved fertility and increased litter size (Abdoli et al., 2016).

Future prospects of precision endocrinology in animal production:

Precision endocrinology is a rapidly growing field in animal production that aims to improve animal health, welfare, and productivity by using precise measurements of hormones and other biomarkers. Here are some future prospects of precision endocrinology in animal production:

1. Improved reproductive management: Reproductive hormones play a crucial role in animal reproduction, and precise measurements of these hormones can help improve reproductive management in livestock. For example, measuring progesterone levels in dairy cows can help identify the optimal time for insemination, leading to improved conception rates and reduced calving intervals (Crowe et al., 2018).

2. Better growth and feed efficiency: Hormones such as growth hormone and insulin-like growth factor (IGF-1) are important regulators of growth and metabolism in animals. Precise measurements of these hormones can help identify animals with superior growth and feed efficiency, allowing farmers to select and breed animals with desirable traits (Velazquez et al., 2008).

3. Improved animal welfare: Hormones such as cortisol and oxytocin are indicators of stress and social bonding, respectively, in animals. Precise measurements of these hormones can help identify animals that are experiencing stress or social isolation, allowing farmers to take corrective actions to improve animal welfare (Neethirajan et al., 2021).

4. Reduced use of antibiotics: Hormones such as

cortisol and progesterone are also involved in the immune response of animals. Precise measurements of these hormones can help identify animals that are at risk of developing infections, allowing farmers to take preventive measures and reduce the need for antibiotics (Neethirajan and Kemp, 2021).

5. Integration with other precision livestock farming technologies: Precision endocrinology can be integrated with other precision livestock farming technologies such as precision feeding, precision health monitoring, and precision genetics to create a comprehensive (Tekın et al., 2021).

Regulatory and ethical considerations

International regulations and guidelines for the use of hormones in animal production

International regulations and guidelines for the use of hormones in animal production vary across different countries and regions. Here are some key points:

1. European union: In the European Union, the use of substances having a hormonal action for growth promotion in farm animals is prohibited (Serratosa et al., 2006).

2. United states: In the United States, the Food and Drug Administration (FDA) has approved the use of growth-promoting hormones such as estradiol, progesterone, testosterone, trenbolone acetate, and zeranol in beef cattle and sheep (Qaid and Abdoun, 2022). However, no steroid hormone implants are approved for growth purposes in dairy cows, veal calves, pigs, or poultry (Drouillard, 2018).

3. Benefits of hormone use: The use of hormonal active growth promoters ("hormones") in farm animals can increase the production of veal and beef significantly, up to 15% (Stephany et al., 2001). Research has demonstrated that hormone treatment improves growth rate, nitrogen retention, and feed conversion efficiency during the five- to six-week period before slaughter (Velle, W.1982).

4. Human health concerns: The former Scientific Committee on Veterinary Measures relating to Public Health (SCVPH) thoroughly re-evaluated the risks to human health from hormone residues in bovine meat and meat products treated with six hormones for growth promotion (Kesler, 1985). In 1999, this independent scientific advisory body concluded that no acceptable daily intake (ADI) could be established for any of these hormones (Alemanno and Capodieci, 2012).

5. Compliance with limits: The levels of hormone residues found in beef originating from the USA are, in the vast majority of cases, below the Maximum Residue Limit as recommended by the FAO/WHO Joint Expert Committee of Food Additives (Stephany et al., 2001). More than 20 countries use growth-promoting hormones regularly and have reduced greenhouse gas emissions, energy use, water use, and reactive nitrogen loss of beef production in comparison to beef raised without growth-promoting hormones (Di Benedetto et al., 2017).

In conclusion, the use of hormones in animal production is a complex issue that involves various factors such as regulations, health risks, and economic importance. While some countries have banned the use of hormones for growth promotion in farm animals, others have approved certain hormones under strict conditions. It is important to consider the potential risks and benefits of using hormones in animal production and to establish safe limits for hormone residues in meat to ensure consumer safety.

Ethical considerations regarding hormone use and animal welfare

Hormone use in animals can raise ethical concerns related to animal welfare. Here are some key considerations:

1. Proper treatment of experimental animals: To avoid undue suffering of animals, it is important to follow ethical considerations during animal studies (Festing and Wilkinson, 2007). This includes providing the best possible care to animals from both ethical and scientific points of view.

2. The "4 R's": The "4 R's" of animal research ethics refer to the principles of Replacement, Reduction, Refinement, and Responsibility (Osinubi, 2013). Responsibility refers to concerns around promoting animal welfare by improving experimental animals' social life, developing advanced scientific methods for objectively determining sentience, consciousness, experience of pain, and intelligence in the animal kingdom, as well as effective involvement in the professionalization of the public discussion on animal ethics (Kiani et al., 2022).

3. Clear rationale and reasoning: Researchers must have a clear rationale and reasoning for the use of animals in a research project. They must have a reasonable expectation of generating useful data from the proposed experiment, and the research study should be designed in such a way that it involves the lowest possible sample size of experimental animals while producing useful results (Kiani et al., 2022).

4. Genetic engineering: Genetic engineering of animals can raise ethical issues, including concerns for animal welfare (Ormandy et al., 2011). Governing bodies have started to develop relevant policies, often calling for increased vigilance and monitoring of potential animal welfare impacts (Ormandy et al., 2011).

5. Wild animal welfare: Research involving wild animals can also raise ethical concerns related to animal welfare (Soulsbury et al., 2020). It is important to consider the welfare of wild animals and the ethics of using them in scientific research (Soulsbury et al., 2020).

6. Guidelines for ethical conduct: The American Psychological Association (APA) has developed guidelines for ethical conduct in the care and use of nonhuman animals in research (Akins and Panicker, 2012). These guidelines are for psychologists working with nonhuman animals and are informed by Section 8.09 of the Ethical Principles of Psychologists and Code of Conduct (Behnke and Jones, 2012). The acquisition, care, housing, use, and disposition of nonhuman animals in research must comply with applicable federal, state, and local laws and regulations, institutional policies, and with international conventions to which the United States is a party (Behnke and Jones, 2012).

Consumer perceptions and attitudes towards hormone-treated animal products

Consumer perceptions and attitudes towards hormone -treated animal products are influenced by various factors, including animal welfare, health concerns, and ethical considerations.

1. Consumer attitudes: Consumer attitudes, subjective norms, and perceived behavioral control have significant and positive effects on their purchase intentions of animal welfare-friendly products (Chang and Chen, 2022).

2. Awareness of animal welfare: Consumers are aware of the pain animals experience in animal agriculture, and this awareness can influence their dietary choices (Fonseca and Sanchez-Sabate, 2022).

3. Health concerns: Study participants acknowledged that what was bad for the animals was ultimately bad for consumers, particularly in relation to the control of disease, and consumer negativity regarding the use of antibiotics in intensive production systems, with all being linked to human health concerns (Clark et al., 2016).

4. Animal welfare labels: Consumers who have greater concern for animal welfare consume animal products less frequently, purchase welfare-friendly products more frequently, and indicate a greater use of welfare-related labels (Clark et al., 2016).

Overall, consumers' perceptions and attitudes towards hormone-treated animal products are complex and influenced by various factors. Animal welfare, health concerns, and ethical considerations are some of the key factors that can influence consumer choices.

Case studies and success stories

Real-world examples of successful endocrine manipulation in animal production

1. Growth promotion: Hormones like estrogen, progesterone, and testosterone have been used to promote growth in beef cattle, dairy cows, and pigs. For instance, estrogenic compounds like diethylstilbestrol (DES) and hexoestrol have been shown to increase weight gain and feed efficiency in beef cattle. The use of testosterone implants in beef cattle has also increased muscle mass and reduced fat deposition (Reddy et al., 2014).

2. Reproductive management: Hormones play a crucial role in managing the reproductive processes of animals. Prostaglandins are used to synchronize estrus in dairy cows, while gonadotropins like follicle-stimulating hormone (FSH) and luteinizing hormone (LH) are used to induce ovulation in mares and cows. Progesterone is used to suppress estrus in mares and synchronize estrus in beef cattle (Bó and Mapletoft, 2014).

3. Disease prevention: Hormones can be used to prevent diseases in animals. Melatonin has been shown to enhance the immune system and reduce the incidence of infectious diseases in sheep and cattle. Growth hormone has improved the immune response in chickens and reduced the incidence of infectious diseases (Leshchinsky and Klasing, 2001).

4. Transgenic animals: Transgenic animals are used to produce specific proteins or traits. Transgenic cows have been developed to produce human lactoferrin in their milk, which has potential applications in infant formula and other food products (Ã et al., 2006).

Comparative analysis of production outcomes using hormone manipulation techniques

1. DES implants: DES implants have been shown to result in an increase of about 12% in gain and an improvement in feed conversion efficiency (FCE) of approximately 10% in animals (Kling et al., 2012).

2. Glucocorticoid manipulation: Glucocorticoid manipulation has been used in survival studies of breeding black-legged kittiwakes (Crossin et al., 2016). However, there are concerns about the safety of hormonal application in farm animal production (Qaid and Abdoun, 2022).

3. Thyroid hormone manipulation: Differential effects of thyroid hormone manipulation and β adrenoceptor agonists have been studied (Mostyn et al., 2008).

In general, hormone manipulation techniques have been shown to improve production outcomes in animal production. However, safety concerns regarding hormonal application in farm animal production exist, and alternative methods to enhance

animal production are being explored (Sillence, 2004).

Lessons learned and implications for future research and practice

Lessons learned

1. Genetic defects of protein folding in the secretory pathway can cause endocrine disorders in animals (Morishita and Arvan, 2021).
2. New evidence obtained in genetically manipulated research animals has challenged the old paradigm of the relationship between growth hormone and reproduction (Dosouto et al., 2019).
3. There are relationships between endocrine traits and life histories in wild animals, but there are also potential pitfalls in studying these traits (Dosouto et al., 2019).
4. A range of techniques allows analysis of the pituitary gland in awake mammalian models in unparalleled detail, complementing large-scale imaging studies (Hoa et al., 2019).
5. Epidemiology and animal experiments have often focused on male reproduction, testicular dysgenesis, and decreased fertility (Skakkebæk et al., 2001).

Implications for future research and practice

1. Researchers should continue to investigate the genetic basis of endocrine disorders in animals, particularly those caused by defects of protein folding in the secretory pathway (Zhu et al., 2002).
2. Future studies should consider the new evidence obtained in genetically manipulated research animals when examining the relationship between growth hormone and reproduction (Aubin-Horth and Renn, 2009).
3. Researchers should be aware of potential pitfalls when studying endocrine traits in wild animals and should take steps to mitigate these risks (Kavlock et al., 1996).
4. The range of techniques available for analyzing the pituitary gland in awake mammalian models should be further explored and developed (Hoa et al., 2019).
5. Future research on endocrine disruptors should consider the potential effects on male reproduction, testicular dysgenesis, and decreased fertility (Skakkebæk et al., 2001).

The lessons learned and implications for future research and practice underscore the need for continued investigation into the genetic and physiological basis of endocrine disorders, as well as the potential risks and benefits of manipulating endocrine systems in animals.

Conclusions and future directions

Key Findings

1. Successful Endocrine Manipulation Techniques: Endocrine manipulation techniques have been applied successfully in animal production. These include using hormones for growth promotion, reproductive management, disease prevention, and the development of transgenic animals to produce specific proteins or traits.

2. Production outcomes: The use of hormones like DES implants has led to increased weight gain and improved feed conversion efficiency in animals. However, there are concerns about the safety of hormonal applications in farm animal production, and alternative methods are being explored.

3. Lessons learned: Genetic defects in the secretory pathway can cause endocrine disorders in animals. New evidence from genetically manipulated research animals has challenged our understanding of the relationship between growth hormone and reproduction. Researchers should consider potential pitfalls when studying endocrine traits in wild animals.

Insights

1. Ethical considerations: The use of hormones in animal production raises ethical concerns related to animal welfare. Proper treatment of experimental animals, adherence to the "4 R's" of animal research ethics, and clear rationale for using animals in research are essential.

2. International regulations: Regulations regarding the use of hormones in animal production vary across different countries and regions. Some countries, like the European Union, prohibit the use of hormonal growth promoters, while others, like the United States, approve specific hormones under strict conditions. Consumer perceptions and attitudes are influenced by factors such as animal welfare, health concerns, and ethical considerations.

3. Precision endocrinology: Precision endocrinology offers promising prospects for improving animal health, welfare, and productivity through the precise measurement of hormones and biomarkers. It can enhance reproductive management, growth, and feed efficiency while reducing the use of antibiotics and improving animal welfare.

4. Health risks: Endocrine-disrupting compounds (EDCs) can interfere with the normal function of the endocrine system and are associated with various health issues, including reproductive problems, early puberty, cancer, and neurobehavioral disorders.

5. Future research: Future research should focus on investigating the genetic basis of endocrine disorders in animals, considering new evidence from genetically manipulated animals, and addressing the relationship between endocrine traits and life histories in wildlife. Additionally, researchers should explore advanced

techniques for analyzing the pituitary gland in awake mammalian models.

In summary, the review highlights the successful applications of endocrine manipulation techniques in animal production, ethical considerations in using hormones, variations in international regulations, the role of precision endocrinology, health risks associated with endocrine-disrupting compounds, and the need for further research to understand and improve endocrine manipulation in animals.

This review on endocrine manipulation in animal production highlights several gaps in knowledge and areas for further research. Here are some of the key gaps and research needs:

1 Safety and long-term effects: While the review discusses the benefits of endocrine manipulation, there is a need for further research to comprehensively assess the long-term safety and potential adverse effects of using hormones and other endocrine-disrupting compounds (EDCs) in animal production. This includes studying the impact on animal health, meat quality, and the environment. Longitudinal studies on the health and welfare of animals exposed to hormone manipulation are necessary.

2. Alternative approaches: The review mentions that there are concerns about the safety of hormonal applications in animal production, which suggests a need for research into alternative approaches to achieve similar or improved production outcomes. Identifying and developing non-hormonal growth promoters and disease prevention methods is essential.

3. Consumer perception and behavior: Further research is needed to understand the factors influencing consumer perceptions and behaviors regarding hormone-treated animal products. This includes studying consumer attitudes toward animal welfare, health concerns, and ethical considerations. Additionally, research can explore the effectiveness of labeling and communication strategies in influencing consumer choices.

4. Ethical considerations: Research should continue to explore ethical considerations in using hormones in animal production. This includes refining ethical guidelines for the treatment of experimental animals, promoting the principles of Replacement, Reduction, Refinement, and Responsibility (the "4 R's"), and addressing concerns related to genetic engineering and research involving wild animals.

6. Genetic and molecular studies: To better understand endocrine disorders and the genetic basis of hormone-related traits, further research is needed to investigate genetic defects in the secretory pathway that can lead to endocrine disorders. This research could provide insights into endocrine-related health issues in animals.

6. Precision endocrinology: The review highlights the potential of precision endocrinology in improving animal health, welfare, and productivity. Future research should focus on developing and validating precise and non-invasive methods for measuring hormones and biomarkers in animals to enhance reproductive management, growth, and animal welfare.

7. Environmental impact: While the review briefly mentions the impact of endocrine manipulation on the environment, there is a need for comprehensive research on how the use of hormones and EDCs in animal production affects soil, water, air, and ecosystems. Studying the environmental risks and sustainability of these practices is crucial.

8. Global comparative analysis: Further research can involve conducting a global comparative analysis of regulations and practices related to hormone use in animal production. This would provide a more in-depth understanding of regional variations and their implications for international trade and consumer preferences.

9. Transgenic animal welfare: As the review mentions the development of transgenic animals, future research should focus on assessing the welfare and ethical considerations related to these genetically modified animals. This research can help establish ethical guidelines and best practices for using transgenic animals in animal production.

10 Health risks of endocrine-disrupting compounds: Research should continue to investigate the health risks associated with endocrine-disrupting compounds (EDCs), particularly their links to diseases such as cancer, reproductive problems, and neurobehavioral disorders. This includes conducting epidemiological studies to better understand the effects of EDC exposure on human and animal health.

In conclusion, this review highlights various knowledge gaps and areas for further research in the field of endocrine manipulation in animal production. Addressing these gaps and conducting further research in these areas will contribute to a better understanding of the effects, safety, and ethical considerations related to hormone use in animal agriculture.

References

Abdoli, R., Zamani, P., Mirhoseini, S. Z., Ghavi Hossein-Zadeh, N., & Nadri, S. (2016). A review on prolificacy genes in sheep. *Reproduction in Domestic Animals = Zuchthygiene, 51*(5), 631–637.

Aubin-Horth, N. A. D. I. A., & Renn, S. C. (2009). Genomic reaction norms: using integrative biology to understand molecular mechanisms of phenotypic plasticity. *Molecular ecology, 18*(18), 3763-3780.

Agrawal, A., Pandey, R. S., and Sharma, B. (2010). Water Pollution with Special Reference to Pesticide Contamination in India. *Journal of Water Resource and Protection, 02*(05), 432–448.

Akins, C. K., & Panicker, S. (2012). *Ethics and regulation of research with nonhuman animals. APA Handbook of Research Methods in Psychology*, Vol 1: Foundations, Planning, Measures, and Psychometrics., 1, 75–82.

Alemanno, A., & Capodieci, G. (2012). Testing the limits of global food governance: The case of ractopamine. *European Journal of Risk Regulation, 3*(3), 400–407.

Alsen, M., Sinclair, C., Cooke, P., Ziadkhanpour, K., Genden, E., & Van Gerwen, M. (2021). Endocrine disrupting chemicals and thyroid cancer: An overview. *Toxics, 9*(1), 1 –26.

Ansar Ahmed, S. (2000). The immune system as a potential target for environmental estrogens (endocrine disrupters): A new emerging field. *Toxicology, 150*(1–3), 191–206. Arcand-Hoy, L. D., & Benson, W. H. (1998). Fish reproduction: An ecologically relevant indicator of endocrine disruption. *Environmental Toxicology and Chemistry, 17*(1), 49–57.

Archer, E., Wolfaardt, G. M., & Wyk, J. H. Van. (2017). Pharmaceutical and personal care products (PPCPs) as endocrine disrupting contaminants (EDCs) in South *African Surface Waters. 43*(4), 684–706.

Asaye, M., Biyazen, H., & Girma, M. (2014). Genetic engineering in animal production : Applications and prospects. *Biochemistry and Biotechnology Research, 2*(2), 12–22.

Ball, P. J. H., & Peters, A. R. (2007). *Reproduction in Cattle: Third Edition*. In Reproduction in Cattle: Third Edition.

Baroiller, J. F., & D'Cotta, H. (2001). Environment and sex determination in farmed fish. *Comparative Biochemistry and Physiology - C Toxicology and Pharmacology, 130*(4), 399–409.

Baroiller, Jean François, & D'Cotta, H. (2016). The reversible sex of gonochoristic fish: Insights and consequences. *Sexual Development, 10*(5–6), 242–266.

Baruselli, P. S., Reis, E. L., Marques, M. O., Nasser, L. F., & Bó, G. A. (2004). The use of hormonal treatments to improve reproductive performance of anestrous beef cattle in tropical climates. *Animal Reproduction Science*, *82–83*, 479–486.

Barzilai, N., Huffman, D. M., Muzumdar, R. H., & Bartke, A. (2012). The critical role of metabolic pathways in aging. *Diabetes, 61*(6), 1315–1322.

Behnke, S. H., & Jones, S. E. (2012). Ethics and ethics codes for psychologists. In S. J. Knapp, M. C. Gottlieb, M. M. Handelsman, & L. D. VandeCreek (Eds.), APA handbook of ethics in psychology, Vol. 1. Moral foundations and common themes. *American Psychological Association, 1*, (pp. 43–74).

Benini, E., Politis, S. N., Nielsen, A., Sørensen, S. R., Tomkiewicz, J., & Engrola, S. (2022). Type of hormonal treatment administered to induce vitellogenesis in European eel influences biochemical composition of eggs and yolk-sac larvae. *Fish Physiology and Biochemistry, 48* (1), 185–200.

Berryman, D. E., Christiansen, J. S., Johannsson, G., Thorner, M. O., & Kopchick, J. J. (2008). Role of the GH/IGF-1 axis in lifespan and healthspan: Lessons from animal models. *Growth Hormone and IGF Research, 18*(6), 455–471.

Berryman, D. E., & List, E. O. (2017). Growth Hormone's effect on adipose tissue: Quality versus quantity. *International Journal of Molecular Sciences, 18*(8), 1–28.

Bharati, A. C., Prasad, B., Mallick, S., Masram, D. S., Kumar, A., & Saxena, G. K. (2023). *Animal and plant hormone*. Handbook of Biomolecules, 151–175.

Bianco, A. C., Maia, A. L., da Silva, W. S., & Christoffolete, M. A. (2005). Adaptive activation of thyroid hormone and energy expenditure. *Bioscience reports, 25*(3-4), 191-208.

Bó, G. A., & Mapletoft, R. J. (2014). Theriogenology Historical perspectives and recent research on superovulation in cattle. *Theriogenology, 81*(1), 38–48.

Bohua, L., Yuexin, W., Yakun, O., Kunlan, Z., Huan, L., & Ruipeng, L. (2023). Ethical framework on risk governance of synthetic biology. *Journal of Biosafety and Biosecurity, 5*(2), 45–56.

Bradford, H., McKernan, C., Elliott, C., & Dean, M. (2022). Consumers' perceptions and willingness to purchase pork labelled 'raised without antibiotics.' *Appetite, 171* (December 2021), 105900.

Brinks, V., Jiskoot, W., & Schellekens, H. (2011). Immunogenicity of Therapeutic Proteins : *The Use of Animal Models*. 2379–2385.

Budd, A. M., Banh, Q. Q., Domingos, J. A., & Jerry, D. R. (2015). Sex control in fish: Approaches, challenges and opportunities for aquaculture. *Journal of Marine Science and Engineering, 3*(2), 329–355.

Campbell, C. G., Borglin, S. E., Green, F. B., Grayson, A., Wozei, E., & Stringfellow, W. T. (2006). Biologically directed environmental monitoring, fate, and transport of estrogenic endocrine disrupting compounds in water: A review. *Chemosphere, 65*(8), 1265–1280.

Chang, M. Y., & Chen, H. S. (2022). Consumer Attitudes and Purchase Intentions in Relation to Animal Welfare-Friendly Products: Evidence from Taiwan. *Nutrients, 14* (21), 1–17.

Chappel, S. C., & Howles, C. (1991). Review: Reevaluation of the roles of luteinizing hormone and follicle-stimulating hormone in the ovulatory process. *Human Reproduction, 6*(9), 1206–1212.

Chatakondi, N. G., Butts, I. A. E., & Dunham, R. A. (2018). Hormone preparation, dosage calculation, and injection techniques for induced spawning of foodfish. *Southern Regional Aquaculture Center*, 0425, 1–6.

Chaves, V. E., Júnior, F. M., & Bertolini, G. L. (2013). The metabolic effects of growth hormone in adipose tissue. *Endocrine, 44*(2), 293–302

Chen, Y., Arsenault, R., Napper, S., & Griebel, P. (2015). Models and methods to investigate acute stress responses in cattle. *Animals, 5*(4), 1268–1295.

Choksi, N. Y., Jahnke, G. D., St. Hilaire, C., & Shelby, M. (2003). Role of Thyroid Hormones in Human and Laboratory Animal Reproductive Health. Birth Defects Research Part B - *Developmental and Reproductive Toxicology, 68*(6), 479–491.

Clark, B., Stewart, G. B., Panzone, L. A., Kyriazakis, I., & Frewer, L. J. (2016). A Systematic review of public attitudes, perceptions and behaviours towards production diseases associated with farm animal welfare. *Journal of Agricultural and Environmental Ethics, 29*(3), 455–478.

Collier, R. J., Dahl, G. E., & Vanbaale, M. J. (2006). Major advances associated with environmental effects on dairy cattle. *Journal of Dairy Science, 89*(4), 1244–1253.

Contreras-Correa, Z. E., Messman, R. D., Swanson, R. M., & Lemley, C. O. (2023). Melatonin in Health and disease: A perspective for livestock production. *Biomolecules, 13* (3).940.

Crawford, A. G., Hurrell, P., Paroschy, K., & Pereira, C. (2017). Pharmaceuticals and other Endocrine Disrupting Compounds in Natural Water Systems.

Crews, D., and Mclachlan, J. A. (2006).Epigenetics, evolution, endocrine disruption, health, and disease. *Endocrinology,* 147(6), s4–s10.

Crossin, G. T., Love, O. P., Cooke, S. J., & Williams, T. D. (2016). Glucocorticoid manipulations in free-living animals: considerations of dose delivery, life-history context and reproductive state. *Functional Ecology, 30*(1), 116-125.

Crowe, M. A., Hostens, M., & Opsomer, G. (2018). Reproductive management in dairy cows - The future. *Irish Veterinary Journal, 71*(1), 1–13.

Devlin, R. H., Sakhrani, D., Tymchuk, W. E., Rise, M. L., & Goh, B. (2009). Domestication and growth hormone transgenesis cause similar changes in gene expression in coho salmon (Oncorhynchus kisutch). Proceedings of the *National Academy of Sciences of the United States of America, 106*(9), 3047–3052.

Di Benedetto, N. A., Corbo, M. R., Campaniello, D., Cataldi, M. P., Bevilacqua, A., Sinigaglia, M., & Flagella, Z. (2017). The role of plant growth promoting bacteria in improving nitrogen use efficiency for sustainable crop production: A focus on wheat. *AIMS Microbiology, 3*(3), 413–434.

Dosouto, C., Calaf, J., Polo, A., Haahr, T., & Humaidan, P. (2019). Growth hormone and reproduction: Lessons learned from animal models and clinical trials. *Frontiers in Endocrinology, 10*, 1–7.

Dourmad, J.-Y., Le Velly, V., Gourdine, J.-L., & Renaudeau, D. (2022). Effect of ambient temperature in lactating sows, a meta-analysis and simulation approach in the context of climate change. *Animal - Open Space, 1*(1), 100025.

Drouillard, J. S. (2018). Current situation and future trends for beef production in the United States of America - A review. *Asian-Australasian Journal of Animal Sciences, 31* (7), 1007–1016.

Edwards, T. A. (2010). Control methods for bovine respiratory disease for feedlot cattle. *Veterinary Clinics of North America - Food Animal Practice, 26*(2), 273–284.

El-Kholy, M. S., El-Hindawy, M. M., Alagawany, M., Abd El-Hack, M. E., & El-Sayed, S. A. E. G. A. E. H. (2017). Dietary Supplementation of Chromium Can Alleviate Negative Impacts of Heat Stress on Performance, Carcass Yield, and Some Blood Hematology and Chemistry Indices of Growing Japanese Quail. *Biological Trace Element Research, 179*(1), 148–157.

Festing, S., & Wilkinson, R. (2007). The ethics of animal research. Talking Point on the use of animals in scientific research. *EMBO Reports, 8*(6), 526–530.

Fonseca, R. P., & Sanchez-Sabate, R. (2022). Consumers' attitudes towards animal suffering: A systematic review on awareness, willingness and dietary change. *International Journal of Environmental Research and Public Health, 19*(23),16372.

Fraga-Corral, M., Ronza, P., Garcia-Oliveira, P., Pereira, A. G., Losada, A. P., Prieto, M. A., Quiroga, M. I., & Simal-Gandara, J. (2022). Aquaculture as a circular bio-economy model with Galicia as a study case: How to transform waste into revalorized by-products. *Trends in Food Science and Technology, 119*, 23–35.

Fraser, D. (1995). Science, Values and Animal Welfare: Exploring the 'Inextricable Connection.' *Animal Welfare, 4* (2), 103–117.

Ghassemi Nejad, J., Ghaffari, M. H., Ataallahi, M., Jo, J. H., & Lee, H. G. (2022). Stress concepts and applications in various matrices with a focus on hair cortisol and analytical methods. *Animals, 12*(22).

González-Bulnes, A., Baird, D. T., Campbell, B. K., Cocero, M. J., García-García, R. M., Inskeep, E. K., López-Sebastián, A., McNeilly, A. S., Santiago-Moreno, J., Souza, C. J. H., & Veiga-López, A. (2004). Multiple factors affecting the efficiency of multiple ovulation and embryo transfer in sheep and goats. *Reproduction, Fertility and Development, 16*(4), 421–435.

Gore, A. C., Crews, D., Doan, L. L., & Merrill, M. La. (2014). *Introduction To Endocrine Disrupting Chemicals (Edcs)* A Guide For Public Interest Organizations And Policy-Makers.

Guiguen, Y., Fostier, A., Piferrer, F., & Chang, C. F. (2010). Ovarian aromatase and estrogens: A important role for gonadal sex differentiation and sex change in fish. *General and Comparative Endocrinology, 165*(3), 352–366.

Hackney, A. C., & Lane, A. R. (2015). *Exercise and the regulation of endocrine hormones.* In Progress in Molecular Biology and Translational Science (1st ed., Vol. 135). Elsevier Inc.

Hlordzi, V., Kuebutornye, F. K. A., Afriyie, G., Abarike, E. D., Lu, Y., Chi, S., & Anokyewaa, M. A. (2020). The use of Bacillus species in maintenance of water quality in aquaculture: A review. *Aquaculture Reports, 18*, 100503.

Hoa, O., Lafont, C., Fontanaud, P., Guillou, A., Kemkem, Y., Kineman, R. D., ... & Mollard, P. (2019). Imaging and manipulating pituitary function in the awake mouse. *Endocrinology, 160*(10), 2271-2281.

Hoga, C. A., Almeida, F. L., & Reyes, F. G. R. (2018). A review on the use of hormones in fish farming: Analytical methods to determine their residues. CYTA - *Journal of Food, 16*(1), 679–691.

Hsu, B. Y., Pakanen, V. M., Boner, W., Doligez, B., Eeva, T., Groothuis, T. G. G., Korpimäki, E., Laaksonen, T., Lelono, A., Monaghan, P., Sarraude, T., Thomson, R. L., Tolvanen, J., Tschirren, B., Vásquez, R. A., & Ruuskanen, S. (2022). Maternally transferred thyroid hormones and life-history variation in birds. *Journal of Animal Ecology, 91*(7), 1489–1506.

Hull, K. L., & Harvey, S. (2014). Growth hormone and reproduction: A review of endocrine and autocrine/ paracrine interactions. *International Journal of Endocrinology*, 2014.

Hume, D. A., Whitelaw, C. B. A., & Archibald, A. L. (2011). The future of animal production: Improving productivity and sustainability. *Journal of Agricultural Science, 149*(S1), 9-16.

Isaksson, O. G. P., Eden, S., & Jansson, J. O. (1985). Mode of action of pituitary growth hormone on target cells. *Annual Review of Physiology, 47*, 483–499.

Jainudeen, M. R., Wahid, H., & Hafez, E. S. E. (2016). Ovulation induction, embryo production and transfer. *Reproduction in Farm Animals*, *3*, 405–430.

Jameson, J. L. (2015). Principles of Endocrinology. *Endocrinology: Adult and Pediatric, 1–2*, 3-15.e1.

Jeong, S. H., Kang, D., Lim, M. W., Kang, C. S., & Sung, H. J. (2013). Risk assessment of growth hormones and antimicrobial residues in meat. *Toxicological Research, 26* (4), 301–313.

Kabir, E. R., Rahman, M. S., & Rahman, I. (2015). A review on endocrine disruptors and their possible impacts on human health. *Environmental Toxicology and Pharmacology, 40* (1), 241–258.

Kadarmideen, H. N., Thompson, R., Coffey, M. P., & Kossaibati, M. A. (2003). Genetic parameters and evaluations from single- and multiple-trait analysis of dairy cow fertility and milk production. *Livestock Production Science, 81*(2–3), 183–195.

Kavlock, R. J., Daston, G. R., Derosa, C., Gray, L. E., Kaattari, S., Lucier, G., Luster, M., Mac, M. J., Maczka, C., Miller, R., Moore, J., Rolland, R., Scott, G., Sheehan, M., Sinks, T., & Tilson, H. A. (1996). Research needs for the risk assessment of health and environmental effects of endocrine disruptors : A Report of the U . S . EPA-sponsored Workshop. 104(August).

Khan, S., Jamal, M. A., Khan, I. M., Ullah, I., Jabbar, A., Khan, N. M., & Liu, Y. (2023). Factors affecting superovulation induction in goats (Capra hericus): An analysis of various approaches. *Frontiers in Veterinary Science*, 10. 1152103.

Khan, S. U., Jamal, M. A., Su, Y., Wei, H. J., Qing, Y., & Cheng, W. (2022). Towards improving the outcomes of multiple ovulation and embryo transfer in sheep, with particular focus on donor superovulation. *Veterinary Sciences, 9*(3), 117.

Kiani, A. K., Pheby, D., Henehan, G., Brown, R., Sieving, P., Sykora, P., Marks, R., Falsini, B., Capodicasa, N., Miertus, S., Lorusso, L., Dondossola, D., Tartaglia, G. M., Ergoren, M. C., Dundar, M., Michelini, S., Malacarne, D., Bonetti, G., Dautaj, A., & Bertelli, M. (2022). Ethical considerations regarding animal experimentation. *Journal of Preventive Medicine and Hygiene, 63*(2), E255–E266.

Kling, P., Jönsson, E., Nilsen, T. O., Einarsdottir, I. E., Rønnestad, I., Stefansson, S. O., & Björnsson, B. T. (2012). The role of growth hormone in growth, lipid homeostasis, energy utilization and partitioning in rainbow trout: interactions with leptin, ghrelin and insulin-like growth factor I. *General and Comparative Endocrinology, 175*(1), 153-162.

Knigh, J. (2021). Endocrine system 1: overview of the endocrine system and hormones. *Nursing Times [Online], 117*(5), 38–42.

Kramer, K., & Meijboom, F. L. B. (2021). Using breeding technologies to improve farm animal welfare: What is the ethical relevance of telos?. *Journal of Agricultural and Environmental Ethics, 34*(1), 2.

Laborde, D., Mamun, A., Martin, W., Piñeiro, V., & Vos, R. (2021). Agricultural subsidies and global greenhouse gas emissions. *Nature Communications, 12*(1), 1–9.

Lee, H. R., Jeung, E. B., Cho, M. H., Kim, T. H., Leung, P. C. K., & Choi, K. C. (2013). Molecular mechanism(s) of endocrine-disrupting chemicals and their potent oestrogenicity in diverse cells and tissues that express oestrogen receptors. Journal *of Cellular and Molecular Medicine, 17*(1), 1–11.

Leshchinsky, T. V., & Klasing, K. C. (2001). Divergence of the inflammatory response in two types of chickens. *Developmental & Comparative Immunology, 25* (7), 629-638.

Li, H., Yang, Y., Hong, W., Huang, M., Wu, M., & Zhao, X. (2020). Applications of genome editing technology in the targeted therapy of human diseases: mechanisms, advances and prospects. Signal *transduction and targeted therapy, 5*(1), 1.

Lloyd, J. K. F. (2017). Minimising stress for patients in the veterinary hospital: Why it is important and what can be done about it. *Veterinary Sciences, 4*(2).

Lucy, M. C., McDougall, S., & Nation, D. P. (2004). The use of hormonal treatments to improve the reproductive performance of lactating dairy cows in feedlot or pasture-based management systems. *Animal Reproduction Science, 82–83*, 495–512.

Lusk, J. L., & Norwood, F. B. (2011). Animal welfare economics. *Applied Economic Perspectives and Policy, 33* (4), 463–483.

Mallozzi, M., Bordi, G., Garo, C., & Caserta, D. (2016). The effect of maternal exposure to endocrine disrupting

chemicals on fetal and neonatal development: A review on the major concerns. *Birth Defects Research Part C - Embryo Today: Reviews, 108*(3), 224–242.

Mantovani, A. (2016). Endocrine disrupters and the safety of food chains. Hormone research in paediatrics, 86(4), 279-288.

Maria Dimova, C., & Stirk, P. M. R. (2019). 済無No Title No Title No Title. 9–25.

Martínez-Miró, S., Tecles, F., Ramón, M., Escribano, D., Hernández, F., Madrid, J., Orengo, J., Martínez-Subiela, S., Manteca, X., & Cerón, J. J. (2016). Causes, consequences and biomarkers of stress in swine: An update. *BMC Veterinary Research, 12*(1), 1–9.

McCormick, S. D., & Romero, L. M. (2017). Conservation endocrinology. BioScience, 67(5), 429–442.

McEwen, B. S. (1992). Steroid hormones: Effect on brain development and function. *Hormone Research, 37*(SUPPL. 3), 1–10.

McFarlane, G. R., Salvesen, H. A., Sternberg, A., & Lillico, S. G. (2019). On-Farm Livestock genome editing using cutting edge reproductive technologies. *Frontiers in Sustainable Food Systems, 3*, 1–7.

Metcalfe, C. D., Bayen, S., Desrosiers, M., Muñoz, G., Sauvé, S., & Yargeau, V. (2022). An introduction to the sources, fate, occurrence and effects of endocrine disrupting chemicals released into the environment. *Environmental research, 207*, 112658.

Moore, K., & Thatcher, W. W. (2006). Major advances associated with reproduction in dairy cattle. *Journal of Dairy Science, 89*(4), 1254–1266.

Morgado, J. N., Lamonaca, E., Santeramo, F. G., Caroprese, M., Albenzio, M., & Ciliberti, M. G. (2023). Effects of management strategies on animal welfare and productivity under heat stress: A synthesis. Frontiers *in Veterinary Science*, 10.

Morishita, Y., & Arvan, P. (2021). HHS Public Access. 1–21.

Mostyn, A., Bos, P. M., Litten, J. C., Laws, J., Symonds, M. E., Clarke, L., Mostyn, A., Bos, P. M., Litten, J. C., Laws, J., & Michael, E. (2008). Differential effects of thyroid hormone manipulation and abundance in adipose tissue and thermoregulation in neonatal pigs ND ES SC RIB. 6278 (March 2016).

Mylonas, C. C., Fostier, A., & Zanuy, S. (2010). Broodstock management and hormonal manipulations of fish reproduction. *General and Comparative Endocrinology, 165*(3), 516–534.

Mylonas, C. C., Fostier, A., Zanuy, S., & Cabanes, R. De. (n.d.). Accepted manuscript.

Nachman, K. E., & Smith, T. J. S. (2015). Hormone Use in Food Animal Production: Assessing Potential Dietary Exposures and Breast Cancer Risk. *Current Environmental Health Reports, 2*(1), 1–14.

Nagy, A., & Malcomson, R. D. G. (2022). *The endocrine system*. Keeling's Fetal and Neonatal Pathology, 677–708.

Nazir, S., Khan, N., Azmat, H., Naveed, S., Ramzan, M. M., & Davies, S. J. (2023). Efficacy of various concentrations of synthetic hormones on the induced breeding of Channa marulius (Sole). Journal *of the World Aquaculture Society, 54*(1), 143–155.

Neethirajan, S., & Kemp, B. (2021). Digital Livestock Farming. *Sensing and Bio-Sensing Research*, 32, 100408.

Neethirajan, S., Reimert, I., & Kemp, B. (2021). Measuring farm animal emotions—sensor-based approaches. *Sensors (Switzerland), 21*(2), 1–23.

Oberbauer, A. M. (2015). Developmental programming: The role of growth hormone. *Journal of Animal Science and Biotechnology, 6*(1), 1–7.

Okeke, E. S., Chukwudozie, K. I., Nyaruaba, R., Ita, R. E., Oladipo, A., Ejeromedoghene, O., Atakpa, E. O., Agu, C. V., & Okoye, C. O. (2022). *Antibiotic resistance in aquaculture and aquatic organisms: a review of current nanotechnology applications for sustainable management*. In Environmental Science and Pollution Research (Vol. 29, Issue 46). Springer Berlin Heidelberg.

Oltenacu, P. A., & Algers, B. (2005). Selection for increased production and the welfare of dairy cows: Are new breeding goals needed? *Ambio, 34*(4–5), 311–315.

Ormandy, E. H., Dale, J., & Griffin, G. (2011). Animal Welfare Bien-être des animaux Genetic engineering of animals : Ethical issues , including welfare concerns. *Canadian Veterinary Journal, 52*(5), 544–550.

Osinubi, A. A. (2013). Animal Experimentation : Contemporary Ethical Considerations. 4(1), 3–13.

Palmer, A. J., Chung, M. Y., List, E. O., Walker, J., Okada, S., Kopchick, J. J., & Berryman, D. E. (2009). Age-related changes in body composition of bovine growth hormone transgenic mice. *Endocrinology, 150*(3), 1353–1360.

Paragliola, R. M., Papi, G., Pontecorvi, A., & Corsello, S. M. (2017). Treatment with synthetic glucocorticoids and the hypothalamus-pituitary-adrenal axis. *International Journal of Molecular Sciences, 18*(10).

Peel, C. J., Fronk, T. J., Bauman, D. E., & Gorewit, R. C. (1983). Effect of exogenous growth hormone in early and late lactation on lactational performance of dairy cows. *Journal of Dairy Science, 66*(4), 776-782.

Perisse, I. V., Fan, Z., Singina, G. N., White, K. L., & Polejaeva, I. A. (2021). Improvements in Gene Editing Technology Boost Its Applications in Livestock. *Frontiers in Genetics, 11,* 1–21.

Persson, I. (2000). Estrogens in the causation of breast, endometrial and ovarian cancers - Evidence and hypotheses from epidemiological findings. *Journal of Steroid Biochemistry and Molecular Biology, 74*(5), 357–364.

Pert, C. B. (1988). *Neuropeptides, the Emotions, and Bodymind_1985. Advances*, 6(8), 8–16.

Piferrer, F. (2001). Endocrine sex control strategies for the feminization of teleost fish. *Aquaculture, 197*(1–4), 229–281.

Pittius, C. W., Hennighausen, L., Lee, E., Westphal, H., Nicols, E., Vitale, J., & Gordon, K. (1988). A milk protein gene promoter directs the expression of human tissue

plasminogen activator cDNA to the mammary gland in transgenic mice. *Proceedings of the National Academy of Sciences of the United States of America, 85*(16), 5874–5878.

Qaid, M. M., & Abdoun, K. A. (2022). Safety and concerns of hormonal application in farm animal production: a review. *Journal of Applied Animal Research, 50*(1), 426–439.

Reddy, B. V., Sivakumar, A. S., Jeong, D. W., Woo, Y. B., Park, S. J., Lee, S. Y., ... & Hwang, I. (2015). Beef quality traits of heifer in comparison with steer, bull and cow at various feeding environments. *Animal Science Journal, 86*(1), 1-16.

Ribaroff, G. A., Wastnedge, E., Drake, A. J., Sharpe, R. M., & Chambers, T. J. G. (2017). Animal models of maternal high fat diet exposure and effects on metabolism in offspring: a meta-regression analysis. *Obesity Reviews, 18*(6), 673–686.

Rogol, A. D., Roemmich, J. N., & Clark, P. A. (2002). Growth at puberty. *Journal of Adolescent Health, 31*(6 Suppl.), 192–200.

Serratosa, J., Blass, A., Rigau, B., Mongrell, B., & Rigau, T. (2006). Residues from veterinary medicinal products , growth promoters and performance enhancers in food-producing animals : a European Union perspective promoters , performance enhancers and veterinary medicinal products : the legal framework. *Revue scientifique et technique, 25*(2), 637–653.

Shahudin, M. S., Ghani, A. A. A., Zamri-Saad, M., Zuki, A. B., Abdullah, F. F. J., Wahid, H., & Hassim, H. A. (2018). The necessity of a herd health management programme for dairy goat farms in Malaysia. *Pertanika journal of tropical agricultural science, 41*(1).

Sillence, M. N. (2004). Technologies for the control of fat and lean deposition in livestock. *The Veterinary Journal. 167*, 242–257.

Skakkebaek, N. E., De Meyts, E. R., & Main, K. M. (2001). Testicular dysgenesis syndrome: an increasingly common developmental disorder with environmental aspects. *Apmis, 109*(S103), S22-S30.

Skórka-Majewicz, M., Goschorska, M., Żwierełło, W., Baranowska-Bosiacka, I., Styburski, D., Kapczuk, P., & Gutowska, I. (2020). Effect of fluoride on endocrine tissues and their secretory functions -- review. *Chemosphere, 260*.

Skoupá, K., Šťastný, K., & Sládek, Z. (2022). Anabolic Steroids in Fattening Food-Producing Animals—A Review. Animals, 12(16).

Soulsbury, C. D., Gray, H. E., Smith, L. M., Braithwaite, V., Cotter, S. C., Elwood, R. W., Wilkinson, A., & Collins, L. M. (2020). The welfare and ethics of research involving wild animals: A primer. *Methods in Ecology and Evolution, 11* (10), 1164–1181.

Squires, E. L. (1993). Use of progestins in open and pregnant mares. *Animal Reproduction Science, 33*(1-4), 183-193.

Stephany, R. W., Gregor, K., Stephany, R., McLachlan, J., & Risbridger, G. (2001). Hormones in meat: Different approaches in the EU and in the USA. *APMIS, Supplement, 109*(103), 357–364.

Tekın, K., Yurdakök-Dıkmen, B., Kanca, H., & Guatteo, R. (2021). Precision livestock farming technologies: Novel direction of information flow. *Ankara Universitesi Veteriner Fakultesi Dergisi, 68*(2), 193–212.

Temple, D., & Manteca, X. (2020). Animal welfare in extensive production systems is still an area of concern. *Frontiers in Sustainable Food Systems, 4*, 545902.

Tilbrook, A. J., & Ralph, C. R. (2018). Hormones, stress and the welfare of animals. *Animal Production Science, 58*(3), 408–415.

Todini, L. (2007). Thyroid hormones in small ruminants: Effects of endogenous, environmental and nutritional factors. *Animal, 1*(7), 997–1008.

Toppari, J., Larsen, J. C., Christiansen, P., Giwercman, A., Grandjean, P., Guillette, L. J., Jégou, B., Jensen, T. K., Jouannet, P., Keiding, N., Leffers, H., McLachlan, J. A., Meyer, O., Müller, J., Rajpert-De Meyts, E., Scheike, T., Sharpe, R., Sumpter, J., & Skakkebæk, N. E. (1996). Male reproductive health and environmental xenoestrogens. *Environmental Health Perspectives, 104(Suppl. 4*), 741–803.

Uthayanan, K., & Sundareswaran, S. (2023). An outline of endocrine disrupting chemicals and the influencing hormones: what the human society ought to know?. *Journal of Drug Delivery and Therapeutics, 13*(5), 66-72.

Velazquez, M. A., Spicer, L. J., & Wathes, D. C. (2008). The role of endocrine insulin-like growth factor-I (IGF-I) in female bovine reproduction. *Domestic Animal Endocrinology, 35*(4), 325–342.

Velle, W. (1982). The use of hormones in animal production. *FAO Animal Production and Health Paper. Oslo, Norway*.

Wakabayashi, Ã, H., Yamauchi, K., & Takase, M. (2006). Lactoferrin research, technology and applications. *International Dairy Journal, 16*, 1241–1251.

West, J. W. (1999). Nutritional strategies for managing the heat-stressed dairy cow. *Journal of Animal Science, 77* Suppl 2(April), 21–35.

Williams, N. H., Cline, T. R., Schinckel, A. P., & Jones, D. J. (1994). The impact of ractopamine, energy intake, and dietary fat on finisher pig growth performance and carcass merit. *Journal of Animal Science, 72*(12), 3152–3162.

Yaron, Z., & Levavi-Sivan, B. (2011). Hormonal control of reproduction and growth, Endocrine regulation of fish reproduction. In encyclopedia of fish physiology: From Genome to Environment: Volume 1-3 (Vols. 1–3). Elsevier Inc.

Yazdan, M. M. S., Kumar, R., & Leung, S. W. (2022). The environmental and health impacts of sSteroids and Hormones in wastewater effluent, as well as wxisting removal technologies: A Review. *Ecologies, 3*(2), 206–224.

Yilmaz, B., Terekeci, H., Sandal, S., & Kelestimur, F. (2020). Endocrine disrupting chemicals: exposure, effects on human health, mechanism of action, models for testing and strategies for prevention. *Reviews in Endocrine and Metabolic Disorders, 21*(1), 127–147.

Zhao, Y., Yang, L., Su, G., Wei, Z., Liu, X., Song, L., Hai, C., Wu, D., Hao, Z., Wu, Y., Zhang, L., Bai, C., & Li, G. (2022). Growth Traits and Sperm Proteomics Analyses of Myostatin Gene-Edited Chinese Yellow Cattle. Life, 12(5).

Zhou, L., Li, M., & Wang, D. (2021). Role of sex steroids in fish sex determination and differentiation as revealed by gene editing. *General and Comparative Endocrinology, 313*(1), 113893.

Zhu, X., Zhou, A., Dey, A., Norrbom, C., Carroll, R., Zhang, C., ... & Steiner, D. F. (2002). Disruption of PC1/3 expression in mice causes dwarfism and multiple neuroendocrine peptide processing defects. *Proceedings of the National Academy of Sciences, 99*(16), 10293-10298.

Zohar, Y., & Mylonas, C. C. (2001). Endocrine manipulations of spawning in cultured fish: From hormones to genes. *Aquaculture, 197*(1–4), 99–136.

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Pasteurella multocida and *Mannheimia haemolytica*; virulence factors, diseases, and notably increasing antibiotic resistance rate among their isolates: a comprehensive review

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ABSTRACT

The current review on *Pasteurella multocida* and *Mannheimia haemolytica* tried to shed light on these two organisms due to their medical and economic importance as well as to their elevating antibiotic resistance rate among the isolates from animals basically cattle, sheep, and goats. In this comprehensive review, we screened both old and recently published works that are available electronically on authorized scientific sites. Here we provide the latest data on those organisms their structure, suitable growth conditions, virulence factors, pathogenesis, their associated diseases, and their distribution along with antibiotic resistance emergence and the possibility of more new emergences of resistant isolates among species of both organisms. Lastly, we reviewed all the old and modern methods for diagnosis, controlling, and preventing the occurrence of diseases caused by these organisms besides studying and reviewing the effective ways to manage antibiotic resistance issues. Our review concluded that more specific research is needed to shed light on phenotype and genotype differences among those organisms, some issues should be subjected to intensive investigations and research focus such as emerging and re-emerging infectious diseases caused by these organisms and antigenic variants between agents. Evaluation of innate and adaptive immunity after infections or vaccinations is important for producing more specific drugs or vaccines in the future.

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Introduction

1. *Pasteurella multocida* (*P. multocida*) and *Mannheimia haemolytica* (*M. haemolytica*) are worldwide spread Gram-negative coccobacilli bacteria that exist in the same family (Pasteurellaceae) that are responsible for many diseases in animal species more intensively in cattle and sheep leading to a huge loss in the livestock sector (Abd-Elsadek et al., 2021). According to WOAH (World Organization for Animal Health), about 20% of bovine deaths come from Pasteurella infections WOAH (2020). Both microrganisms are found on the surface of the respiratory tract as commensal microflora but can

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proliferate and act as an opportunistic pathogen in case of association with other illnesses or any condition that could predispose infection by dropping the immune system defense mechanisms (Abbas et al., 2023; Hsuan et al., 1999).

Different strains of *P. multocida* are multi-species organisms which causes very serious illness in animals like hemorrhagic septicemia (HS) in cattle and buffalo, fowl cholera, atrophic rhinitis in swine, snuffles in rabbits, enzootic calf pneumonia and shipping fever (bovine respiratory disease). Although some of these diseases are classified as multifactorial diseases (Mahrous et al., 2022); till now no epidemiological study clearly explained the role of *P. multocida*. Pasteurellosis is one of the zoonotic diseases; Human cases of Pasteurellosis usually are due to bites or scratches by pets-animals or by getting contact with contaminated animal food or other belongings (Hanafy et al., 2022; Omaleki et al., 2016). *M. haemolytica* formerly had been classified as *Pasteurella haemolytica* type A until Angen and his colleagues had reclassified it in a separate genus (Angen et al., 1999a). Bovine respiratory disease (also known as shipping fever) is a major disease that comes as a result of infection with *M. haemolytica* and other etiologies in addition to septicemia and pneumonia in sheep and goats, also sheep mastitis has been reported to be caused by *M. haemolytica*. Some studies suggest that *M. haemolytica* can form biofilm on epithelial cells of the respiratory system (Amat, 2019).

2. Historical background

The organism had been identified for the first time by French chemist Louis Pasteur in 1880 when he had worked on fowl cholera, so named after his name as Patsteurella (Pasteur, 1880). According to recent classification *P. multocida* has been grouped into three subtypes they are; *P. multocida* subsp. *multocida*, *P. multocida* subsp. *gallicida*, and *P. multocida* subsp. *Septica*. *P. multocida* divided to five different serotypes (A, B, D, E, and F) based on capsular polysaccharides such as hyaluronic acid, heparin, arabinose, mannose, galactose and chondroitin. These serotypes again divided according to their lipopolysaccharides to 16 different serovars from (1 to 16) (Harper et al., 2006). *M. haemolytica* previously had been isolated for the first time from bovine pneumonia in the 1920s and classified as *Pasteurella haemolytica* to be distinguished from none haemolytic one (Jones, 1921).. *P. haemolytica* had two distinct biotypes: A biotype, which ferments arabinose, and T biotype, which ferments trehalose. The T biotype was later reclassified as *P. trehalosi* and eventually placed into a separate genus called *Bibersteinia trehalosi* (Blackall et al., 2007).

Angen and his colleagues re-identified *P. haemolytica* to be *M. haemolytica* (Serotypes 1, 2, 5–9, 12–14, 16, and 17). *M. haemolytica* serotypes seem to be closely related to each other, especially S1, S2, and S6 on serologic and genetic bases, but sing molecular techniques for specific genes has revealed the differences between these strains (Angen et al., 1999b; Davies & Lee, 2004).

3. Bacteriology and growth conditions

P. multocida and *M. haemolytica* are Gram-negative, non-motile, non-spore-forming small facultative coccobacillus both of them are members of the family Pasteurellaceae. Organisms grow well at 37°C on blood agar and also can be grown on dextrose-starch, casein-sucrose-yeast (CSY), chocolate, Mueller-Hinton, or brain heart infusion (BHI) agar, however, there is no growth on MacConkey agar. *M. haemolytica* is producing β-hemolysis on blood agar (Garzon et al., 2023; Rice et al., 2007).

Most clinical isolates of *P. multocida* are catalase, oxidase, indole, and ornithine decarboxylase positive. Also, isolates can ferment sucrose and glucose without gas production and reduce nitrate to nitrite on biochemical bases. *M. haemolytica* fail to ferment D-mannose which is first step to differentiate it from *P. multocida* (Carter & Cole Jr, 2012).

4. Virulence factors

Tremendous virulence factors are possessed by *P. multocida* and *M. haemolytica*. As shown in Table 1

Table 1. Some important immunogenic factors of both *P. multocida* and *M. haemolytica*

Factor	Origin	Mechanism	Immunogenicity action
Capsule	Bacterial surface	Antiphagocytic and resistance of lysis	Weak
Leukotoxin	Secreted	Leukocyte necrosis	Strong
LPS	Bacterial cell wall	Proinflammatory	Lipid A is strong
OmpP2	Outer membrane	Biofilm formation and adhesion	Weak
OmpA	Outer membrane	Adherence to lactoferrin	Strong
PlpE	Outer membrane	Unknown	Strong
Biofilm (*M. haemolytica*)	Surface proteins (adhesins)	Antibiotic resistance and escape immune response	Generally weak

these factors are similar to each other due to their mutual origin, however, some genetic differences between those factors like molecular weight, protein synthesis pathways, and mode of action have been disclosed (De la Mora et al., 2007).

4. 1. *P. multocida*

4.1.1. Capsule

P. multocida has five different capsular groups according to the capsular polysaccharide they possess. The genes encoding for these strains was assumed to be located in a single region on the genome until studies done by DeAngelis and White showed that capsules of strain A and B were encoded from different region, so they concluded that may refer to farther capsule variation in future (DeAngelis & White, 2004; Mirtneh et al., 2022). The main role of the capsule is the resistance to phagocytosis and resistance to complement-mediated lysis (innate immunity) mainly through expressing hydrophilic substrate (Boyce et al., 2000; Guan et al., 2020).

4.1.2. *Pasteurella multocida* toxin (PMT)

This toxin is about 146 - kDa protein encoded on lysogenic bacteriophage existing only in the genome of the toxin-producing strains (type D strain; the causative agent of atrophic rhinitis in swine). The toxin is released upon bacterial lysis and inters host cells via receptor-mediated endocytosis (Pullinger et al., 2004). The role of PMT in pathogenesis is the activation of some proteins in the cytoplasm of host cells such as G proteins, (Gq and G12/13) and Cβ. These interactions between protein pathways result in activation of specific signal transduction pathways which lead to cytoskeletal changes, inhibition of osteoblast maturation and activation of the mitogen activating protein. PMT may also obstacle the migration of dendritic cells to lymph nodes (Kitadokoro et al., 2007).

4.1.3. Iron acquisition proteins

Iron is a very important compound for survival of bacteria; *P. multocida* has many different ways to acquire iron from host depots such as ferric hydroxides, hem, and transferrin. Acquiring iron requires a specific outer membrane receptor and a periplasmic-binding protein. Siderophores are the keyword in the iron acquisition process; these compounds are normally possessed by many microorganisms. It can be classified into three structural types: hydroxy carboxylate, catecholate, or hydroxamate. Only one siderophore, named multocidin, is unique to *P. multocida* (Boyce et al., 2010). Recent study has shown that lacking of some iron acquisition protein genes such as HgbA and HgbB genes may lead to forming of nontypical *P. multocida*, eventually, *P. multocida* may change or lose its capsule due to shortness or absence of iron acquisition proteins (Balevi et al., 2023).

4.1.4. Lipopolysaccharide (LPS)

LPS generally is a very important component of Gram-negative bacteria rich with variability in polymeric O-antigen repeats, but with exception of *P. multocida*; there is a lack of O-antigen repeats. LPS of *P. multocida* is varied from one strain to another for example LPS of strain A differs from other strains LPS. Also, LPS fails to induce the same immune response in two different animal species and therefore no heterogenous immunity can be induced (Harper et al., 2011; Mombeni et al., 2021).

4.1.5. Fimbriae and adhesin

Full genome sequencing of P. multocia pm70 had been carried out, it revealed that all types possess in their genome many genes such as (ptfA, flp1, and flp2) that encode for different types of fimbriae (May et al., 2001). Although *P. multocida* genetically has different genes and different types of fimbriae, they functionally do the same role in pathogenesis by attaching to surfaces of the respiratory tract and adhering to host cells (colonization and invasion) (Yanthi et al., 2021).

4.1.6. Sialic Acid metabolism

Sialic acid has several roles in the pathogenesis of *P. multocida* including stabilization of membranes and regulation of transmembrane receptors function. Bacteria can utilize host sialic acid as a carbon and nitrogen source by sialidases enzyme. Existing of sialic acid may enhance the ability of bacteria to avoid the immune system of the host by blocking some receptors or altering some molecules features (Vimr et al., 2004).

4.1.7. Hyaluronidase

Hyaluronidase enzyme assumed to play important role in pathogenesis of *P. multocida* usually produced by type B strain that causing hemorrhagic septicemia in buffaloes and cattle. HA is thought to function as a "spreading factor" by degrading hyaluronic acid on host cells, thus allowing the spread of bacteria in tissues (Carter & Chengappa, 1980). Type A and B strain especially B: 2 strain is the only type that produces this enzyme so some researchers suggest it may be a useful tool for diagnosing and differentiating type B: 2 strain of *P. multocida* (Chung et al., 2001; Raj et al., 2023).

4.1.8. Outer membrane proteins (OMPs)

OMPs of *P. multocida* was identified firstly as one of five important immunogens able to evoke an immune response, it is mass about 37kDa; monoclonal antibody produced against this protein enhanced immunity of rabbit and protected from homologous

challenge; but for heterologous strains, some limitations have been noted. The main role of these outer membrane proteins is facilitating adherence of invading organisms to host cell surfaces. The major outer membrane proteins of *P. multocida* are OmpH, OmpA, Oma87, Pm1069, iron related proteins, and Tbp (transferrin binding protein) (Gogoi et al., 2018), recently (Zhao et al., 2021) approved that pcgD outer membrane has a critical role in P. multocida infection.

Biofilm formation has been reported for the first time by (Petruzzi et al., 2017). They confirmed the ability of *P. multocida* serogroup A to produce a type of biofilm in vitro, produced biofilm is smooth and thicker than biofilm produced by other encapsulated bacteria. In the same trial, they suggested that capsular polysaccharide (CPS) may interfere with biofilm formation by blocking the adherence of bacteria to the surfaces or by preventing the EPS matrix from encasing large numbers of bacterial. In a different study (Petruzzi et al., 2018) experimentally found that *P. multocida* was able to produce biofilm in vivo on avian pulmonary tissues and suggested that it may be correlate to chronic infections of fowl cholera.

4.2. *Mannheimia haemolytica* and LPS

M. haemolytica produces many types of capsular antigens it differs according to serotype of the organism for example S1, S9, and S12 but generally they evoke host immune responses besides it is main role in avoiding phagocytosis by macrophages. LPS extremely necessary for *M. haemolytica* pathogenesis it has a classical endotoxic activity able to stimulate pro-inflammatory mediator production and inflammation specially O-antigen which is known to be the most immunogenic component of LPS (Table 1). activation of these inflammatory substances results in damaging endothelium layers and causing vascular leakage (Confer & Ayalew, 2013; Kamarulrizal et al., 2022).

4.2.1. Outer membrane proteins (OMPs)

This compound structure of OMPs maintains the hemostasis of bacterium by controlling the influx and efflux of nutrients in addition to coordinating signals transduction. There are different types of outer membrane proteins with different functions, basically they act as adhesin factors or iron acquisition proteins, the function of some proteins is still unknown. Examples of these proteins are PlpE, PlpF, OmpA, and OmpP2 (Avalos-Gómez et al., 2020).

Members of the OmpA family of proteins resemble a good model for vaccine production for several bacteria. *M. haemolytica* OmpA is an approximately 30 kDa, heat-modifiable, it was found to be a highly immunogenic protein with porin activity. Members of the OmpA protein family share some features such as homology with heat-modifiable OMPs protein from numerous bacteria (Confer & Ayalew, 2013; Ujvari et al., 2019). OmpA protein has adhesin properties and recently had been identified as a binding factor to lactoferrin (Zhang et al., 2016). Although a lot of these proteins are not immunogenic, they potentially trigger immune response for producing opsonizing antibodies which afforded protective against *M. haemolytica*. Transferrin binding proteins B family also remove iron from transferrin, mostly outer membrane immunogens are strong immunogens (Samaniego-Barrón et al., 2016).

4.2.2. Adhesins

M. haemolytica adhesins include a 68 kDa glycoprotein, N-acetyl-D-glucosamine (MhA) that responsible for adherence to epithelial cells of trachea and activates the oxidative burst of host neutrophils through glycoprotein receptor, it has major role in colonization of respiratory tract surfaces. *M. haemolytica* produces this adhesin more intensively when cultivated at 41°C (Wynn & Clawson, 2022; Zhang et al., 2016) in addition to this many types of antigens act as adhesins such as OmpA, lipoprotein1, Filamentous hemagglutinin and fimbriae (Kisiela & Czuprynski, 2009).

4.2.3. Toxins

M. haemolytica is known to produce leukotoxin (LKT) since it associated with infection in bovine respiratory disease where serious damage on host macrophages and neutrophils has been reported (Maheswaran et al., 1992). Four genes are coding for leukotoxin (lktC, lktA, lktB, and lktD) lktA codes for the structural toxin, and lktC codes for activation, whereas products of lktB and lktD are coding for secretion of toxin (Rice et al., 2007). LKT is the most important virulence factor in *M. haemolytica* – induced pneumonia. LKT is also responsible for hemolysis in vitro, toxin is produced by all serotypes of the bacterium except the mutant one (LKT – deficient mutant) (Confer & Ayalew, 2018).

4.2.4. Biofilm formation

Biofilm concept refers to a type of microorganism arrangement, often in two different levels the first one is attachment of microorganisms to each other and secondly attaching to adjuvant surfaces here adherent bacteria become embedded within a slimy extracellular matrix mainly in shape of extracellular polymeric substances (EPSs) (Montes García et al., 2018; Olson et al., 2002). These biofilms protect the bacterial cells against the host immune response and antibiotic treatment bacteria within a biofilm are more resistant to antibiotics than planktonic cells.

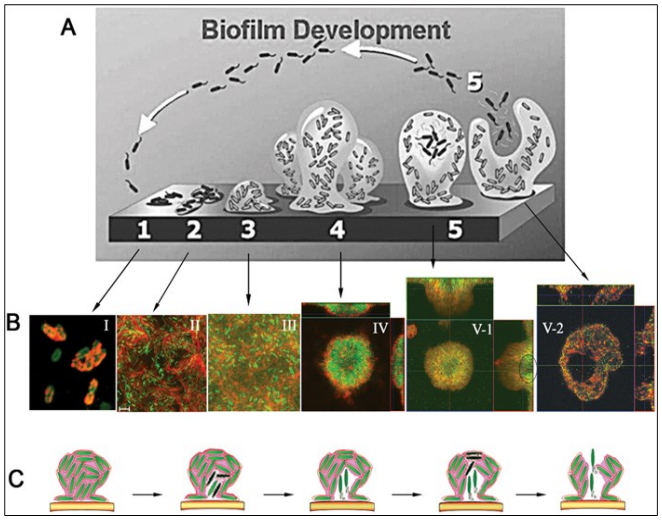

Figure 1. Stages of biofilm development.
Initial attachment, (2) Irreversible attachment, (3) Maturation I, (4) Maturation II, and (5) Dispersion. Each stage of development in the diagram is paired with a photomicrograph of a developing P. aeruginosa biofilm created by P. Dirckx, K. Sauer, and D. Davies (100).

Production of biofilm in *M. haemolytica* has been reported in bovine respiratory disease (Boukahil & Czuprynski, 2016; Kandimalla et al., 2022).

4.2.5. Produced enzymes

M. haemolytica secreted numerous enzymes that participated somehow in pathogenesis. Neuraminidase (sialidase) is a major extracellular protein associated with many bacterial species; it acts on sialic acid residues of host mucosal sialo-glycoprotein exposing underlying carbohydrate moieties used for bacterial adhesion. Neuraminidase has a role in enhancing bacterial adherence to cell surfaces that have been affected by this enzyme action.

M. haemolytica genes also code for O-sialoglycoprotease enzyme which hydrolyzes peptide bonds within glycoproteins. Studies found that homologs of the protein were detected in several Gram-negative bacteria; however, secretion in the form of O-sialoglycoprotease was restricted to *M. haemolytica* serotypes (Klima et al., 2018). Protease recently has been detected on supernatant from *M. haemolytica* culture it is responsible for cleaving of host immunoglobulins, especially IgG1into 39,12 and 7 kDa bands whereas no effect on IgG2 has been recorded (Kotelnikova et al., 2016a).

5. Pathogenesis

5.1. *P. multocida* pathogenesis

Although *P. multocida* possesses a huge set of virulence factors the mechanism of action for some of them is still anonymous. Induced diseases and susceptible hosts are so variable with strong specificity it may be due to strain, adaptation, possession and expression of specific virulence factors. Also host factors such as anatomic features and innate immunity, above all the ecology factors which partially neglected in studies on *P. multocida* infections (Harper et al., 2006; Peng et al., 2019).

P. multocida is an opportunistic pathogen that normally colonizes surfaces of the respiratory tract of hosts so it directly moves down into tissues after association with suitable predisposing factors. Studies on fowl cholera suggest that mucosal membranes may act as a portal of entry, the same for open wounds (Pattison et al., 2007).

Firstly *P. multocida* attaches to host cells using adhesins, which are surface proteins that bind to specific receptors on the host cell surface. Secondly one of the crucial steps in the pathogenesis of systemic disease is resistance of phagocytosis by bacterial capsule; the capsule has two major roles in pathogenesis; one is producing hyaluronidase enzyme that degrades the hyaluronic acid of the host cell to facilitate entering into tissues leading to necrosis and cellulitis, and the other one is avoiding lysis through complement system proteins. Also one of important steps in *P. multocida* pathogenesis it is ability to invoked a high inflammatory response when growing in any parenchymatous tissue this damage usually is local as abscesses (Diallo & Frost, 2000; Smallman et al., 2024); found evidence for multiplication of *P. multocida* on blood in vivo. However, some strains survive in vitro in serum with active complement proteins and they think growing in serum did not necessary correlate with virulence in avian strain of *P. multocida*. In fowl cholera and hemorrhagic septicemia usually, animals death is attributed to spreading petechiae on serosal and epicardial surfaces, which indicates consumptive coagulopathy common to many endotoxemias.

P. multocida produces various toxins, dermonecrotic toxin (in atrophic rhinitis in pigs), leukotoxin, these toxins can damage host cells, disrupt the immune response, and promote bacterial survival and dissemination, beside that also *P. multocida* can modulating cytokine production that result in can manipulate the immune response to its advantage (Boyce et al., 2010; Sahoo et al., 2020).

5.2. *M. haemolytica* pathogenesis

M. haemolytica has a variety of virulence factors involved in pathogenesis process as a general mechanism of these factors resemble its counterparts in other Gram-negative bacteria, few differences may exist depending upon bacteria-host interaction mechanisms. Infection with *M. haemolytica* is

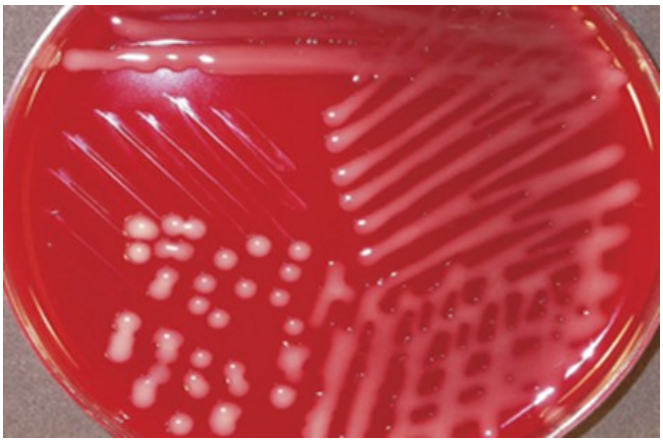

Figure 2. P. multocida on blood agar. Photo taken from atlas.sund.ku.dk

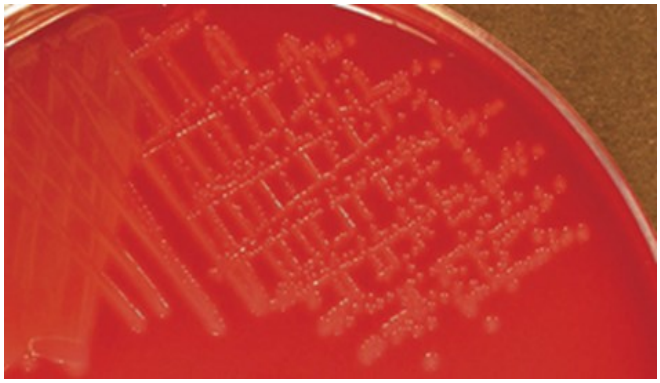

Figure 3. *M. haemolytica* on blood agar. (101)

reported to be related specifically with ruminants (Confer & Ayalew, 2018). Classic immunogens such as capsule, LPS, toxins and neuraminidase are major factors contributing in clinical manifestation of *M. haemolytica* infections (Rice et al., 2007).

Routes of entering same as for *P. multocida* through respiratory tract after association with viral diseases and/or other illness that pave the road for their proliferation and invasion of tissues. Unlike *P. multocida*, *M. haemolytica* has limitations in infected hosts and produced clinical cases (Gelasakis et al., 2015). Capsule and surface proteins are very important in colonization of bacteria. Penetration of epithelial cells is facilitated by secretion of neuraminidase enzyme which is an extracellular protein present in many bacterial species. Its role involves cleaving sialic acid residues from host mucosal sialoglycoproteins, which exposes underlying carbohydrate structures that bacteria use for adhesion. This mechanism is crucial for bacterial attachment to host cells and tissues (Zhao et al., 2023). *M. haemolytica* neuraminidase was demonstrated to be a large, approximately 160 kDa, extracellular, enzyme produced by different serotypes, mainly during stationary growth phase (Highlander, 2001; Klima et al., 2014) Neuraminidase is produced in vivo in *M. haemolytica*-infected cattle as evidenced by the rise in anti-neuraminidase antibodies during infection (Briggs et al., 2021; Confer & Ayalew, 2018).

Several proteases were recently identified in the culture supernatant of *M. haemolytica* S2, and those were primarily cysteine proteases or metalloproteases (Rico et al., 2017).

Proteases that can break down IgG have been found in Streptococcus pyogenes, *Pseudomonas aeruginosa*, *Proteus mirabilis*, and Staphylococcus *aureus*. Although a search of the genome databases of 10 *M. haemolytica* did not reveal a specific IgG protease, the presence of multiple endopeptidases suggests the potential for IgG cleavage. Furthermore, (Ayalew et al., 2017)) identified a potential IgA

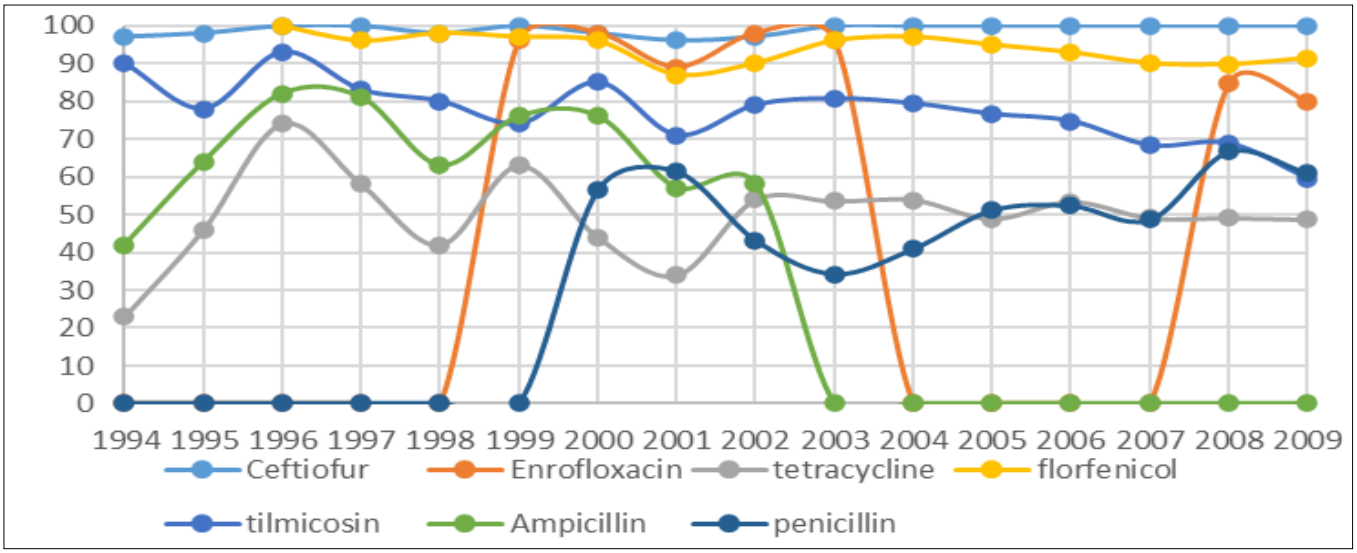

Figure 4. Susceptibility of *Pasteurella multocida* and *Mannheimia haemolytica* to some antibiotics within fifteen years

protease in *M. haemolytica* culture supernatant using proteomic analyses. IgA proteases in other bacteria are typically associated with autotransporter molecules, facilitating bacterial invasion of mucosal surfaces and evasion of host defenses. Bacterial IgA proteases are also known to be immunogenic, triggering the production of local and systemic antibodies in infected hosts (Kotelnikova et al., 2016b). The gene encoding sialoglycoprotease and its enzymatic activity were linked to various serotypes of *M. haemolytica*, with similar proteins found in several Gram-negative bacteria. However, the secretion of O-sialoglycoprotease was specific to *M. haemolytica* serotypes Vaccination of calves with a recombinant sialoglycoprotease-fusion protein induced antibody production against the protein (Shewen et al., 2003). Antibodies against sialoglycoprotease were detected in the sera of cattle challenged with live *M. haemolytica* (Lee et al., 1994; McGill & Sacco, 2020). The exact role of sialoglycoprotease in respiratory pathogenesis remains unclear. Studies have shown that it can cleave cell surface glycoproteins such as CD34 (present on hematopoietic progenitors and endothelium), CD43 (leukosialin found on leukocytes), CD44 (a receptor for hyaluronic acid involved in cell adhesion), CD45 (a leukocyte common antigen involved in signal transduction), and platelet selectin (Ramírez-Rico et al., 2024).

Leukotoxin has been subjected to intensive studies since in vitro damage by *M. haemolytia* toxin had been reported on bovine neutrophils. Leukotoxin type A lyse cells mainly through pores formation; leads to the efflux of K+ and influx of Ca2+ leading to swelling and eventual cell lysis, apoptosis and mitochondrial dysfunction (Atapattu & Czuprynski, 2005; Hsuan et al., 1999). Dominant chemokines in production of pro-inflammatory response are cytokines, such as tumor necrosis factor-α (TNF-α), interleukin (IL)-1β and IL-8. Researchers suggest that LPS has basic role in cytotoxicity of macrophages and monocytes (Jeyaseelan et al., 2000).

Biofilm formation is one *M. haemolytica* tools to persist and invade respiratory system. Conducted in vitro trials was found that OmpA adhesin is the main factor that facilitates attachment of *M. haemolytica* to plastic surfaces so OmpA considers the first step toward biofilm formation (Boukahil & Czuprynski, 2015) and (Kisiela & Czuprynski, 2009) reported in their study that lipoprotein 1 (Lpp1) also has a role in attachment of *M. haemolytica* to bovine epithelial cells. (Boukahil & Czuprynski, 2015); took advantage of this knowledge (adhesins role in biofilm formation) to design blocking substances such as monosaccharides to compete with bacterial adhesins in attaching to host cells surfaces this resulted in inhibition of bacterial adhesins and obstacles biofilm formation.

6. Epidemiology

6.1. *P. multocida*

Theodore Kitt in 1885 first described the causative agent called Bacterium bipolare multocidum, later Bacillus bovisepticus by Flugge in 1896. In 1900 Lignieres proposed the name *Pasteurella*, Pasteurella *haemolytica* was given by Newsom and Cross in 1932 and this has also been well accepted (Rehmtulla & Thomson, 1981). Incidence of *P. multocida* is high globally and so distribution of serotypes and serovars of this organism. Despite intensive studies on this pathogen neither developed countries nor undeveloped counties have ultimate way to remove clinical manifestations of this pathogen. Outbreaks redundantly have been reported in tropical regions more specifically in Asia and Africa as hemorrhagic septicemia and fowl cholera diseases which are caused by *P. multocida* strain B and strain A respectively (Moustafa et al., 2015).

Morbidity and mortality of shipping fever average approximately 14 and 1% respectively (USDA National Animal Health Monitoring System (NAHMS) (Storz et al., 2000) revealed in their study the important role of coronavirus in shipping fever wherein two different outbreaks high percentage of association of respiratory bovine coronavirus with shipping fever had been reported as 61% and 74%. Their study recommended that vaccination and management methods against shipping fever should consider and include this virus as main factors in the infection.

Diseases of *P. multocida* in livestock are varying in clinical features and animal species, most important diseases besides (BRDC) are hemorrhagic septicemia (HS) in cattle and buffaloes, fowl cholera in poultry, atrophic rhinitis in pigs and snuffles in rabbits, pneumonic and septicemic Pasteurellosis. Generally, Pasteurellosis has roughly 100% mortality in endemic areas of Africa and Asia (OIE 2009). Deaths usually occur in older calves and young adults alike. In non-endemic areas, massive epizootics may occur with a high morbidity rate may reach up to 100% if treatment is not applied at an early stage of disease onset (Benkirane & De Alwis, 2002). As for Fowl cholera it is a very serious disease with a mortality rate reach to 90% disease had been recorded in Europe since the 18th century. Outbreaks of fowl cholera associated with high mortality are typically seen in laying flocks (Singh et al., 2014). Several studies carried out on diversity of *P. multocida* associated with fowl cholera outbreaks; the findings of such epidemiological studies

vary in their outcome, and some outbreaks are associated with a single genotype for several years (Singh et al., 2013). However, outbreaks with multiple genotypes have been reported in turkeys this contradiction some reports suggest problems in Heddleston serotyping method (reproducibility or stability). Overall, the evidence of study carried by (Singh et al., 2014). Researchers strongly suggested a single strain of *P. multocida* causing these repeated outbreaks of fowl cholera. They identified *P. multocida* by PCR then serotyped isolates using the Heddleston scheme and genotyped using both multilocus sequence typing (MLST) method and an enterobacterial repetitive intergenic consensus (ERIC)-PCR method. Kumar and his colleagues hypothesized that the variation in the distribution and occurrence of *P. multocida* infections in different agroclimatic regions of India includes different species of animals and avian species. They also observed outbreaks throughout the year regardless of the season. Their findings contradict the notion that HS mostly occurred during the monsoon or post-monsoon period and this is consistent with the previous findings (Kumar et al., 2004; Loneragan, 2001). Globally serotype B is second dominant strain in fields after type A strain of *P. multocida*. Hemorrhagic septicemia causing strain in Africa is prevalently caused by B strain and strain E is most dominant in Asia but some studies showed that both B and E are isolated from Africa and Asia from clinical cases of *P. multocida* infections (Smith et al., 2021).

In Europe, although HS is uncommon diseases which firstly been reported in pigs in Spain; *P. multocida* has been intensively studied from clinical to advance genetic approach levels especially in bovine respiratory complex (Borge et al., 2011). All epidemiological studies assure that *P. multocida* is a ubiquitous organism that lives as commensal on surfaces of respiratory tracts of animals acts as an opportunistic pathogen cannot clinically induce diseases alone unless in presence of predisposing factors such as Mycoplasma species most relevant with calf pneumonia infections *M. haemolytica*, *H. somni coronavirus, adenovirus* and Bovine syncytial virus (Autio et al., 2007; Hirose et al., 2003). Outbreak of bovine haemorrhagic septicaemia caused by *P. multocida* type B has been reported for first tinme by (Cuevas et al., 2020). Investigation of nontypical of *P. multocida* has been carried by (Sakmanoğlu et al., 2021) Surprisingly they found the nontypical strains number is higher than previously reported numbers. They found out of 92 isolates of *P. multocida* 34 are type A:3A as the most dominant strain type about (36.95%) all isolated were from animals with a respiratory disease which may imply to hidden role of nontypical *P. multocida* in pathogenesis too.

6.2. *Mannheimia haemolytica*

M. haemolytica in comparison with *P. multocida*; it tends to be moderate in severity with few numbers of diseases although recently an increase in annual incidence has been mentioned. In an epidemiological study conducted by (Biesheuvel et al., 2021) in Netherlands, morbidity rate reached 65% in dairy cows while in veal calves reached 20.6%. They attributed death in highly productive dairy cows to acute pleuropneumonia caused by *M. haemolytica*. This type of pneumonia results in death often within 24 hours. While in veal calves *M. haemolytica* infections cause fatal polyserositis, characterized by acute pleuritis, peritonitis, and pericarditis (Timsit et al., 2016). There are several serotypes of *M. haemolytica* Serotype 1 (S1) is most commonly isolated from diseased cattle or lesions of pneumonia while serotypes 2 and 6 are a common cause of sheep pneumonia (Klima et al., 2014). Serotype 6 is associated with BRD cases approximately 20% of the time or less. Whereas S2 is often isolated from the nasal passages in high concentration in healthy non-stressed cattle but infrequently may causes bovine pneumonia (Odendaal & Henton, 1995). *M. haemolytica* has been reported as the causative agent of mastitis in sheep with a prevalence rate reaching 40% in sporadic cases, this implies the significance of this bacterium in ovine mastitis some reports assume it may be similar to or even greater than that of classical agent of mastitis (*Staphylococcus aureus*) (Omaleki et al., 2011; Omaleki et al., 2016). Incidence rate of clinical mastitis is less than 5% in sheep and goats and increases to more than 20% in outbreaks associated with particular predisposing factors. *M. haemolytica* is also causing septicemia in young animals and pneumonia in animals at different ages. Reports of mastitis in sheep caused by saying *M. haemolytica* can be more transmitted horizontally transmission to lambs via suckling (Omaleki et al., 2016).

7. Diagnosis

P. multocida and *M. haemolytica* originated from the same family of Pasteurellaceae, both of them settle the upper and lower respiratory tract surfaces of an animal (Kisiela & Czuprynski, 2009). Moreover, diseases caused by them resemble each other so almost same diagnosis procedures are applied for them. The first step toward perfect diagnosis it is the isolation of the bacterium from the clinically ill animal through culturing on different media and later

identification through molecular methods (Dziva et al., 2008). Sampling depends upon disease type and whether a serological tests or culturing is going to be carried out for normal culturing usually samples like, lung, liver, spleen, kidney and intestine swaps from wounds also can be collected and cerebral fluids are useful if animal has septicemia. Prior to culturing, a direct examination can be conducted by creating an impression smear of the liver to observe bacteria under a microscope, using stains such as Giemsa or Wright's. Both Pasteurella and Mannheimia grow readily in trypticase soy, blood or dextrose agar plates at 35-37°C. *P. multocida* on blood agar plate usually is mucoid, 1 to 3 mm in diameter, and nonhemolytic colonies. Whereas *M. haemolytica* on blood agar plates is grey, medium sized colonies with zones of β-haemolysis (Carter & Cole Jr, 2012). As for serological tests serum and whole blood can be used. Serological approaches are mostly used for the assessment of herd immunity after vaccination campaigns in addition to research purposes. Routinely used tests are ELISA, disk diffusion test, rapid whole blood agglutination and serum plate agglutination. Moreover, indirect hemagglutination test (IHA) is considered the most serological test used for diagnosis of *M. haemolytica* infections and serotyping of field isolates (Carter & Cole Jr, 2012; WOAH, 2020). Also advanced techniques such as immunofluorescent microscope, in-situ hybridization (ISH) and polymerase chain reaction (PCR) are applied for more specific identifications (Townsend et al., 2001). They have developed multiplex PCR as a rapid alternative approach to the conventional capsular serotyping system of Henderson. Overall, PCR tests and molecular typing techniques have their benefits, they are most effective when used in combination with other methods and integrated into a broader investigative approach.

8. Transmission

Diseases caused by those pathogens are respiratory system restricted so the transmission of the infections from animal to animal or among birds easily occurs during Pasteurella and Mannheimia outbreaks. Chronically infected, asymptomatic carrier chickens or turkeys are considered to be the main sources of disease in fowl cholera infections this besides other carrier such as wild birds or other mammals (dogs, cats, and rodents). Fowl cholera infection does not seem to be vertical transmittable through the egg (Christensen et al., 2008). Generally, infections caused by *P. multocida* and *M. haemolytica* transimitted either by direct contact or from surrounding environment contaminated with coughing and excretions which could be nasal discharges, mouth saliva or lacrimation (D'Amico et al., 2022). These bacteria can survive long enough to be spread by contaminated water, feed, shoes, clothes and other equipment in the farms (Magyar & Lax, 2014). *M. haemolytica* in mastitis infection can be transmitted through milk secretions that contaminate the surrounding environment and to lambs through suckling, also infection can be transmitted ascendingly from lambs to dams if there are some abrasions or wounds on udder surface or teats (Kannangara et al., 2020; Omaleki et al., 2016).

9. Antibiotic Resistance

Antibiotic resistance by bacteria of an animal origin especially form animal raised for milk or meat consumption purposes shape a critical situation due to correlation with human health in general and possibility of new emergence of antibiotic resistant bacteria in human medicine field. Although not all *P. multocida* possess plasmids in some isolates plasmids with different sizes have been identified. Mostly these plasmids harbor resistance genes to multiple antibiotics (Hirsh et al., 1981; San Millan et al., 2011). Most commonly these resistance to antibiotics such as erythromycin, b-lactams, tetracycline, fluoroquinolones, novobiocin chloramphenicol, streptomycin, sulfonamides and thiamphenicol (Arif & Champlin, 1998). Generally antibiotic resistance plasmids of Pasteurellaceae members are transferrable among the family members (Bahr et al., 2021; San Millan et al., 2011; Shayegh et al., 2009). Studies carried by (Kadlec et al., 2011) and (Michael et al., 2012) showed that *P. multocida* strain 36950 isolated from a case of bovine respiratory disease (BRD), resist all antibiotics that commonly used to control BRD (Hirsh et al., 1981). Further genome sequencing of this isolate revealed that antibiotic resistance genes have been found on mobilizable or conjugative elements integrated into the chromosomes of the strain. Also studies showed increasing of antibiotic resistance by *M. haemolytica* to very high percentage of resistance to routinely used antibiotics, commonly *M. haemolytica* resist tilmicosin oxytetracycline, spectinomycin, erythromycin (McClary et al., 2011; Schink et al., 2022), Tetracycline and ampicillin. Obviously this wide range of resistance to different classes of antibiotics indicate that *M. haemolytica* is multidrug resistant; although the resistance is relative and differ according to region of research, and type of breeding system and feeding system and antibiotics usage status in the farms or feedlots, still ability of more antibiotic resistance emergence is high especially to antibiotics routinely used in BRD treatment (Ashrafi et al., 2022; Lubbers & Hanzlicek, 2013).

10. Prevention and control

Generally, respiratory diseases caused by and *P. multocida M. haemolytica* are preventable diseases; although its prevention may be a long and complicated process but is achievable. The process depends mainly on good management of the animal farms and good veterinary practice by veterinarians and assistant staff (Yeates, 2012). For treatment always antibiotics are the first choice to reduce the secondary infection and control animal parameters, Cephalothin of first-generation of cephalosporin and third-generation of cephalosporin antibiotics basically ceftiofur, both reported to be very effective (Nagai et al., 2019; Welsh et al., 2004). Oxytetracycline, streptomycin and rifampicin are found to be a good choice, also surgical treatment for atrophic rhinitis can be carried out (Lizarazo et al., 2006; Van Driessche et al., 2018).

Vaccination plays an important role in protection of livestock, poultry, and indirectly mankind, vaccine decreases incidence of diseases among herds of animals in endemic regions or areas of outbreaks. Since (Pasteur,1880) worked on a vaccine against fowl cholera causing *P. multocida* till today so many vaccine trials have been tried out and a lot of them succeeded in evoking the immune system with long term protection and marginal side effects, most of them are commercially available nowadays (Dabo et al., 2007; Elsayed et al., 2021). A wide range of vaccines have been introduced to control respiratory infections caused by these two-organisms starting from bacterins, live attenuated, killed, subunit, recombinant and DNA vaccines, each of these vaccines model has its specific features with different immune response produced by host animals (Ahmad, 2014; Wubet et al., 2019). In fowl cholera live attenuated vaccine has long cross-serotype protection when administered in food or water but it considers unsafe compared to bacterins which affords safety short-term, serotype-specific immunity (Chung et al., 2005). One of limitations in vaccination programs against *P. multocida* and *M. haemolytica* is multi-causative agent in bovine respiratory diseases and calf pneumonia, in these complex cases introducing one vaccine against specific causative agent is not enough for producing full protection, although some studies showed that vaccination with single vaccine may protect herds. In 1975 (Ismail et al., 2023; Thomson et al., 1975) found that vaccination of cattle with efficacious *M. haemolytica* vaccines prior to shipment potentially increased specific antibody titers and reduces shipping fever pneumonia. Also, control of respiratory disease in newborn calf may fail due to either failure of passive transfer of maternal antibodies through colostrum or due to unspecific immune repose from newborn calf which possesses native immune cells (Furman-Fratczak et al., 2011). In calf the most important cause of vaccination failure is the maternal antibodies which react against introduce vaccine and neutralize them so timing of vaccination is critical step. It should not be soon after birth not later after declining of maternal antibodies titers which may result in a period of vulnerability before newborn calf develop their own immune response (Detmer & Glenting, 2006; Tabatabaei et al., 2007). Therefore, measuring of maternal antibodies is very important before starting any vaccination program Moreover, using of unspecific adjuvant also can play vital role in failure of animal immunization (Roth, 1999). In addition, vaccination may fail due to so many reasons such as stress, malnutrition, concurrent illness and immunosuppression status which lead to imperfect immune response; also immaturity of immune system is one of these reasons. Vaccines also fail to evoke the immune system due to interference between multiple vaccine those introduced concurrently in last differences between vaccine strain and field strains should be considered when administrate vaccines (Adler et al., 1999; Harland & Potter, 1992; Mostaan et al., 2021).

11. Conclusion

Principles of one health must be applied in dealing with diseases caused by Pasteurella and Mannheimia in general. Moreover, the industry must potentially act with the diseases as an animal welfare issue not just a matter of profit and loss, and more importantly they should apply the experimentally approved to be an excellent choice of drug or vaccine. More specific research needed to shed light on phenotype and genotype differences among those organisms. Although a full genetic map has been successfully achieved still more genomic studies are needed to reveal the origin of virulence diversity, host specificity and predilection side of bacteria. Likewise developing an ideal vaccine against those bacteria also is one of future research challenges. According to available literature specifically some issues should be subjected to intensive investigations and research focus such as emerging and re-emerging infectious diseases and antigenic variants between agents. Evaluation of innate and adaptive immunity after infections or vaccinations is important for producing more specific drugs or vaccines in the future. Some vaccine strains have been in use for more than five decades so new innovative vaccines using new technologies are demanded. Lastly that shortage in diagnostic field tests is also one of the challenges faces scientists nowadays.

References

Abbas, A., Khan, R. T. H., Asghar, A. Y., Shafeeq, M., & Rahim, A. (2023). Pneumonic Pasteurellosis: Role of Pasteurella multocida and Mannheimia haemolytica in respiratory disease of cattle.*Research Journal of Agricultural Sciences*, 2(1), 94-110.

Abd-Elsadek, E., Mostafa, A. E., & Abouelkhair, A. (2021). Molecular studies on Pasteurella multocida in ducks. *Journal of Current Veterinary Research, 3*(1), 1-9.

Adler, B., Bulach, D., Chung, J., Doughty, S., Hunt, M., Rajakumar, K., Serrano, M., Van Zanden, A., Zhang, Y., & Ruffolo, C. (1999). Candidate vaccine antigens and genes in Pasteurella multocida. *Journal of biotechnology, 73*(2-3), 83-90.

Ahmad, A. M. (2014). Efforts towards the development of recombinant vaccines against *Pasteurella multocida*. *Science World Journal, 9*(2), 1-7.

Amat, S. (2019). *Bovine respiratory disease in feedlot cattle: Antimicrobial resistance in bovine respiratory bacterial pathogens and alternative antimicrobial approaches*. In Bacterial Cattle Diseases. IntechOpen.

Angen, Ø., Mutters, R., Caugant, D. A., Olsen, J. E., & Bisgaard, M. (1999a). Taxonomic relationships of the [Pasteurella] haemolytica complex as evaluated by DNA-DNA hybridizations and 16S rRNA sequencing with proposal of Mannheimia haemolytica gen. nov., comb, nov., Mannheimia granulomatis comb. nov., Mannheimia glucosida sp. nov., Mannheimia ruminalis sp. nov. and Mannheimia varigena sp. nov. *International Journal of Systematic and Evolutionary Microbiology, 49(*1), 67-86.

Angen, Ø., Mutters, R., Caugant, D. A., Olsen, J. E., & Bisgaard, M. (1999b). Taxonomic relationships of the [Pasteurella] haemolytica complex as evaluated by DNA-DNA hybridizations and 16S rRNA sequencing with proposal of Mannheimia haemolytica gen. nov., comb. nov., Mannheimia granulomatis comb. nov., Mannheimia glucosida sp. nov., Mannheimia ruminalis sp. nov. and Mannheimia varigena sp. nov. International *Journal of Systematic and Evolutionary Microbiology, 49*(1), 67-86.

Arif, M., & Champlin, F. (1998). Adaptive acquisition of novobiocin resistance in Pasteurella multocida strains of avian origin. *Veterinary Research Communications, 22*, 445-455.

Ashrafi, F., Azari, A. A., & Fozouni, L. (2022). Prevalence and Antibiotic Resistance Pattern of Mannheima haemolytica and Pasteurella multocida Isolated from Cattle Lung Samples from an Industrial Abattoir: A Study from Northeastern Iran. *Iranian Journal of Veterinary Medicine, 16*(4).

Atapattu, D. N., & Czuprynski, C. J. (2005). Mannheimia haemolytica leukotoxin induces apoptosis of bovine lymphoblastoid cells (BL-3) via a caspase-9-dependent mitochondrial pathway. *Infection and Immunity, 73*(9), 5504-5513.

Autio, T., Pohjanvirta, T., Holopainen, R., Rikula, U., Pentikäinen, J., Huovilainen, A., Rusanen, H., Soveri, T., Sihvonen, L., & Pelkonen, S. (2007). Etiology of respiratory disease in non-vaccinated, non-medicated calves in rearing herds. *Veterinary Microbiology, 119*(2-4), 256-265.

Avalos-Gómez, C., Reyes-López, M., Ramírez-Rico, G., Díaz-Aparicio, E., Zenteno, E., González-Ruiz, C., & de la Garza, M. (2020). Effect of apo-lactoferrin on leukotoxin and outer membrane vesicles of Mannheimia haemolytica A2. *Veterinary Research, 51*(1), 1-13.

Ayalew, S., Confer, A. W., Hartson, S. D., Canaan, P. J., Payton, M., & Couger, B. (2017). Proteomic and bioinformatic analyses of putative Mannheimia haemolytica secretome by liquid chromatography and tandem mass spectrometry. *Veterinary Microbiology, 203*, 73-80.

Bahr, A., Salib, F., Soliman, Y., & Amin, M. (2021). Multi-drug resistant Pasteurella multocida and Mannheimia haemolytica strains isolated from different hosts affected by pneumonic pasteurellosis in Egypt. *Advances in Animal and Veterinary Sciences , 9*(3), 356-364.

Balevi, A., Ilban, A., Uslu, A., Sayin, Z., Gok, A., Padron, B., Toslak, E., & Erganis, O. (2023). The enigmatical manipulators in the capsule synthesis of Pasteurella multocida: Iron acquisition proteins. *Eurasian Journal of Veterinary Sciences, 39*(3), 139-143.

Benkirane, A., & De Alwis, M. (2002). Haemorrhagic septicaemia, its significance, prevention and control in Asia. *Veterinarni Medicina Praha, 47*(8), 234-240.

Biesheuvel, M., van Schaik, G., Meertens, N., Peperkamp, N., van Engelen, E., & van Garderen, E. (2021). Emergence of fatal Mannheimia haemolytica infections in cattle in the Netherlands. *The Veterinary Journal, 268*, 105576.

Blackall, P., Bojesen, A. M., Christensen, H., & Bisgaard, M. (2007). Reclassification of [Pasteurella] *trehalosi* as *Bibersteinia trehalosi* gen. nov., comb. nov. International *Journal of Systematic and Evolutionary Microbiology, 57* (4), 666-674.

Borge, C., Barranco, I., Márquez del Cid, J., Rodrıguez-Guerra, M., Carbonero, A., Carrasco, L., & Perea, A. (2011). *Outbreak of acute septicaemia by Pasteurella multocida type B in pigs reared in extensive system in Spain*. Proceedings of the European Symposium of Porcine Health and Management, Helsinki, Espoo,

Boukahil, I., & Czuprynski, C. J. (2015). Characterization of Mannheimia haemolytica biofilm formation in vitro. *Veterinary Microbiology, 175*(1), 114-122.

Boukahil, I., & Czuprynski, C. J. (2016). Mannheimia haemolytica biofilm formation on bovine respiratory epithelial cells. *Veterinary Microbiology, 197*, 129-136.

Boyce, J. D., Chung, J. Y., & Adler, B. (2000). Pasteurella multocida capsule: composition, function and genetics. *Journal of Biotechnology, 83*(1-2), 153-160.

Boyce, J. D., Harper, M., Wilkie, I., & Adler, B. (2010). *Pasteurella*. In Pathogenesis of bacterial infections in animals (pp. 325-346). John Wiley & Sons.

Briggs, R. E., Billing, S. R., Boatwright Jr, W. D., Chriswell, B. O., Casas, E., Dassanayake, R. P., Palmer, M. V., Register, K. B., & Tatum, F. M. (2021). Protection against

vaccination with modified-live Mannheimia haemolytica expressing Mycoplasma antigens. *Microbial Pathogenesis*, *161*, 105159.

Carter, G., & Chengappa, M. (1980). Hyaluronidase production by type B Pasteurella multocida from cases of hemorrhagic septicemia. *Journal of Clinical Microbiology, 11*(1), 94-96.

Carter, G. R., & Cole Jr, J. R. (2012). *Diagnostic procedure in veterinary bacteriology and mycology*. Academic Press.

Christensen, J. P., Bojesen, A. M., & Bisgaard, M. (2008). Fowl cholera. *Poultry Diseases, 6*, 149-154.

Chung, J. Y., Wilkie, I., Boyce, J. D., & Adler, B. (2005). Vaccination against fowl cholera with acapsular Pasteurella multocida A: 1. *Vaccine, 23*(21), 2751-2755.

Chung, J. Y., Wilkie, I., Boyce, J. D., Townsend, K. M., Frost, A. J., Ghoddusi, M., & Adler, B. (2001). Role of capsule in the pathogenesis of fowl cholera caused by Pasteurella multocida serogroup A. *Infection and Immunity, 69*(4), 2487-2492.

Confer, A. W., & Ayalew, S. (2013). The OmpA family of proteins: roles in bacterial pathogenesis and immunity. *Veterinary Microbiology, 163*(3-4), 207-222.

Confer, A. W., & Ayalew, S. (2018). Mannheimia haemolytica in bovine respiratory disease: immunogens, potential immunogens, and vaccines. *Animal Health Research Reviews, 19*(2), 79-99.

Cuevas, I., Carbonero, A., Cano, D., Pacheco, I. L., Marín, J. C., & Borge, C. (2020). First outbreak of bovine haemorrhagic septicaemia caused by Pasteurella multocida type B in Spain–Short communication. *Acta Veterinaria Hungarica, 68*(1), 8-11.

D'Amico, F., Casalino, G., Bozzo, G., Camarda, A., Lombardi, R., Dimuccio, M. M., & Circella, E. (2022). Spreading of Pasteurella multocida infection in a pet rabbit breeding and possible implications on healed bunnies. *Veterinary Sciences, 9*(6), 301.

Dabo, S., Taylor, J., & Confer, A. (2007). Pasteurella multocida and bovine respiratory disease. *Animal Health Research Reviews, 8*(2), 129-150.

Davies, R. L., & Lee, I. (2004). Sequence diversity and molecular evolution of the heat-modifiable outer membrane protein gene (ompA) of Mannheimia (Pasteurella) haemolytica, Mannheimia glucosida, and Pasteurella trehalosi. *Journal of Bacteriology, 186*(17), 5741-5752.

De la Mora, A., Suárez-Güemes, F., Trigo, F., Gorocica, P., Solórzano, C., Slomianny, M.-C., Agundis, C., Pereyra, M. A., & Zenteno, E. (2007). Purification of the receptor for the N-acetyl-D-glucosamine specific adhesin of Mannheimia haemolytica from bovine neutrophils. *Biochimica et Biophysica Acta (BBA)-General Subjects, 1770*(10), 1483-1489.

DeAngelis, P. L., & White, C. L. (2004). Identification of a distinct, cryptic heparosan synthase from Pasteurella multocida types A, D, and F. *Journal of Bacteriology, 186* (24), 8529-8532.

Detmer, A., & Glenting, J. (2006). Live bacterial vaccines–a review and identification of potential hazards. *Microbial Cell Factories, 5*(1), 1-12.

Diallo, I. S., & Frost, A. J. (2000). Survival of avian strains of Pasteurella multocida in chicken serum. *Veterinary Microbiology, 72*(1-2), 153-161.

Dziva, F., Muhairwa, A. P., Bisgaard, M., & Christensen, H. (2008). Diagnostic and typing options for investigating diseases associated with Pasteurella multocida. *Veterinary Microbiology, 128*(1-2), 1-22.

Elsayed, M. S. A. E., Eldsouky, S. M., Roshdy, T., Said, L., Thabet, N., Allam, T., Mohammed, A., Nasr, G. M., Basiouny, M. S., & Akl, B. A. (2021). Virulence determinants and antimicrobial profiles of Pasteurella multocida isolated from cattle and humans in Egypt. *Antibiotics, 10*(5), 480.

Furman-Fratczak, K., Rzasa, A., & Stefaniak, T. (2011). The influence of colostral immunoglobulin concentration in heifer calves' serum on their health and growth. Journal *of Dairy Science, 94*(11), 5536-5543.

Garzon, A., Hoyos-Jaramillo, A., Hustad, S., Byrne, B. A., Fritz, H. M., Lehenbauer, T. W., Aly, S., & Pereira, R. (2023). In vitro evaluation of the effect of transport medium, temperature, and time on the recovery of Mannheimia haemolytica and Pasteurella multocida. *JDS communications, 4*(3), 214-218.

Gelasakis, A., Mavrogianni, V., Petridis, I., Vasileiou, N., & Fthenakis, G. (2015). Mastitis in sheep–The last 10 years and the future of research. *Veterinary Microbiology, 181* (1-2), 136-146.

Gogoi, A., Sharma, R., Saikia, G., Borah, P., Tamuly, S., Deka, P., Barkalita, L., & Ahmed, S. (2018). Comparative immunogenicity of outer membrane vesicles (OMVS) and outer membrane proteins (OMPS) of Pasteurella multocida capsular type a of pig. *International Journal of Communication Systems , 6*(5), 302-306.

Guan, L., Zhang, L., Xue, Y., Yang, J., & Zhao, Z. (2020). Molecular pathogenesis of the hyaluronic acid capsule of Pasteurella multocida. *Microbial Pathogenesis, 149*, 104380.

Hanafy, M., Elhelw, R., Soliman, S., & Marouf, S. (2022). Phenotypic and genotypic characterization of PasteurIella species isolated from camels in Egypt. *Advances in Animal and Veterinary Sciences , 10*(2), 298-306.

Harland, R. J., & Potter, A. A. (1992). The effect of subunit or modified live bovine herpesvirus-1 vaccines on the efficacy of a recombinant Pasteurella haemolytica vaccine for the prevention of respiratory disease in feedlot calves. *The Canadian Veterinary Journal, 33*(11), 734.

Harper, M., Boyce, J. D., & Adler, B. (2006). Pasteurella multocida pathogenesis: 125 years after Pasteur. *FEMS Microbiology Letters, 265*(1), 1-10.

Harper, M., Cox, A. D., Adler, B., & Boyce, J. D. (2011). Pasteurella multocida lipopolysaccharide: the long and the short of it. *Veterinary Microbiology, 153*(1-2), 109-115.

Highlander, S. K. (2001). Molecular genetic analysis of virulence in Mannheimia (Pasteurella) haemolytica. Front

Hirose, K., Kobayashi, H., Ito, N., Kawasaki, Y., Zako, M., Kotani, K., Ogawa, H., & Sato, H. (2003). Isolation of Mycoplasmas from nasal swabs of calves affected with respiratory diseases and antimicrobial susceptibility of their isolates. *Journal of Veterinary Medicine, Series B, 50* (7), 347-351.

Hirsh, D. C., Martin, L., & Rhoades, K. (1981). Conjugal transfer of an R-plasmid in Pasteurella multocida. *Antimicrobial Agents and Chemotherapy, 20*(3), 415-417.

Hsuan, S., Kannan, M. S., Jeyaseelan, S., Prakash, Y., Malazdrewich, C., Abrahamsen, M., Sieck, G., & Maheswaran, S. (1999). Pasteurella haemolyticaleukotoxin and endotoxin induced cytokine gene expression in bovine alveolar macrophages requires NF-κB activation and calcium elevation. *Microbial pathogenesis, 26*(5), 263-273.

Ismail, M. T. A., El-Moneim, A., Mohamed, M., Soliman, H., & Ibrahim, F. (2023). Preparation and evaluation of a novel combined inactivated vaccine against Pasteurellosis and E. coli infection in Sheep. *Benha Veterinary Medical Journal, 45*(2), 121-125.

Jeyaseelan, S., Hsuan, S., Kannan, M. S., Walcheck, B., Wang, J., Kehrli, M., Lally, E., Sieck, G., & Maheswaran, S. (2000). Lymphocyte function-associated antigen 1 is a receptor for Pasteurella haemolytica leukotoxin in bovine leukocytes. *Infection and Immunity, 68*(1), 72-79.

Jones, F. (1921). A study of Bacillus bovisepticus. *The Journal of Experimental Medicine, 34*(6), 561-577.

Kadlec, K., Brenner Michael, G., Sweeney, M. T., Brzuszkiewicz, E., Liesegang, H., Daniel, R., Watts, J. L., & Schwarz, S. (2011). Molecular basis of macrolide, triamilide, and lincosamide resistance in Pasteurella multocida from bovine respiratory disease. *Antimicrobial Agents and Chemotherapy, 55*(5), 2475-2477.

Kamarulrizal, M. I., Chung, E. L. T., Jesse, F. F. A., Paul, B. T., Azhar, A. N., Lila, M. A. M., Salleh, A., Abba, Y., & Shamsuddin, M. S. (2022). Changes in selected cytokines, acute-phase proteins, gonadal hormones and reproductive organs of non-pregnant does challenged with Mannheimia haemolytica serotype A2 and its LPS endotoxin. *Tropical Animal Health and Production, 54*(3), 1-16.

Kandimalla, K., Awati, B., Putty, K., Ram, V. K. S., Yella, N. R., Patil, N. A., Bhoyar, R., Choudapur, M. K., Karate, A., & Lunavat, G. (2022). Evaluation of biofilm formation capacity of Pasteurella multocida and its relationship with antibiotic resistance. *Indian Journal of Veterinary Sciences & Biotechnology, 18*(5), 68-74.

Kannangara, D. W., Pandya, D., & Patel, P. (2020). Pasteurella multocida infections with unusual modes of transmission from animals to humans: a study of 79 cases with 34 nonbite transmissions. *Vector-Borne and Zoonotic Diseases, 20*(9), 637-651.

Kisiela, D. I., & Czuprynski, C. J. (2009). Identification of Mannheimia haemolytica adhesins involved in binding to bovine bronchial epithelial cells. *Infection and immunity, 77*(1), 446-455.

Kitadokoro, K., Kamitani, S., Miyazawa, M., Hanajima-Ozawa, M., Fukui, A., Miyake, M., & Horiguchi, Y. (2007). Crystal structures reveal a thiol protease-like catalytic triad in the C-terminal region of Pasteurella multocida toxin. *Proceedings of the National Academy of Sciences, 104*(12), 5139-5144.

Klima, C. L., Alexander, T. W., Hendrick, S., & McAllister, T. A. (2014). Characterization of Mannheimia haemolytica isolated from feedlot cattle that were healthy or treated for bovine respiratory disease. *Canadian journal of veterinary research, 78*(1), 38-45.

Klima, C. L., Zaheer, R., Cook, S. R., Rasmussen, J., Alexander, T. W., Potter, A., Hendrick, S., & McAllister, T. A. (2018). In silico identification and high throughput screening of antigenic proteins as candidates for a Mannheimia haemolytica vaccine. *Veterinary Immunology and Immunopathology, 195*, 19-24.

Kotelnikova, O., Zinchenko, A., Vikhrov, A., Alliluev, A., Serova, O., Gordeeva, E., Zhigis, L., Zueva, V., Razgulyaeva, O., & Melikhova, T. (2016a). Serological analysis of immunogenic properties of recombinant meningococcus IgA1 protease-based proteins. *Bulletin of Experimental Biology and Medicine, 161*(3), 391-394.

Kotelnikova, O., Zinchenko, A., Vikhrov, A., Alliluev, A., Serova, O., Gordeeva, E., Zhigis, L., Zueva, V., Razgulyaeva, O., & Melikhova, T. (2016b). Serological analysis of immunogenic properties of recombinant meningococcus IgA1 protease-based proteins. Bulletin *of Experimental Biology and Medicine, 161*, 391-394.

Kumar, A., Shivachandra, S., Biswas, A., Singh, V., Singh, V. P., & Srivastava, S. (2004). Prevalent serotypes of Pasteurella multocida isolated from different animal and avian species in India. *Veterinary Research Communications, 28* (8), 657-667.

Lee, C. W., Shewen, P. E., Cladman, W. M., Conlon, J., Mellors, A., & Lo, R. (1994). Sialoglycoprotease of Pasteurella haemolytica A1: detection of antisialoglycoprotease antibodies in sera of calves. *Canadian Journal of Veterinary Research, 58*(2), 93.

Lizarazo, Y. A. V., Ferri, E. F. R., de la Fuente, A. J. M., & Martín, C. B. G. (2006). Evaluation of changes in antimicrobial susceptibility patterns of Pasteurella multocida subsp multocida isolates from pigs in Spain in 1987–1988 and 2003–2004. American *Journal of Veterinary Research, 67*(4), 663-668.

Loneragan, G. H. (2001). *Acute interstitial pneumonia, bovine respiratory disease complex and potential pneumotoxicity in feedlot cattle*. Colorado State University.

Lubbers, B. V., & Hanzlicek, G. A. (2013). Antimicrobial multidrug resistance and coresistance patterns of Mannheimia haemolytica isolated from bovine respiratory disease cases—a three-year (2009–2011) retrospective analysis. *Journal of Veterinary Diagnostic Investigation, 25* (3), 413-417.

Magyar, T., & Lax, A. (2014). *00106-Bacteria: Pasteurella multocida*. In History, Science and Methods (pp. 476-479). Elsevier.

Maheswaran, S., Weiss, D., Kannan, M., Townsend, E., Reddy, K., Whiteley, L., & Srikumaran, S. (1992). Effects of Pasteurella haemolytica A1 leukotoxin on bovine neutrophils: degranulation and generation of oxygen-derived free radicals. *Veterinary immunology and immunopathology, 33*(1-2), 51-68.

Mahrous, E., Abd Al-Azeem, M., Wasel, F., & Younis, W. (2022). Molecular detection and characterization of pasteurella multocida isolated from rabbits. *Journal of Animal Health and Production, 10*(1), 1-9.

May, B. J., Zhang, Q., Li, L. L., Paustian, M. L., Whittam, T. S., & Kapur, V. (2001). Complete genomic sequence of Pasteurella multocida, Pm70. Proceedings *of the National Academy of Sciences, 98*(6), 3460-3465.

McClary, D. G., Loneragan, G. H., Shryock, T. R., Carter, B. L., Guthrie, C. A., Corbin, M. J., & Mechor, G. D. (2011). Relationship of in vitro minimum inhibitory concentrations of tilmicosin against Mannheimia haemolytica and Pasteurella multocida and in vivo tilmicosin treatment outcome among calves with signs of bovine respiratory disease. *Journal of the American Veterinary Medical Association, 239*(1), 129-135.

McGill, J. L., & Sacco, R. E. (2020). The immunology of bovine respiratory disease: recent advancements. *Veterinary Clinics of North America: Food Animal Practice, 36*(2), 333-348.

Michael, G. B., Kadlec, K., Sweeney, M. T., Brzuszkiewicz, E., Liesegang, H., Daniel, R., Murray, R. W., Watts, J. L., & Schwarz, S. (2012). ICE Pmu1, an integrative conjugative element (ICE) of Pasteurella multocida: structure and transfer. *Journal of Antimicrobial Chemotherapy, 67*(1), 91-100.

Mirtneh, Y., Vemulapati, B., Takele, A., Martha, Y., Teferi, D., Alebachew, B., Getaw, D., & Esayas, G. (2022). Phenotypic and Molecular Characterization of the Capsular Serotypes of Pasteurella multocida Isolated from Pneumonic Cases of Cattle in Ethiopia. *Agricultural Science Digest, 42*(3).

Mombeni, E. G., Gharibi, D., Ghorbanpoor, M., Jabbari, A. R., & Cid, D. (2021). Toxigenic and non-toxigenic Pasteurella multocida genotypes, based on capsular, LPS, and virulence profile typing, associated with pneumonic pasteurellosis in Iran. *Veterinary Microbiology, 257*, 109077.

Montes García, J., Vaca, S., Delgado, N. L., Uribe-García, A., Vázquez, C., Sánchez Alonso, P., Xicohtencatl Cortes, J., Cruz Cordoba, A., & Negrete Abascal, E. (2018). Mannheimia haemolytica OmpP2-like is an amyloid-like protein, forms filaments, takes part in cell adhesion and is part of biofilms. *Antonie Van Leeuwenhoek, 111*(12), 2311-2321.

Mostaan, S., Ghasemzadeh, A., Asadi Karam, M. R., Ehsani, P., Sardari, S., Shokrgozar, M. A., Abolhassani, M., & Nikbakht Brujeni, G. (2021). Pasteurella multocida PlpE protein polytope as a potential subunit vaccine candidate. *Vector-Borne and Zoonotic Diseases, 21*(11), 870-874.

Moustafa, A. M., Seemann, T., Gladman, S., Adler, B., Harper, M., Boyce, J. D., & Bennett, M. D. (2015). Comparative genomic analysis of Asian haemorrhagic septicaemia-associated strains of Pasteurella multocida identifies more than 90 haemorrhagic septicaemia-specific genes. *PLoS One, 10*(7), e0130296.

Nagai, K., Otomaru, K., Ogawa, R., Oishi, S., Wataya, K., Honkawa, Y., Iwamoto, Y., Ando, T., Hyakutake, K., & Shirahama, H. (2019). Effect of combined vaccination for Pasteurella multocida, Mannheimia haemolytica, and Histophilus somni to prevent respiratory diseases in young Japanese Black calves in the field. Journal *of Veterinary Medical Science, 81*(9), 1355-1358.

Odendaal, M., & Henton, M. M. (1995). The distribution of Pasteurella haemolytica serotypes among cattle, sheep and goats in South Africa and their association with disease. *Onderstepoort Journal of Veterinary Research, 62*(4):223-226.

Olson, M. E., Ceri, H., Morck, D. W., Buret, A. G., & Read, R. R. (2002). Biofilm bacteria: formation and comparative susceptibility to antibiotics. *Canadian Journal of Veterinary Research, 66*(2), 86.

Omaleki, L., Browning, G. F., Allen, J. L., & Barber, S. R. (2011). The role of Mannheimia species in ovine mastitis. *Veterinary Microbiology, 153*(1-2), 67-72.

Omaleki, L., Browning, G. F., Allen, J. L., Markham, P. F., & Barber, S. R. (2016). Molecular epidemiology of an outbreak of clinical mastitis in sheep caused by Mannheimia haemolytica. *Veterinary Microbiology, 191*, 82-87.

Pasteur, L. (1880). The attenuation of the causal agent of fowl cholera. *Comptes rendus de l'Académie des Sciences 91*, 673-680.

Pattison, M., McMullin, P., Bradbury, J. M., & Alexander, D. (2007). *Poultry diseases*. Elsevier Health Sciences.

Peng, Z., Wang, X., Zhou, R., Chen, H., Wilson, B. A., & Wu, B. (2019). Pasteurella multocida: genotypes and genomics. Microbiology and *Molecular Biology Reviews, 83(*4), 10.1128/mmbr. 00014-00019.

Petruzzi, B., Briggs, R. E., Swords, W. E., De Castro, C., Molinaro, A., & Inzana, T. J. (2017). Capsular polysaccharide interferes with biofilm formation by Pasteurella multocida serogroup A. *MBio, 8*(6), e01843-01817.

Petruzzi, B., Dalloul, R. A., LeRoith, T., Evans, N. P., Pierson, F. W., & Inzana, T. J. (2018). Biofilm formation and avian immune response following experimental acute and chronic avian cholera due to *Pasteurella multocida*. *Veterinary Microbiology, 222*, 114-123.

Pullinger, G. D., Bevir, T., & Lax, A. J. (2004). The Pasteurella multocida toxin is encoded within a lysogenic bacteriophage. Molecular microbiology, 51(1), 255-269.

Raj, R., Kumar Sharma, A., & Kumar Arora, A. (2023). Pasteurella multocida effectively utilizes hyaluronic acid to facilitate its continuous growth-short comminication. *Veterinarski Arhiv, 93*(5), 549-558.

Ramírez-Rico, G., Martinez-Castillo, M., Ruiz-Mazón, L., Meneses-Romero, E. P., Palacios, J. A. F., Díaz-Aparicio, E., Abascal, E. N., & de la Garza, M. (2024). Identification,

Mannheimia haemolytica A2. International *journal of molecular sciences, 25*(2), 1289.

Rehmtulla, A., & Thomson, R. (1981). A review of the lesions in shipping fever of cattle. *The Canadian Veterinary Journal, 22*(1), 1.

Rice, J., Carrasco-Medina, L., Hodgins, D., & Shewen, P. (2007). Mannheimia haemolytica and bovine respiratory disease. *Animal Health Research Reviews, 8*(2), 117-128.

Rico, G. R., Martínez-Castillo, M., González-Ruíz, C., Luna-Castro, S., & de la Garza, M. (2017). Mannheimia haemolytica A2 secretes different proteases into the culture medium and in outer membrane vesicles. *Microbial Pathogenesis, 113*, 276-281.

Roth, J. A. (1999). Mechanistic bases for adverse vaccine reactions and vaccine failures. *Advances in Veterinary Medicine, 41*, 681.

Sahoo, M., Baloni, S., Thakor, J. C., Dinesh, M., Bhutediya, J., Qureshi, S., Dhama, K., Dubal, Z. B., Singh, K., & Singh, R. (2020). Localization of Pasteurella multocida antigens in the brains of pigs naturally infected with Pasteurellosis revealing a newer aspect of pathogenesis. *Microbial pathogenesis, 140*, 103968.

Sakmanoğlu, A., Uslu, A., Sayin, Z., Karyeyen, Y., GÖLEN, G. S., İLBAN, A., Padron-Perez, B., Tekindal, M. A., & ERGANİŞ, O. (2021). Investigation of the nontypical Pasteurella multocida strains obtained from multiple sources, regions, and times: an unexpected increase was detected. *Turkish Journal of Veterinary and Animal Sciences, 45*(5), 814-824.

Samaniego-Barrón, L., Luna-Castro, S., Piña-Vázquez, C., Suárez-Güemes, F., & de la Garza, M. (2016). Two outer membrane proteins are bovine lactoferrin-binding proteins in Mannheimia haemolytica A1. *Veterinary Research, 47*(1), 1-15.

San Millan, A., Giufré, M., Escudero, J. A., Hidalgo, L., Gutierrez, B., Cerquetti, M., & Gonzalez-Zorn, B. (2011). Contribution of ROB-1 and PBP3 mutations to the resistance phenotype of a β-lactamase-positive amoxicillin/clavulanic acid-resistant Haemophilus influenzae carrying plasmid pB1000 in Italy. Journal *of Antimicrobial Chemotherapy, 66*(1), 96-99.

Schink, A. K., Hanke, D., Semmler, T., Brombach, J., Bethe, A., Lübke-Becker, A., Teske, K., Müller, K. E., & Schwarz, S. (2022). Novel multiresistance-mediating integrative and conjugative elements carrying unusual antimicrobial resistance genes in Mannheimia haemolytica and Pasteurella multocida. *Journal of Antimicrobial chemotherapy, 77*(7), 2033-2035.

Shayegh, J., Mikaili, P., Sharaf, J. D., & Rastgu, A. (2009). Antimicrobial resistance evaluation of Iranian ovine and bovine Pasteurella multocida. *Journal of Animal and Veterinary Advances, 8*(9), 1753-1756.

Shewen, P. E., Lee, C. W., Perets, A., Hodgins, D. C., Baldwin, K., & Lo, R. Y. (2003). Efficacy of recombinant sialoglycoprotease in protection of cattle against pneumonic challenge with Mannheimia (Pasteurella) haemolytica A1. *Vaccine, 21*(17-18), 1901-1906.

Singh, R., Blackall, P. J., Remington, B., & Turni, C. (2013). Studies on the presence and persistence of Pasteurella multocida serovars and genotypes in fowl cholera outbreaks. *Avian Pathology, 42*(6), 581-585.

Singh, R., Remington, B., Blackall, P., & Turni, C. (2014). Epidemiology of fowl cholera in free range broilers. *Avian Diseases, 58*(1), 124-128.

Smallman, T. R., Perlaza-Jiménez, L., Wang, X., Korman, T. M., Kotsanas, D., Gibson, J. S., Turni, C., Harper, M., & Boyce, J. D. (2024). Pathogenomic analysis and characterization of Pasteurella multocida strains recovered from human infections. *Microbiology Spectrum*, e03805-03823.

Smith, E., Miller, E., Aguayo, J. M., Figueroa, C. F., Nezworski, J., Studniski, M., Wileman, B., & Johnson, T. (2021). Genomic diversity and molecular epidemiology of Pasteurella multocida. *PLoS One, 16*(4), e0249138.

Storz, J., Purdy, C. W., Lin, X., Burrell, M., Truax, R. E., Briggs, R. E., Frank, G. H., & Loan, R. W. (2000). Isolation of respiratory bovine coronavirus, other cytocidal viruses, and Pasteurella spp from cattle involved in two natural outbreaks of shipping fever. *Journal of the American Veterinary Medical Association, 216*(10), 1599-1604.

Tabatabaei, M., Jula, G. M., Jabbari, A., & Esmailzadeh, M. (2007). Vaccine efficacy in cattle against hemorrhagic septicemia with live attenuated aroA mutant of Pasteurella multocida B: 2 strain. *Journal of Cell and Animal Biology, 1*(4), 062-065.

Thomson, R., Chander, S., Savan, M., & Fox, M. (1975). Investigation of factors of probable significance in the pathogenesis of pneumonic pasteurellosis in cattle. *Canadian Journal of Comparative Medicine, 39*(2), 194.

Timsit, E., Holman, D. B., Hallewell, J., & Alexander, T. W. (2016). The nasopharyngeal microbiota in feedlot cattle and its role in respiratory health. *Animal Frontiers, 6*(2), 44-50.

Townsend, K. M., Boyce, J. D., Chung, J. Y., Frost, A. J., & Adler, B. (2001). Genetic organization of Pasteurella multocida cap loci and development of a multiplex capsular PCR typing system. *Journal of Clinical Microbiology, 39*(3), 924-929.

Ujvari, B., Makrai, L., & Magyar, T. (2019). Virulence gene profiling and ompA sequence analysis of Pasteurella multocida and their correlation with host species. *Veterinary Microbiology, 233*, 190-195.

Van Driessche, L., Bokma, J., Gille, L., Ceyssens, P.-J., Sparbier, K., Haesebrouck, F., Deprez, P., Boyen, F., & Pardon, B. (2018). Rapid detection of tetracycline resistance in bovine Pasteurella multocida isolates by MALDI Biotyper antibiotic susceptibility test rapid assay (MBT-ASTRA). *Scientific Reports, 8*(1), 13599.

Vimr, E. R., Kalivoda, K. A., Deszo, E. L., & Steenbergen, S. M. (2004). Diversity of microbial sialic acid metabolism. *Microbiology and Molecular Biology Reviews, 68*(1), 132-153.

Welsh, R. D., Dye, L. B., Payton, M. E., & Confer, A. W. (2004). Isolation and antimicrobial susceptibilities of bacterial pathogens from bovine pneumonia: 1994–2002. *Journal*

of Veterinary Diagnostic Investigation, 16(5), 426-431.

WOAH. (2020). Epidemiology diagnosis prevention and control references; World organisation for animal health. In.

Wubet, W., Bitew, M., Mamo, G., Gelaye, E., Tesfaw, L., Sori, H., Zewdie, T., & Abayneh, T. (2019). Evaluation of inactivated vaccine against fowl cholera developed from local isolates of Pasteurella multocida in Ethiopia. *African Journal of Microbiology Research, 13*(27), 500-509.

Wynn, E. L., & Clawson, M. L. (2022). Differences between predicted outer membrane proteins of Pasteurella multocida, Histophilus somni, and genotype 1 and 2 Mannheimia haemolytica strains isolated from cattle. *Genome, 65*(2), 115-121.

Yanthi, N., Herlina, N., Agung, P., & Dewi, K. (2021). Recombinant expression protein of Type 4 Fimbrial gene (ptfA) of Pasteurella multocida. *IOP Conference Series*: *Earth and Environmental Science,*

Yeates, J. (2012). *Animal welfare in veterinary practice*. John Wiley & Sons.

Zhang, X., Yang, T., Cao, J., Sun, J., Dai, W., & Zhang, L. (2016). Mucosal immunization with purified OmpA elicited protective immunity against infections caused by multidrug-resistant Acinetobacter baumannii. *Microbial Pathogenesis, 96*, 20-25.

Zhao, C., Hu, X., Qiu, M., Bao, L., Wu, K., Meng, X., Zhao, Y., Feng, L., Duan, S., & He, Y. (2023). Sialic acid exacerbates gut dysbiosis-associated mastitis through the microbiota-gut-mammary axis by fueling gut microbiota disruption. *Microbiome, 11*(1), 78.

Zhao, X., Shen, H., Liang, S., Zhu, D., Wang, M., Jia, R., Chen, S., Liu, M., Yang, Q., & Wu, Y. (2021). The lipopolysaccharide outer core transferase genes pcgD and hptE contribute differently to the virulence of Pasteurella multocida in ducks. *Veterinary Research, 52*(1), 1-13.

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Determination of collagen and pH measurement in beef: Modern laboratory techniques

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ABSTRACT

Determining the pH value and collagen value in beef is very important in terms of healthy and quality nutrition. Collagen value is of great importance in meat-like products in order to offer healthy products to consumers. Today, various measurement techniques are used to measure pH value and collagen value. However, the success and reliability of each measurement varies. Therefore, more sensitive and reliable measurement methods need to be developed. Within the scope of this study, modern techniques used in the food industry to measure the pH value and collagen value of cut meat were examined. The reliability and acceptance level of each technique varies. This research aims to contribute to the development of more accurate methods for measuring collagen and pH values. In this way, it is aimed to increase the quality of beef products and offer healthier and more delicious products to consumers.

Keywords: pH, beef, collagen, measurement, techniques

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Introduction

The increasing human population in the world causes people to migrate from rural to urban areas. As a result, the demand for animal foods is gradually increasing (FAO, 1998). People meet their nutritional needs by consuming protein-rich foods, and animal foods such as meat (cattle, sheep, goats, poultry, pigs, fish and seafood/shellfish), milk and eggs are the main ones (Geletu et al., 2021).

Meat and meat products are the most valuable animal products and sources of high quality protein due to their amino acid composition. While the need for protein is increasing day by day, the rich protein content in these products offers an important source for a healthy diet. For the food industry, the safety of products and consumer satisfaction are as important as meeting protein needs. In the food industry, collagen content and pH value are two important parameters that determine the quality of beef products. Collagen is one of the building blocks of meat tissue and is a critical component that determines the texture, tenderness and durability of meat. Therefore, determining the right amount of collagen is a factor that affects the quality and cooking characteristics of meat. In addition, pH value is another important parameter affecting meat quality. pH value indicates the acidity level of meat and affects its color, taste and storage time. Correct pH values ensure that the meat remains fresh and durable and offers a better product to the consumer.

Beef is an important source of protein consumed worldwide and consumer demands are consistently high. Therefore, ensuring quality control in beef production is a critical imperative for the industry. Collagen and pH are two determining factors that

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affect success in this process. Too little or too much collagen can adversely affect the texture of the meat and provide an unpleasant experience for the consumer. Likewise, an incorrect pH value can shorten the shelf life of the meat and reduce the consumability of the product. Therefore, accurately measuring and regulating collagen and pH in cattle is a fundamental step to improve the quality of meat products and gain the trust of consumers.

In this study, we will examine in detail why the measurement of collagen and pH in cattle is important and the laboratory techniques used to determine these parameters. Laboratory methods are indispensable tools to optimize the quality of bovine meat products and to provide safe and healthy products in the food industry.

1.1. Collagen: structure, variety and role in food products

Collagen is the most abundant protein in mammals and birds and is found in all tissues, especially skin, tendons and bone. Connective tissue, which is mainly composed of collagen, serves to support, separate, protect and provide bedding for vascular and neural tissue and prevents excessive elongation of the muscle and damage to its contractile structure (Weston et al., 2002). Collagen is found in various parts of the muscle. Within the muscle, it acts to coordinate and transmit the forces generated in individual muscle fibers so that a reaction occurs as a result. In the form of tendons, collagen connects muscle to bone and plays a role in moving a body part through muscle contraction (Fratzl, 2008). Naturally synthesized collagen molecules consist of three long helicoid chains of amino acid residues with non-helical terminals at both ends. At least 46 unique polypeptide chains have been found in collagen from various animals (Matinong et al., 2022).

Collagen chains are most commonly composed of a Gly-X-Y motif. Here, Gly represents the amino acid glycine, while X and Y usually refer to the amino acids proline and 4-hydroxyproline, respectively. This motif is different from other ECM components (Matinong et al. 2022). The α chains of different types of collagen differ in composition depending on the frequency of repetition and the length of the segment containing the Gly-X-Y motif, whether it is interrupted or continuous, and the amino acid residues occurring at the X and Y positions (Holmes et al., 2018).

The arrangement of polypeptide chains and the diversity of terminals give both fibrillar and non-fibrillar collagen types their distinctiveness. Collagen types vary in conformations resulting in a distribution of different lengths of helices and non-helical segments. These criteria are used to group collagens into various groups. General groups include fibrillar collagens, FACIT (fibril-associated collagens with interrupted triple helices), FACIT-like collagen, basement membrane collagen, beaded filament collagen, transmembrane collagen, short-chain collagen and unclassified collagen (Sherman et al., 2015). At least 29 types of collagen are currently recognized (Sorushanova et al., 2021). Fibrillar collagens are the most abundant ECM proteins in vertebrates and provide stability, connectivity and form to tissues and organs. The most abundant fibrillar collagen in most tissues is type I collagen. It is found primarily on the fibrillar surfaces of skin and bones and in connective tissues. Collagen type I has a rod-like structure consisting of three helical chains. It has a molecular weight of approximately 300 kDa, a length of 280 nm, a diameter of 1.4 nm and contains approximately 1020 amino acid residues per chain (Matinong et al., 2022).

There are also some commercial collagen-based by-products used in various meats and meat products, including hides (skins) and skin trimmings, tendon, bovine shank, sinus remnants and bones separated manually or mechanically from bovine, porcine and poultry carcasses (Zarkadas and Maloney, 1998). Inauthentic meat products may include the substitution of meat from a high-value species with meat from a lower-value species, the augmentation of a meat product with connective tissue or fat (so that these ingredients are present in greater quantities than those naturally associated with the meat used), and the use of non-meat proteins or other substances. Many countries therefore specify the maximum allowable level of collagen in comminuted meat products, such as Protected Designation of Origin (PDO) and Protected Geographical Indication (PGI) products (Messia et al., 2008).

Collagen content in slaughtered beef products may vary depending on various factors. In one study, it was found that the total collagen content in the longissimus lumborum (LL) muscle of cattle breeds such as Limousine and Charolais was around 287-368 mg/100 g, while the insoluble collagen content was 215-278 mg/100 g (Szałkowska and Modzelewska-Kapituła, 2017).

Another study by Modzelewska-Kapituła et al. (2016) examined the effect of crossbreeding on collagen solubility and tenderness in beef. The researchers found that crossbreeding had an effect on collagen content and solubility, as well as on the shear strength and eating quality of beef. This suggests that crossbreeding can affect the collagen properties and

tenderness of beef.

A meta-analysis conducted by Blanco et al. (2013) investigated the influence of animal and management factors on collagen properties in beef. The study revealed that the development of total and insoluble collagen content with degree of maturity (DOM) differed between dairy and beef breeds, especially in bulls. However, the relationships between collagen content and DOM were not precise enough for prediction. The study suggested the development of a dynamic mechanistic model to better understand changes in collagen content in beef.

Bruce and Roy (2009) investigated the production factors affecting the contribution of collagen to beef toughness. They found that factors such as age of the animal at slaughter, steroid and beta-adrenergic agonist use and breed of cattle can affect the contribution of collagen to beef quality. In particular, mature collagen cross-link pyridinoline (PYR) concentrations were positively correlated with cutting force and age at slaughter.

1.2. Importance of pH value in determining meat quality

The pH level of meat is one of the most important criteria in determining quality. While the pH level in meat is 7.3 when the animal is alive, the pH level drops to 7.0 after the animal is slaughtered and bled. The decrease in oxygen level after slaughter and the increase in lactic acid formed as a result of aneorobic glucose in the muscles cause the pH level in the meat to decrease. Within the first 1 hour after slaughter, the pH value of the meat drops to between 5.6-6.2. As a result of the decrease in pH, the meat absorbs more water and becomes crisp. It is a known fact that the rearing conditions of the animals and the ill-treatment at the time of slaughter and the resulting stress are effective on the pH values measured after slaughter. Among these treatments, stress (beating, handling, exercise), electrical stimulation and stunning before slaughter are known to affect the pH levels of meat. It is also known that the quality of feeding applied to the animal is also a factor affecting the pH level after slaughter. Animals fed high energy diets have lower pH levels compared to animals fed low energy diets (Şireli, 2018).

We have stated that the pH value in beef can be affected by various factors. In one study, a relationship was found between the increase in the number of days in the feedlot and the increase in carcass weight and the increase in the temperature predicted at pH 6 and the occurrence of high rigor temperature (Warner et al., 2014). In another study, it was observed that pH decreased significantly as the number of freeze-thaw cycles increased, indicating a deterioration in the physicochemical quality of bovine muscle (Rahman et al., 2015). Research has also shown that instrumental precision measurements such as pH and shear force can be used to predict beef eating quality with a high degree of accuracy. However, the success of these predictions can vary greatly and R2 values for sensory precision can be as low as 0.01 (Farmer and Farrell, 2018). Furthermore, pH values of beef patties did not show clear trends and were not significantly affected by time (Gómez et al., 2014). In terms of meat quality, the expression of heat shock proteins has been associated with both the sensory quality and meat quality characteristics of highly marbled beef (Oh et al., 2019). Heat shock proteins play a role in protecting cells from stress and can affect the tenderness and flavor of beef.

Modern laboratory tests used in the field of food engineering

Some important modern laboratory techniques used in food engineering to determine collagen and pH in beef are listed below:

1.3.a. Near infrared spectroscopy (NIRS): Near infrared spectroscopy (NIRS) is increasingly used to monitor fermentation processes using NIR absorption spectroscopy ranging from 12-820 to 4000 cm-1. Chemical structures contribute to the characteristic position, shape and size of the analyte's absorption bands. Nutrients, metabolites, product formation or biomass concentrations can be monitored simultaneously with process-related changes in the NIR spectra of complex culture media. NIRS is a frequently used non-destructive technique for collagen determination in the meat industry (Hills, 2017; Monago-Maraña et al., 2021).

1.3.b. Fluorescence Spectroscopy: Fluorescence spectroscopy is used to determine the changes that occur in food products as a result of technological process and storage. This method can determine various properties of foods (such as functional component, composition, nutritional component) without the use of chemical reagents. White and red meat products have a composition rich in protein, polyunsaturated fatty acids, vitamins and minerals. However, the quality of red and white meat deteriorates rapidly during storage due to microbial growth, oxidation and enzymatic autolysis. Therefore, rapid analysis methods are important to ensure food safety and quality (Karoi and Blecker, 2011; Ankaralıgil and Güneşer, 2021).

1.3.c. FTIR-ATR Spectroscopy: FTIR, also known as the Fourier transform method, is a chemical analytical

method that measures the wavelength of light against its infrared intensity. FTIR-ATR method is used in quantitative and qualitative analysis in food research. This method allows for rapid evaluation of ingredients to ensure quality, safety and traceability in foods. The scale of infrared spectroscopy can be extended for applications such as food classification, sorting, authentication, tracking of contaminants and adulteration. FTIR-ATR has been used to determine fatty acid content in meat and meat products. This method has the ability to determine the ratio of saturated fatty acids to monounsaturated and polyunsaturated fatty acids (Lucarini et al., 2018).

1.3.d. High Performance Liquid Chromatography (HPLC): HPLC is an efficient technology used in analytical chemistry for the separation, identification and quantification of components. This technique is based on the principle of moving a pressurized liquid solvent (mobile phase) at high pressure through a column filled with a solid adsorbent material (stationary phase). Each component experiences unique molecular interactions with the adsorbent material, and since these interactions are different for each component, the components have different flow rates, which allow them to separate as they exit the column. Analysis is supported by multiple detectors such as a variable wavelength ultraviolet (UV) detector, fluorescence detector and refractive index detector (RID). HPLC is a versatile and widely preferred technique in industry that can quantify target components using calibrations prepared with standard substances (Sheppard and O'Dell, 2003; Shockcor, 2017).

1.3.e. pH meter: A pH meter is a critical tool used to measure levels of acidity or alkalinity. For example, the specially designed Skin pH Meter® PH 905 is used to measure the pH of the skin and includes a special electrode to increase skin contact (Ariffin and Hasham, 2020). pH meters were used in a study evaluating the efficacy of proton pump inhibitor (PPI) injections by measuring the pH of gastric fluids (Diesner et al., 2016).

The pH meter is an important tool in research and analysis (Ariffin and Hasham, 2020). In the food industry, pH meters are used to measure the pH of different food products and for quality control. These modern techniques offer valuable assistance to food engineers in the quality, nutritional value and safety of beef (Sheppard and O'Dell, 2003; Shockcor, 2017).

Conclusion

In the food industry, it is important to provide consumers with high quality and healthy products. This high product quality is a combination of a number of factors, including the amount of collagen in meat. Meat contains collagen, a connective tissue protein, which has a decisive effect on its texture and durability. The amount of collagen, its maturation state and pH value are factors that directly affect the tenderness and flavor of meat. Collagen provides structure and durability by forming intermolecular cross-links between the muscle fibers in the meat. These bonds change during the processing of meat with temperature. Collagen fibrils shrink as they heat up, which causes the loss of water in the meat, resulting in meat that contains less water and is less tender. However, this process also serves to hold the muscle fibers of collagen together to preserve the texture of the meat after cooking.

Collagen is more abundant in older animals and mature cross-links increase with age, resulting in tougher meat than in younger animals. Therefore, the content of collagen, its maturation state and pH value is an important factor to determine the quality of meat products. Furthermore, the post-mortem degradation of collagen and the use of collagenase enzymes are also used to control the tenderness of meat. These enzymes are used to alter the structure of collagen to provide the desired tenderness and texture.

In the food industry, the detection, analysis and pH determination of collagen plays a critical role in improving the quality of meat products and providing consumers with more flavorful and tender meat. Factors such as collagen content, maturation status and pH value are important determinants of the quality of meat products and therefore need to be carefully monitored. There is therefore a great interest in developing tools for the rapid determination of collagen in meat and for controlling its pH value.

In this study, modern laboratory techniques used for collagen determination and pH measurement in cattle were discussed. These techniques include tools such as near infrared spectroscopy (NIRS), fluorescence spectroscopy, FTIR-ATR spectroscopy, high pressure liquid chromatography (HPLC) and pH meters. These techniques play an important role in assessing and analyzing the quality of meat products, and their ability to precisely measure critical properties such as collagen content and pH value has been highly successful in ensuring quality control in beef production and providing consumers with more palatable, healthy and durable meat products. Nowadays, modern laboratory techniques are frequently used in the meat industry to improve product quality. Our study makes an important contribution to improve the quality standard of meat products and meet consumer demands.

References

Ankaralıgil, P., & Güneşer, B. (2021) Importance of Instrumental Analysis Techniques in Determination of Functional Food Components. *European Journal of Science and Technology,* 28, 251-258.

Ariffin, N., Hasham, R. (2020) Assessment of non-invasive techniques and herbal-based products on dermatological physiology and intercellular lipid properties. *Heliyon, 6*(5): e03955.

Blanco, M., Jurie, C., Micol, D., Agabriel, J., Picard, B., & Garcia-Launay, F. (2013). Impact of animal and management factors on collagen characteristics in beef: a meta-analysis approach. *Animal, 7*(7), 1208-1218.

Bruce, H., & Roy, B. (2009) Production factors affecting the contribution of collagen to beef toughness. *Journal of Animal Science, 97*(5), 2270-2278.

Diesner, S. C., Bergmayr, C., Pfitzner, B., Assmann, V., Krishnamuthy, D., Starkl, P., Endesfelder, D., Rothballer, M., Welzl, G., Rattei, T., Eiwegger, T., Szépfalusi, Z., Fehrenbach, H., Jensen-Jarolim, E., Hartmann, A., Pali-Schöll, I., & Untersmayr, E. (2016) A distinct microbiota composition is associated with protection from food allergy in an oral mouse immunization model. *Clinical Immunology, 173*, 10-18.

Farmer, L., & Farrell, D. (2018). Review: beef-eating quality: a european journey. *Animal,12*(11), 2424-2433.

Food and Agriculture Organization of the United Nations (1998). FAO Production Yearbook (p.233). Rome, Italy.

Fratzl, P. (2008). *Collagen: structure and mechanics, an introduction.* In Collagen: structure and mechanics (pp. 1-13). Boston, MA: Springer US.

Geletu, U. S., Usmael, M. A., Mummed, Y. Y., & Ibrahim, A. M. (2021). Quality of cattle meat and its compositional constituents. *Veterinary Medicine International,* 1-9

Gómez, I., Beriain, M., Sarriés, M., Insausti, K., & Mendizabal, J. (2014). Low-fat beef patties with augmented omega-3 fatty acid and cla levels and influence of grape seed extract. *Journal of Food Science, 79*(11), S2368-S2376.

Hills, A. E. (2017). Spectroscopy in biotechnology research and development. *Encyclopedia of Spectroscopy and Spectrometry*, 198-202.

Holmes, D. F., Lu, Y., Starborg, T., & Kadler, K. E. (2018). Collagen fibril assembly and function. *Current Topics in Developmental Biology, 130*, 107-142

Karoui, R., & Blecker, C. (2011). Fluorescence spectroscopy measurement for quality assessment of food systems-a review. *Food and Bioprocess Technology, 4*, 364-386.

Lucarini, M, Durazzo, A, Del Pulgar, J. S., Gabrielli, P, & Lombardi-Boccia, G. (2018). Determination of fatty acid content in meat and meat products: The FTIR-ATR approach. *Food Chemistry, 267*, 223-230.

Matinong, A. M. E., Chisti, Y., Pickering, K. L., & Haverkamp, R. G. (2022). Collagen extraction from animal skin. *Biology, 11*(6), 905.

Messia, M. C., Di Falco, T., Panfili, G., & Marconi, E. (2008). Rapid determination of collagen in meat-based foods by microwave hydrolysis of proteins and HPAEC–PAD analysis of 4-hydroxyproline. *Meat Science, 80*(2), 401-409.

Modzelewska-Kapituła, M., Nogalski, Z., & Kwiatkowska, A. (2016). The influence of crossbreeding on collagen solubility and tenderness of Infraspinatus and Semimembranosus muscles of semi-intensively reared young bulls. *Animal Science Journal, 87*(10), 1312-1321.

Monago-Maraña, O., Wold, J. P., Rødbotten, R., Dankel, K. R., & Afseth, N. K. (2021). Raman, near-infrared and fluorescence spectroscopy for determination of collagen content in ground meat and poultry by-products. *Lwt, 140*, 110592.

Oh, E., Lee, B., & Choi, Y. (2019). Associations of heat-shock protein expression with meat quality and sensory quality characteristics in highly marbled longissimus thoracis muscle from hanwoo steers categorized by warner-bratzler shear force value. *Foods, 8*(12), 638.

Rahman, M., Hossain, M., Rahman, S., Amin, M., & Oh, D. (2015). Evaluation of physicochemical deterioration and lipid oxidation of beef muscle affected by freeze-thaw cycles. *Korean Journal for Food Science of Animal Resources, 35*(6), 772-782.

Sheppard, A. J., & O'Dell, R. G. (2003). Cholesterol properties and determination. *Encyclopedia of Food Sciences and Nutrition*, 1220-1226.

Sherman, V. R., Yang, W., & Meyers, M. A. (2015). The materials science of collagen. *Journal of the mechanical behavior of biomedical materials, 52*, 22-50

Shockcor, J. P. (2017). Hplc-nmr, pharmaceutical applications. *Encyclopedia of Spectroscopy and Spectrometry*, 141-151.

Sorushanova, A., Skoufos, I., Tzora, A., Mullen, A. M., & Zeugolis, D. I. (2021). The influence of animal species, gender and tissue on the structural, biophysical, biochemical and biological properties of collagen sponges. *Journal of Materials Science: Materials in Medicine, 32*, 1-12

Szałkowska, A., & Modzelewska-Kapituła, M. (2017). Collagen profile and tenderness of strip loin and silverside originated from Polish Holstein-Friesian bulls of the black and white variety. *Acta Alimentaria, 46*(3), 378-383.

Şireli, H. D. (2018). Traditional methods and new techniques used in determining meat quality in carcasses. *Journal of Dicle University Institute of Science and Technology, 7*(3), 126-132.

Warner, R. D., Dunshea, F. R., Gutzke, D., Lau, J., & Kearney, G. (2014). Factors influencing the incidence of high rigor temperature in beef carcasses in Australia. *Animal Production Science, 54*(4), 363-374.

Weston, A. R., Rogers, R. W., & Althen, T. G. (2002). The role of collagen in meat tenderness. The *Professional Animal Scientist, 18*(2), 107-111.

Zarkadas, C. G., & Maloney, S. A. (1998). Assessment of the protein quality of the smooth muscle myofibrillar and connective tissue proteins of chicken gizzard. *Poultry Science, 77*(5), 770-779.

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Pet microbiota and its relationship with obesity

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ABSTRACT

The incidence of obesity in pets appears to be increasing in line with the increasing incidence of obesity in humans, and leads to decreased life expectancy. Obesity, which is considered a multifactorial disease caused by excessive adiposity, leads to a decrease in quality of life and serious health problems. It is known that there is an increase in the incidence of respiratory disorders, cardiological disorders, metabolic and endocrine problems, orthopedic diseases and some types of cancer in obese cats and dogs. There are many factors in the formation of obesity. One of these factors is the balance of the microbiota in gut. Many studies have shown that the microbiota affects critical steps in the formation of obesity and there are strong relationships between dietary content, microbiota, and obesity. In particular, high-fat diets are known to increase microbiome composition in terms of gram-negative bacterial strains and trigger dysbiosis. Again, in cases where dysbiosis occurs, the levels of volatile fatty acids also vary and lead to undesirable results through hormonal mechanisms. This condition, which causes hyperphagia, hypertriglyceridemia and insulin resistance, increases the incidence of obesity and diabetes mellitus. The ratio of Firmicutes and Bacteroidetes, which are among the largest phylae of the microbiota, shows serious differences when compared in underweight and obese animals. In this article, these relationships between microbiota and obesity are reviewed.

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Introduction

Obesity constitutes an important health problem in pets as well as in humans in developed countries. Most researchers agree that the incidence of obesity in the pet population, as in humans, is increasing and leading to a decrease in quality of life. A study conducted in 11 European countries reveals that 30-70% of pet dogs and 20-50% of dog owners are overweight (Muñoz-Prieto et al., 2018). Recent studies have shown that the prevalence of obesity in dogs in developed countries varies between 25% and 44% (Pegram et al., 2021). In the United Kingdom, obesity was the seventh most common disease reported in dogs in 2014, and in 2021 obesity was the third most common disease, after periodontal disease and otitis externa (O'Neill et al., 2021). According to the results of the Pet Obesity Research published by the Association for the Prevention of Pet Obesity in 2022, it was reported that 59% of dogs and 61% of cats were classified as overweight or obese (Anonymous, 2022). Energy imbalance is the main reason for the development of obesity in cats and dogs. In this context, excessive dietary intake or insufficient energy

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use leads to a state of positive energy balance, resulting in excess fat storage in the body (Kopelman, 2000). However, some pets may be classified as overweight due to markedly reduced energy expenditure due to neutering and reduced activity levels even if there is no increase in energy intake. Cats are generally regarded as being overweight when their body weight is at least 10-20%, obese 20% above ideal weight (Roudebush et al., 2008a).

Obesity alters the metabolic and endocrine function of adipose tissue, resulting in increased release of fatty acids, hormones, and pro-inflammatory molecules that contribute to obesity-related complications (Okada et al., 2017). Obesity-associated diseases include diabetes, insulin resistance, lipid profile abnormalities, orthopedic diseases, cardiorespiratory diseases, neoplasia, and shortened life expectancy (Roudebush et al., 2008b). In a lifelong study conducted with 48 Labrador Retriever dogs, it was found that the median life expectancy was significantly higher in the experimental group fed 25% fewer calories compared to the control group. In addition, it was determined that the onset of chronic health problems such as osteoarthritis was later in the experimental group consumed less calories (Kealy et al., 2002). In a study conducted with 1457 cats, it was revealed that obese or overweight cats were 3.9 times more likely to develop diabetes mellitus and 4.9 times more likely to develop lameness complaints than thin and lean cats (Scarlett & Donoghue, 1998).

Many factors are involved in the development of obesity, including inadequate lifestyle, neuronal and hormonal mechanisms, imbalance between energy intake and expenditure, and genetic and epigenetic factors. White adipose tissue is the most well-known type of fat where triglycerides are stored and lipids are mobilized for systemic use when other tissues need energy. Although once thought of as a passive fuel depot, white adipose tissue is now an active endocrine organ that provides feedback from the adipose tissue and communicates with the brain and peripheral tissues by secreting a wide variety of hormones and protein factors called adipokines, including leptin (Trayhurn, 2005).

Microbiota in cats and dogs

The gut microbiota can be defined as the total population of microbial species living in the digestive tract. From birth, a diverse community of microorganisms, including bacteriophages, bacteria, archaea, viruses, and eukaryotic microorganisms, use the digestive system as a host. This community of microorganisms makes an important contribution to the digestion and utilization of foods in the gastrointestinal tract. The mammalian gastrointestinal microbiota is diverse and complex, consisting of at least hundreds, perhaps thousands, of interdependent or competing species that are often poorly characterized (Ley et al., 2008). Microbial density varies greatly throughout the gut. While the stomach and small intestine contain a small number of bacteria, the large intestine has a much more dense population. Bacterial components are the largest in this population and provide basic digestive functions such as fermentation of fibers (Sender et al., 2016). In one study, molecular analysis using 16S rRNA sequencing revealed that Firmicutes, Fusobacteria, Bacteroidetes and Proteobacteria commonly reside in the gastrointestinal tract of dogs (Handl et al., 2011). According to studies conducted in dog feces, it has been observed that there is a core microbiota consisting of Firmicutes, Bacteroidetes and Fusobacteria (Hand et al., 2013). These phyla include bacteria belonging to the Clostridia and Bacilli classes, such as Faecalibacterium, which produce short chain fatty acids (SCFA), and Lactobacillus, which have probiotic properties by producing lactic acid (Pilla & Suchodolski, 2021a). In another study, it was shown that in the gastrointestinal tract of cats, Firmicutes and Proteobacteria, with the predominance of the Bacteroidetes phylum, are common, while archaea, fungi and viruses are found in much lower amounts (Tun et al., 2012). The dominant bacterial phyla in cat feces are Firmicutes, Bacteroidetes and Proteobacteria, respectively (Barry et al., 2012). High amounts of obligate anaerobic bacteria in the feces of cats are considered abnormal in dogs and humans (Johnston et al., 2001). While bacterial diversity in cats varies less between individuals, it is known that individual microbiota diversity in dogs is higher (Handl et al., 2011). The bacterial load, especially in the large intestine, is closely related to energy homeostasis, fat metabolism and obesity. The gut microbiota is also thought to play a role in regulating food intake by affecting metabolic function and hormones that stimulate areas of the brain associated with eating behavior (Hildebrandt et al., 2009).

The most important mediators that enable the microbiota to exert its effects on host health are bacterial metabolites such as tryptophan metabolites, SCFA and secondary bile acids (Lavelle & Sokol, 2020). SCFAs, especially acetate, propionate and butyrate, are products of bacterial fermentation of dietary fibers. It has been shown that acetate and propionate are mainly produced by the Bacteroidetes phylum, while butyrate is predominantly produced by the

Firmicutes phylum (Ismail et al., 2011). SCFAs contribute to intestinal health by acting as energy substrates for colonic epithelial cells, maintaining epithelial barrier integrity, regulating energy metabolism, and providing an anti-inflammatory effect (Koh et al., 2016). In addition, by regulating the satiety signal and intestinal movements, they lower the intestinal pH and provide the formation of an antimicrobial environment against pH-sensitive enteropathogens (Cherrington et al., 1991, Rowland et al., 2018). In addition to being an energy source for colonocytes in mice (Hartstra et al., 2015), butyrate has been shown to be effective against diet-mediated obesity without causing hypophagia (Lin et al., 2012a). It is known that propionate contributes to gluconeogenesis (Lin et al., 2012b), reduces cholesterol synthesis and increases leptin gene expression (Harris et al., 2012).

One of the ways that the microbiota exerts its effects is through lipopolysaccharides (LPS). LPS, an endotoxin found in the cell membrane of gram-negative bacteria, provides the immunomodulation activity of the microbiota. They are powerful activators of the inflammatory response and cause the inflammatory response to occur even in small amounts (de Vos et al., 2022). LPSs that activate Toll-like receptor 4 (TLR-4) induce antigen-presenting cell activation in this way. Thus, by establishing a connection between innate immunity and acquired immune response, a response to microbial factors occurs and signalling cascades for damaged tissue repair are activated (Poltorak et al., 1998). One of the microbiota's contributions to digestion is through secondary bile acids. Primary bile acids that escape the enterohepatic bile cycle undergo modifications such as deconjugation and dehydroxylation by bacteria in the intestine (de Vos et al., 2022). As a result, secondary bile acids are formed, changing the bioavailability and bioactivity of the bile cycle and their effects on the metabolic pathways in which they are involved (de Aguiar Vallim et al., 2013).

It is extremely important for the microbiota to be in an individual-specific order and balance to maintain general health. Imbalance within the microbiota is called dysbiosis and potentially leads to the formation of pathologies (Carding et al., 2015). Dysbiosis has been linked to inflammatory bowel disease, acute diarrhea, autoimmune diseases, various cancers, and obesity in humans (Rojo et al., 2017). The state of dysbiosis is thought to promote adiposity and lead to obesity through several different mechanisms. These include changes in satiety signals in the brain, regulation of hormones originating from the gastrointestinal tract, and affecting lipid metabolism in white adipose tissue and liver (Tremaroli & Bäckhed, 2012).

Dietary content and connection to microbiota

Gastrointestinal microbiota may vary depending on diet, eating habits and nutritional content. The bacterial population, which may vary depending on the substrate present in the intestinal tract, is primarily affected by the nutritional compositions that make up the diet. While changes in bacterial taxa require major changes in macronutrients in the diet, changes in micronutrients are required for bacterial metabolites and microbiota function changes (Pilla & Suchodolski, 2021b). While cats are obligate carnivores whose diets must be high in protein, dogs are metabolically omnivorous and can digest and metabolize higher amounts of carbohydrates than cats (Deng & Swanson, 2015). This difference in digestive systems is one of the factors that shape the diversity in the microbiota of cats and dogs. Microbiota changes depending on nutrients; It leads to a decrease in the synthesis of SCFAs, which protect the intestinal epithelial barrier integrity, reduce inflammation and increase the expression of hormones that suppress hunger. In addition, adipocyte expression is inhibited, resulting in dyslipidemia. It is argued that this situation will result in conditions such as low-grade chronic inflammation and obesity (Amabebe et al., 2020a). In this context, one of the most important factors affecting diversity is protein:carbohydrate ratio. In a study comparing the effects of a diet containing medium levels of protein and carbohydrates (MPMC) and two diets containing high levels of protein and low levels of carbohydrates (HPLC) on the microbiota in kittens, it was observed that the number of Actinobacteria in the feces was higher and the number of Fusobacteria was lower in kittens fed MPMC. Fecal Fusobacterium count was found to be highest in kittens fed by HPLC (Hooda et al., 2013). In another study conducted in 8 healthy adult cats, the effects of dietary protein levels on the microbiota were examined. While the bacterial similarity index was 66.7% in the group fed with a medium-level protein diet, this value was 40.6% in the group fed with a high-level protein diet. It was observed that Bifidobacterium populations were higher in cats fed a diet containing moderate protein than in the other group, while Clostridium perfringens populations were higher in the group fed a diet containing high levels of protein. The results of this study indicate that the amount of protein in the diet can cause dramatic changes in microbiota, and the necessity of probiotic/ prebiotic supplements should be considered in cats

fed high protein diets (Lubbs et al., 2009). In a similar study conducted with canine subjects, it was shown that C. perfringens increased while the Bifidobacterium phylum decreased (Zentek et al., 2003). Similar studies have also been conducted on humans. In human subjects undergoing diet trials such as high protein-low carbohydrate or high protein-medium carbohydrate, fecal SCFA and butyrate concentrations have been shown to decrease in the group with reduced carbohydrate levels (Duncan et al., 2008). Supporting the results of this study, in another study, it was observed that fecal output decreased in the obese group on a high-fat diet, and the total SCFA amount, butyrate concentrations and Bifidobacteria count in the stool were lower in subjects fed diets containing different levels of carbohydrates and fat. It is thought that changes in the microbiota may be related to various gastrointestinal diseases (Brinkworth et al., 2009).

Diets containing high fat-carbohydrate make the microbiota rich in bacteria associated with pathogenicity such as Firmicutes (Clostridium), Prevotella and Methanobrevibacter, while beneficial bacteria such as Bacteroides, Bifidobacterium, Lactobacillus and Akkermansia are lacking (Amabebe et al., 2020b). Firmicutes:Bacteroidetes ratio is used to evaluate the increased amount of body fat and susceptibility to obesity (Dreyer & Liebl, 2018). It is thought that a microbiota composition that supports obesity may increase fatness by obtaining more energy from diet (Turnbaugh et al., 2008). It was observed that the amount of the Actinobacteria phylum increased while the amount of the Bacteroidetes phylum decreased significantly in a study investigating the effects of a diet containing moderate protein and high fiber on the microbiota of 8 neutered male obese cats with limited feeding. Likewise, it was concluded that Prevotella bacteria decreased significantly with weight loss (Pallotto et al., 2018). In a study in which a commercial weight loss diet was applied to 22 obese cats for 24 weeks, in the first sampled microbiota composition was observed to be the most dominant phylum Firmicutes, followed by Bacteroidetes. After restricted feeding with a commercial diet containing high protein and high fiber content, it was observed that there were decreases in 7 bacterial species belonging to the Firmicutes phylum. Additionally, decreases in concentrations of fecal metabolites including SCFA, branched-chain fatty acids, phenol, and indoles have been reported. As a result of this study, it is understood that restricted feeding along with dietary content may have an effect on the microbiota due to the decreasing amount of substrate for the colonic microbiota (Opetz et al., 2023). One of the effects of diet content on obesity and general health status is through LPSs. High fat content in the diet and uncontrolled increases in body weight cause high intestinal permeability, increasing the circulating plasma LPS level. Thus, the metabolic endotoxemia situation is occurred (Cani et al., 2007).

It is known that probiotic supplements given with the diet have positive effects on health. A decrease in the number of Clostridium spp. and Enterococcus faecalis in the feces was observed in 15 cats in which Lactobacillus acidophilus supplement was used. In addition, while plasma endotoxin concentrations decreased, an increase in phagocytic capacity was demonstrated in peripheral granulocytes. These results are evidence that probiotic supplements added to the diet create positive systemic changes in addition to immunomodulatory effects (Marshall-Jones et al., 2006).

Relationship between microbiota and obesity

High-fat diets alter the composition of the microbiome in a way that increases the proliferation of gram-negative bacterial strains, namely Bacteroidetes. This causes increased production of LPS, a component of the Gram-negative cell membrane, and increased intestinal permeability. High levels of LPS and SCFA production activate TLR-4, which selectively binds LPS and induces low-grade inflammation that plays a role in the development of obesity (Graham et al., 2015). TLR-4 activation also results in the regulation of inflammatory pathways that contribute to insulin resistance and increased adiposity (Petrich et al., 2004). A study using rats fed a high-fat diet found an increase in acetate conversion rate and glucose-stimulated insulin secretion proportional to total calories consumed. It was concluded that increased acetate concentrations originate from the gut microbiota and drive parasympathetic nervous system activation, resulting in pancreatic β-cell stimulation and increased secretion of ghrelin, an appetite-stimulating hormone (Perry et al., 2016).

A study on the relationship between obesity and microbiota was also conducted using twin human individuals and mice. Fecal samples from human twins discordant for obesity were transplanted into germ-free mice. In a study conducted on mice, microbiota of obese individuals transported to lean control groups and the results showed that there was a significant increase in adiposity without an increase in food consumption. This study showed that obesity can be transmitted through microbiota modulation (Ridaura et al., 2013). In another study involving 10 obese and 10 normal weight dogs, microbiome analysis was

performed in feces. It was understood that the most abundant phyla in both groups were Firmicutes and Bacteroidetes, followed by Fusobacteria, Proteobacteria and Actinobacteria. However, when the two groups were compared, significant differences were observed in the microbiome composition. While a higher number of Firmicutes was observed in obese dogs compared to normal dogs, Bacteroidetes was found in lower numbers. The ratio of Firmicutes to Bacteroidetes was significantly lower in normal weight dogs compared to obese dogs (Thomson et al., 2022). In another study conducted by Moinard et al. (2020), it was shown that the Firmicutes:Bacteroidetes ratio increased in dogs on a high-fat diet, and this was accompanied by a decrease in insulin sensitivity and changes in epithelial permeability (Moinard et al., 2020). It was also observed that this level decreased in dogs with weight loss. Considering the studies conducted on dogs, it appears that changes in the Firmicutes:Bacteroidetes ratio contribute to the development and maintenance of obesity in dogs, in line with humans and other animals. Martínez-Cuesta et al. (2021) it has been suggested that the change in this ratio leads to the induction of specific metabolic pathways involved in SCFA production and, as a result, causes an increase in fat tissue in the individual (Martínez-Cuesta et al., 2021).

In another study on the effect of obesity on intestinal microbiota diversity, the composition of the fecal microbiota was evaluated in 22 lean and 21 obese domestic dogs, as well as five research dogs fed ad libitum and four research dogs as a lean control group. As a result, Firmicutes, Fusobacteria and Actinobacteria were the dominant bacterial phyla, and Actinobacteria phylum and Roseburia genus were significantly more abundant in obese domestic dogs. In research dogs, it was observed that the order Clostridiales increased significantly under ad libitum feeding (Handl et al., 2013).

Another study was conducted to investigate the anti-obesity and hypocholesterolemic effects of Bifidobacteria animalis DY-64, a lactic acid bacterium isolated from the human intestine, with 40 male Sprague-Dawley rats. The rats were divided into four groups, respectively, and four rations were created with control diet, B. animalis supplement added to the control diet, high-fat-high-cholesterol diet, and B. animalis supplement added to the high-fat-high-cholesterol diet, and a feeding protocol was applied for 4 weeks. At the end of the experiment, when the group fed with a high-fat-high-cholesterol diet was compared with the group fed with a diet supplemented with B. animalis; It was understood that the increase in body weight, liver and fat tissue weights was greater than the former. It was observed that serum total cholesterol, LDL-cholesterol and leptin levels, which were significantly higher in the group fed with a high-fat-high-cholesterol diet than in the control group, decreased in the fatty diet group supplemented with B. animalis. These results indicate that B. animalis DY-64, isolated from human intestine, exerts hypocholesterolemic effects by reducing serum and liver cholesterol levels and plays a role in preventing obesity caused by high-fat diet (Choi et al., 2013). Similarly rats fed a high-fat diet and a control diet were supplemented with L. rhamnosus for 13 weeks. Then, body weights, insulin sensitivity, and expression of genes related to glucose and lipid metabolism were examined. As a result, a decrease in weight gain and increased insulin sensitivity were observed in the group fed with a high-fat diet, while no change was observed in the group fed with a control diet. Significant adiponectin production had been also reported. These results suggest that supplementing the diet with L. rhamnosus improves insulin sensitivity and reduces lipid accumulation by stimulating adiponectin secretion (S.-W. Kim et al., 2013). An attempt has also been made to establish a link between body condition and intestinal diversity in relation to obesity. In this context, a study conducted with 24 healthy 2-year-old Beagle dogs focused on this connection. When fecal analyses were performed in dogs fed a commercial diet based on metabolic properties, it was understood that this main microbiota in the intestine was distributed differently according to body condition score. In subjects with high body condition scores, Firmicutes was the most common bacterial species, followed by Bacteroidetes, Fusobacteria, Proteobacteria and Actinobacteria. It was observed that taxonomic diversity was higher when the body condition score was 3 but decreased when the score increased to 4-6. Fusobacteria species were observed to increase especially at condition score 6 and higher. Thus, the idea that microbiome diversity may have an opposite relationship with fitness has emerged (Chun et al., 2020). In a study conducted in Italy, 16 dogs with a body condition score of 7 and above out of 9 formed the obese group, while 15 dogs with a body condition score of 4 and 5 out of 9 formed the control group. The average overall weight loss percentage after calorie restrictions with the commercial obesity diet was 12.9% of the initial body weight. At the end of the study, total protein, C-reactive protein, haptoglobin and reactive oxygen compounds in the blood were higher in the obese group than in the lean group. In the feces, biogenic

amines such as putrescine, cadaverine, spermine and spermidine were found to be more in the obese group and the amount of Firmicutes decreased and the amount of Bacteriodes increased in the obese group. This study ultimately provided evidence that obese dogs suffer from a subclinical inflammatory state characterized by higher levels of certain inflammatory markers and an accompanying higher total antioxidant capacity (Vecchiato et al., 2023).

It is known that there are significant microbiota changes in obese cats compared to thin cats (Kieler et al., 2016). According to the results of the research, Fusobacteria was found more in thin cats and Actinobacteria in obese cats, but the difference was not significant (Li & Pan, 2020). In another study conducted with cats, different result was obtained from the results observed in humans and rodents. Significant decreases were observed in the microbiome of obese cats compared to normal cats, which suggests a dysbiosis state in obese cats. The most common phylum in obese cats was Bacteroidetes with a rate of 40.9%, while Firmicutes came in second with 27.9%. It was concluded that the Firmicutes/ Bacteroidetes ratio was significantly low (Ma et al., 2022). Result suggests that, unlike humans and rodents, high Bifidobacterium levels may be associated with obesity. Accordingly, probiotic and prebiotic supplements to be used for weight management should be well designed. Consistent with the results of the aforementioned study, an another study showed that the Firmicutes phylum was found at higher levels in neutered thin cats than in the neutered obese group, while the Bacteroidetes phylum was significantly lower (Fischer et al., 2017).

The results of a study conducted with mice on the effects of SCFAs is also pointed out to a microbiota effect. In the study investigating the mechanism behind the anorectic effect of prebiotic fibers, the relationship of acetate, with appetite control was emphasized. Two groups of rats fed a high-fat diet supplemented with highly digestible inulin and a high-fat diet supplemented with low-digestible cellulose were subjected to various analyses after 8 weeks. The results showed that mice fed the inulin-supplemented diet gained significantly less weight and consume less food than the other group. It was determined that there was a significant increase in acetate in the colonic content (Frost et al., 2014). In another study, mice were supplemented with prebiotics and synbiotics from birth until the 42nd day and were then placed on a high-fat diet for 8 weeks. The results showed that adult insulin sensitivity and dyslipidemia improved and the most significant changes in gene expression were in the ileum, where Bifidobacterium is concentrated and authors concluded that early-life synbiotics protect mice from excessive fat accumulation caused by a high-fat diet (Mischke et al., 2018).

Conclusion

Obesity is a common metabolic dysfunction disease that is highly associated with the homeostasis of the gut microbiota. Evaluating the changes in the intestinal microbiota of obese pets and the physiological consequences resulting from these changes has an important place in the universal fight against obesity. The intestinal microbiota paves the way for obesity by regulating energy absorption, central appetite, fat storage and triggering chronic inflammation. Similarly, there are also strong connections between dysbiosis and obesity. At the phylum level, the increased Firmicutes/Bacteroidetes ratio is thought to be an important feature of the intestinal microbiota in obesity. The relationships between the composition and diversity of the intestinal microbiota and metabolic diseases such as obesity also emerge as a target for the prevention and treatment of these diseases. Considering all these, more studies are needed to elucidate the relationship between obesity and microbiota.

References

Ahima, R. S., Prabakaran, D., Mantzoros, C., Qu, D., Lowell, B., Maratos-Flier, E., & Flier, J. S. (1996). Role of leptin in the neuroendocrine response to fasting. *Nature, 382* (6588), 250–252.

Amabebe, E., Robert, F. O., Agbalalah, T., & Orubu, E. S. F. (2020a). Microbial dysbiosis-induced obesity: role of gut microbiota in homoeostasis of energy metabolism. *British Journal of Nutrition, 123*(10), 1127–1137.

Amabebe, E., Robert, F. O., Agbalalah, T., & Orubu, E. S. F. (2020b). Microbial dysbiosis-induced obesity: role of gut microbiota in homoeostasis of energy metabolism. *British Journal of Nutrition, 123*(10), 1127–1137.

Anonymous. (2022). Association for Pet Obesity Prevention the Global Pet Obesity Initiative Position Statement. Https://Www.Petobesityprevention.Org/2022.

Barry, K. A., Middelbos, I. S., Vester Boler, B. M., Dowd, S. E., Suchodolski, J. S., Henrissat, B., Coutinho, P. M., White, B. A., Fahey, G. C., & Swanson, K. S. (2012). Effects of dietary fiber on the feline gastrointestinal metagenome. *Journal of Proteome Research, 11*(12), 5924–5933.

Brinkworth, G. D., Noakes, M., Clifton, P. M., & Bird, A. R. (2009). Comparative effects of very low-carbohydrate, high-fat and high-carbohydrate, low-fat weight-loss diets on bowel habit and faecal short-chain fatty acids and bacterial populations. *British Journal of Nutrition, 101*(10), 1493.

Cani, P. D., Amar, J., Iglesias, M. A., Poggi, M., Knauf, C., Bastelica, D., Neyrinck, A. M., Fava, F., Tuohy, K. M., Chabo, C., Waget, A., Delmée, E., Cousin, B., Sulpice, T., Chamontin, B., Ferrières, J., Tanti, J.-F., Gibson, G. R., Casteilla, L., … Burcelin, R. (2007). Metabolic endotoxemia initiates obesity and insulin resistance. *Diabetes, 56*(7), 1761–1772.

Carding, S., Verbeke, K., Vipond, D. T., Corfe, B. M., & Owen, L. J. (2015). Dysbiosis of the gut microbiota in disease. *Microbial Ecology in Health & Disease, 26*(0). 26191.

Cherrington, C. A., Hinton, M., Pearson, G. R., & Chopra, I. (1991). Short-chain organic acids at pH 5.0 kill Escherichia coli and Salmonella spp. without causing membrane perturbation. *Journal of Applied Bacteriology, 70*(2), 161–165.

Choi, S.-H., Lee, M.-Y., Jhon, D.-Y., Choi, Y.-I., & Lee, J.-J. (2013). Antiobesity and Cholesterol-Lowering Effects of Bifidobacteria animalis DY-64 in Rats Fed a High-Fat/High-Cholesterol Diet. *Korean Journal for Food Science of Animal Resources, 33*(6), 701–707.

Chun, J. L., Ji, S. Y., Lee, S. D., Lee, Y. K., Kim, B., & Kim, K. H. (2020). Difference of gut microbiota composition based on the body condition scores in dogs. *Journal of Animal Science and Technology, 62*(2), 239–246.

de Vos, W. M., Tilg, H., Van Hul, M., & Cani, P. D. (2022). Gut microbiome and health: mechanistic insights. *Gut, 71*(5), 1020–1032.

de Aguiar Vallim, T. Q., Tarling, E. J., & Edwards, P. A. (2013). Pleiotropic roles of bile acids in metabolism. *Cell Metabolism, 17*(5), 657–669.

Deng, P., & Swanson, K. S. (2015). Gut microbiota of humans, dogs and cats: current knowledge and future opportunities and challenges. *British Journal of Nutrition, 113*(S1), S6–S17.

Dreyer, J. L., & Liebl, A. L. (2018). Early colonization of the gut microbiome and its relationship with obesity. Human *Microbiome Journal, 10*, 1–5.

Duncan, S. H., Lobley, G. E., Holtrop, G., Ince, J., Johnstone, A. M., Louis, P., & Flint, H. J. (2008). Human colonic microbiota associated with diet, obesity and weight loss. *International Journal of Obesity, 32*(11), 1720–1724.

Fischer, M. M., Kessler, A. M., Kieffer, D. A., Knotts, T. A., Kim, K., Wei, A., Ramsey, J. J., & Fascetti, A. J. (2017). Effects of obesity, energy restriction and neutering on the faecal microbiota of cats. *British Journal of Nutrition, 118*(7), 513–524.

Frost, G., Sleeth, M. L., Sahuri-Arisoylu, M., Lizarbe, B., Cerdan, S., Brody, L., Anastasovska, J., Ghourab, S., Hankir, M., Zhang, S., Carling, D., Swann, J. R., Gibson, G., Viardot, A., Morrison, D., Louise Thomas, E., & Bell, J. D. (2014). The short-chain fatty acid acetate reduces appetite via a central homeostatic mechanism. *Nature Communications, 5*(1), 3611.

Graham, C., Mullen, A., & Whelan, K. (2015). Obesity and the gastrointestinal microbiota: a review of associations and mechanisms. *Nutrition Reviews, 73*(6), 376–385.

Hand, D., Wallis, C., Colyer, A., & Penn, C. W. (2013). Pyrosequencing the canine faecal microbiota: Breadth and depth of biodiversity. *PLoS ONE, 8*(1), e53115.

Handl, S., Dowd, S. E., Garcia-Mazcorro, J. F., Steiner, J. M., & Suchodolski, J. S. (2011). Massive parallel 16S rRNA gene pyrosequencing reveals highly diverse fecal bacterial and fungal communities in healthy dogs and cats. *FEMS Microbiology Ecology, 76*(2), 301–310.

Handl, S., German, A. J., Holden, S. L., Dowd, S. E., Steiner, J. M., Heilmann, R. M., Grant, R. W., Swanson, K. S., & Suchodolski, J. S. (2013). Faecal microbiota in lean and obese dogs. *FEMS Microbiology Ecology, 84*(2), 332–343.

Harris, K., Kassis, A., Major, G., & Chou, C. J. (2012). Is the Gut Microbiota a new factor Contributing to obesity and its metabolic disorders? *Journal of Obesity, 2012*, 1–14.

Hartstra, A. V., Bouter, K. E. C., Bäckhed, F., & Nieuwdorp, M. (2015). Insights into the role of the microbiome in obesity and type 2 diabetes. *Diabetes Care, 38*(1), 159–165.

Hildebrandt, M. A., Hoffmann, C., Sherrill–Mix, S. A., Keilbaugh, S. A., Hamady, M., Chen, Y., Knight, R., Ahima, R. S., Bushman, F., & Wu, G. D. (2009). High-fat diet determines the composition of the murine gut microbiome independently of obesity. *Gastroenterology, 137*(5), 1716-1724.e2.

Hooda, S., Vester Boler, B. M., Kerr, K. R., Dowd, S. E., & Swanson, K. S. (2013). The gut microbiome of kittens is affected by dietary protein:carbohydrate ratio and associated with blood metabolite and hormone concentrations. *British Journal of Nutrition, 109*(9), 1637–1646.

Ismail, N. A., Ragab, S. H., ElBaky, A. A., Shoeib, A. R. S., Alhosary, Y., & Fekry, D. (2011). Frequency of Firmicutes and Bacteroidetes in gut microbiota in obese and normal weight Egyptian children and adults. *Archives of Medical Science, 3*, 501–507.

Johnston, K. L., Swift, N. C., Forster-van Hijfte, M., Rutgers, H. C., Lamport, A., Ballàvre, O., & Batt, R. M. (2001). Comparison of the bacterial flora of the duodenum in healthy cats and cats with signs of gastrointestinal tract disease. *Journal of the American Veterinary Medical Association, 218*(1), 48–51.

Kealy, R. D., Lawler, D. F., Ballam, J. M., Mantz, S. L., Biery, D. N., Greeley, E. H., Lust, G., Segre, M., Smith, G. K., & Stowe, H. D. (2002). Effects of diet restriction on life span and age-related changes in dogs. *Journal of the American Veterinary Medical Association, 220*(9), 1315–1320.

Kieler, I. N., Mølbak, L., Hansen, L. L., Hermann-Bank, M. L., & Bjornvad, C. R. (2016). Overweight and the feline gut microbiome – a pilot study. Journal *of Animal Physiology and Animal Nutrition, 100*(3), 478–484.

Kim, S.-W., Park, K.-Y., Kim, B., Kim, E., & Hyun, C.-K. (2013). Lactobacillus rhamnosus GG improves insulin sensitivity and reduces adiposity in high-fat diet-fed mice through enhancement of adiponectin production. *Biochemical and Biophysical Research Communications, 431*(2), 258–263.

Koh, A., De Vadder, F., Kovatcheva-Datchary, P., & Bäckhed, F. (2016). From dietary fiber to host physiology: Short-Chain Fatty Acids as Key Bacterial Metabolites. Cell, 165 (6), 1332–1345.

Kopelman, P. G. (2000). Obesity as a medical problem. *Nature, 404*(6778), 635–643.

Lavelle, A., & Sokol, H. (2020). Gut microbiota-derived metabolites as key actors in inflammatory bowel disease. *Nature Reviews Gastroenterology & Hepatology, 17*(4), 223–237.

Ley, R. E., Hamady, M., Lozupone, C., Turnbaugh, P. J., Ramey, R. R., Bircher, J. S., Schlegel, M. L., Tucker, T. A., Schrenzel, M. D., Knight, R., & Gordon, J. I. (2008). Evolution of mammals and their gut microbes. *Science, 320*(5883), 1647–1651.

Li, Q., & Pan, Y. (2020). Differential Responses to Dietary Protein and Carbohydrate Ratio on Gut Microbiome in Obese vs. Lean Cats. *Frontiers in Microbiology*, 11.

Lin, H. V., Frassetto, A., Kowalik Jr, E. J., Nawrocki, A. R., Lu, M. M., Kosinski, J. R., Hubert, J. A., Szeto, D., Yao, X., Forrest, G., & Marsh, D. J. (2012b). Butyrate and propionate protect against diet-Induced obesity and regulate gut hormones via free fatty acid receptor 3-independent mechanisms. *PLoS ONE, 7*(4), e35240.

Lubbs, D. C., Vester, B. M., Fastinger, N. D., & Swanson, K. S. (2009). Dietary protein concentration affects intestinal microbiota of adult cats: a study using DGGE and qPCR to evaluate differences in microbial populations in the feline gastrointestinal tract. *Journal of Animal Physiology and Animal Nutrition, 93*(1), 113–121.

Ma, X., Brinker, E., Graff, E. C., Cao, W., Gross, A. L., Johnson, A. K., Zhang, C., Martin, D. R., & Wang, X. (2022). Whole-genome shotgun metagenomic sequencing reveals distinct gut microbiome signatures of obese cats. *Microbiology Spectrum, 10*(3). e00837-22.

Marshall-Jones, Z. V., Baillon, M.-L. A., Croft, J. M., & Butterwick, R. F. (2006). Effects of Lactobacillus acidophilus DSM13241 as a probiotic in healthy adult cats. *American Journal of Veterinary Research, 67*(6), 1005–1012.

Martínez-Cuesta, M. C., del Campo, R., Garriga-García, M., Peláez, C., & Requena, T. (2021). Taxonomic characterization and short-chain fatty acids production of the obese microbiota. *Frontiers in Cellular and Infection Microbiology, 11*. 598093.

Mischke, M., Arora, T., Tims, S., Engels, E., Sommer, N., van Limpt, K., Baars, A., Oozeer, R., Oosting, A., Bäckhed, F., & Knol, J. (2018). Specific synbiotics in early life protect against diet-induced obesity in adult mice. *Diabetes, Obesity and Metabolism, 20*(6), 1408–1418.

Moinard, A., Payen, C., Ouguerram, K., André, A., Hernandez, J., Drut, A., Biourge, V. C., Suchodolski, J. S., Flanagan, J., Nguyen, P., & Leray, V. (2020). Effects of high-fat diet at two energetic levels on fecal microbiota, colonic barrier, and metabolic parameters in dogs. *Frontiers in Veterinary Science, 7*. 566282.

Muñoz-Prieto, A., Nielsen, L. R., Dąbrowski, R., Bjørnvad, C. R., Söder, J., Lamy, E., Monkeviciene, I., Ljubić, B. B., Vasiu, I., Savic, S., Busato, F., Yilmaz, Z., Bravo-Cantero, A. F., Öhlund, M., Lucena, S., Zelvyte, R., Aladrović, J., Lopez-Jornet, P., Caldin, M., … Tvarijonaviciute, A. (2018). European dog owner perceptions of obesity and factors associated with human and canine obesity. *Scientific Reports, 8*(1), 13353.

Okada, Y., Kobayashi, M., Sawamura, M., & Arai, T. (2017). Comparison of visceral fat accumulation and metabolome markers among cats of varying BCS and novel classification of feline obesity and metabolic syndrome. *Frontiers in Veterinary Science, 4. 17.*

O'Neill, D. G., James, H., Brodbelt, D. C., Church, D. B., & Pegram, C. (2021). Prevalence of commonly diagnosed disorders in UK dogs under primary veterinary care: results and applications. *BMC Veterinary Research, 17*(1), 69.

Opetz, D. L., Oba, P. M., Kostiuk, D., Kelly, J., & Swanson, K. S. (2023). Effects of weight loss and feeding specially formulated diets on the body composition, blood metabolite profiles, voluntary physical activity, and fecal metabolites and microbiota of overweight cats. *Journal of Animal Science, 101. skad332.*

Pallotto, M. R., de Godoy, M. R. C., Holscher, H. D., Buff, P. R., & Swanson, K. S. (2018). Effects of weight loss with a moderate-protein, high-fiber diet on body composition, voluntary physical activity, and fecal microbiota of obese cats. *American Journal of Veterinary Research, 79*(2), 181–190.

Pegram, C., Raffan, E., White, E., Ashworth, A. H., Brodbelt, D. C., Church, D. B., & O'Neill, D. G. (2021). Frequency, breed predisposition and demographic risk factors for overweight status in dogs in the UK. *Journal of Small Animal Practice, 62*(7), 521–530.

Perry, R. J., Peng, L., Barry, N. A., Cline, G. W., Zhang, D., Cardone, R. L., Petersen, K. F., Kibbey, R. G., Goodman, A. L., & Shulman, G. I. (2016). Acetate mediates a microbiome–brain–β-cell axis to promote metabolic syndrome. *Nature, 534*(7606), 213–217.

Petrich, B. G., Eloff, B. C., Lerner, D. L., Kovacs, A., Saffitz, J. E., Rosenbaum, D. S., & Wang, Y. (2004). Targeted Activation of c-Jun N-terminal Kinase in Vivo Induces Restrictive Cardiomyopathy and Conduction Defects. Journal of Biological *Chemistry, 279*(15), 15330–15338.

Pilla, R., & Suchodolski, J. S. (2021a). The Gut Microbiome of Dogs and Cats, and the Influence of Diet. *Veterinary Clinics of North America: Small Animal Practice, 51*(3), 605–621.

Pilla, R., & Suchodolski, J. S. (2021b). The Gut Microbiome of Dogs and Cats, and the Influence of Diet. *Veterinary Clinics of North America: Small Animal Practice, 51*(3), 605–621.

Poltorak, A., He, X., Smirnova, I., Liu, M.-Y., Huffel, C. Van, Du, X., Birdwell, D., Alejos, E., Silva, M., Galanos, C., Freudenberg, M., Ricciardi-Castagnoli, P., Layton, B., & Beutler, B. (1998). Defective LPS signaling in C3H/HeJ and C57BL/10ScCr mice: Mutations in Tlr4 Gene. *Science, 282* (5396), 2085–2088.

Ridaura, V. K., Faith, J. J., Rey, F. E., Cheng, J., Duncan, A. E., Kau, A. L., Griffin, N. W., Lombard, V., Henrissat, B., Bain, J. R., Muehlbauer, M. J., Ilkayeva, O., Semenkovich, C. F., Funai, K., Hayashi, D. K., Lyle, B. J., Martini, M. C., Ursell, L. K., Clemente, J. C., … Gordon, J. I. (2013). Gut microbiota from twins discordant for obesity modulate metabolism in mice. *Science, 341*(6150).

Rojo, D., Méndez-García, C., Raczkowska, B. A., Bargiela, R., Moya, A., Ferrer, M., & Barbas, C. (2017). Exploring the human microbiome from multiple perspectives: factors altering its composition and function. FEMS *Microbiology Reviews, 41*(4), 453–478.

Roudebush, P., Schoenherr, W. D., & Delaney, S. J. (2008a). An evidence-based review of the use of therapeutic foods, owner education, exercise, and drugs for the management of obese and overweight pets. *Journal of the American Veterinary Medical Association, 233*(5), 717 –725.

Roudebush, P., Schoenherr, W. D., & Delaney, S. J. (2008b). An evidence-based review of the use of therapeutic foods, owner education, exercise, and drugs for the management of obese and overweight pets. *Journal of the American Veterinary Medical Association, 233*(5), 717 –725.

Rowland, I., Gibson, G., Heinken, A., Scott, K., Swann, J., Thiele, I., & Tuohy, K. (2018). Gut microbiota functions: metabolism of nutrients and other food components. *European Journal of Nutrition, 57*(1), 1–24.

Scarlett, J. M., & Donoghue, S. (1998). Associations between body condition and disease in cats. *Journal of the American Veterinary Medical Association, 212*(11), 1725–1731.

Sender, R., Fuchs, S., & Milo, R. (2016). Revised estimates for the number of human and bacteria cells in the body. *PLOS Biology, 14*(8), e1002533.

Thomson, P., Santibáñez, R., Rodríguez-Salas, C., Flores-Yañez, C., & Garrido, D. (2022). Differences in the composition and predicted functions of the intestinal microbiome of obese and normal weight adult dogs. *Peer J, 10*, e12695.

Trayhurn, P. (2005). Adipose Tissue in Obesity—An Inflammatory Issue. Endocrinology, 146(3), 1003–1005.

Tremaroli, V., & Bäckhed, F. (2012). Functional interactions between the gut microbiota and host metabolism. *Nature, 489*(7415), 242–249.

Tun, H. M., Brar, M. S., Khin, N., Jun, L., Hui, R. K.-H., Dowd, S. E., & Leung, F. C.-C. (2012). Gene-centric metagenomics analysis of feline intestinal microbiome using 454 junior pyrosequencing. *Journal of Microbiological Methods, 88* (3), 369–376.

Turnbaugh, P. J., Bäckhed, F., Fulton, L., & Gordon, J. I. (2008). Diet-Induced Obesity Is Linked to Marked but Reversible Alterations in the Mouse Distal Gut Microbiome. *Cell Host & Microbe, 3*(4), 213–223.

Vecchiato, C. G., Golinelli, S., Pinna, C., Pilla, R., Suchodolski, J. S., Tvarijonaviciute, A., Rubio, C. P., Dorato, E., Delsante, C., Stefanelli, C., Pagani, E., Fracassi, F., & Biagi, G. (2023). Fecal microbiota and inflammatory and antioxidant status of obese and lean dogs, and the effect of caloric restriction. *Frontiers in Microbiology, 13*. 1050474.

Zentek, J., Marquart, B., Pietrzak, T., Ballèvre, O., & Rochat, F. (2003). Dietary effects on bifidobacteria and Clostridium perfringens in the canine intestinal tract. *Journal of Animal Physiology and Animal Nutrition, 87*(11–12), 397–407.

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Recent advances in assisted reproductive technologies of feline reproduction

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ABSTRACT

Many wildcat species are threatened with extinction, rare or vulnerable due to habitat destruction and poaching. In addition, Ankara and Van domestic cat species originating from Türkiye are in danger of extinction and are under protection. Thus, the requirement for assisted reproductive techniques in b oth domestic and nondomestic cat species has been increasing in recent years. Assisted Reproductive Technologies (ART) such as in vitro maturation, in vitro fertilization, embryo transfer, and cloning in domestic cats (Felis catus) provide a useful and suitable model for the conservation of endangered cat species. Domestic cats can be recipients for embryo transfer and recipient cytoplasm for nuclear transfer from various small wildcat species. Thanks to ART, it is possible to ensure the continuation of the generation by producing in vitro embryos or by making intra or inter-species clones from wild cats that have lost their reproductive functions or even died recently. Many inherited genetic disorders have been identified in cats that are similar to humans. Due to their genetic closeness, they have recently begun to be used as animal models in some therapeutic studies on humans, especially on kidney and nervous system diseases. In the early years, in vitro study results were less successful than in farm animals but in recent years ART's such as in vitro embryo production, embryo transfer, cloning, and transgenesis have made significant progress in domestic of domestic and wild cats. This review includes the assisted reproductive technologies applied in recent years and the results obtained in domestic cat and felines.

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Introduction

Although there is currently no species-specific culture medium, significant advances have been made in assisted reproductive technologies in domestic cats since the birth of the first kitten produced from IVF-derived embryos was born in 1988 (Goodrowe et al., 1988). This review will give information about the estrous cycle in cats, and recent ART's such as in vitro oocyte maturation, in vitro fertilization, embryo transfer, semen storing, cloning, and transgenic studies, which have rapidly developed in recent years.

1. Estrus Cycle of Cat

Free-living female cats show seasonal polyestrus. This season in the northern hemisphere begins in January when the day length begins to increase and melatonin pressure begins to disappear and intensifies in spring. Estrus continues to decrease towards the summer and lasts until autumn. Melatonin hormone has an anti-gonadal effect in cats, and the period of anestrus is between October and December when the dark period becomes longer. Cats living at home may show estrus

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and ovulation can only occur by stimulating endogenous LH secretion through natural/artificial mating reflex or exogenous hormone applications. If there is no mating or any exogenous stimulation or hormone administration, the follicles developed in the ovaries are resorbed after about a week and cats enter interestrus, which is a calm period. After 2-3 weeks of interestrus, cats come into estrus again. This cycle continues throughout the season. Even if a non-fertile mating occurs, rapidly rising progesterone levels decrease to basal levels only after 40-45 days, and this period is called pseudo-pregnancy. After a fertile mating, the resulting embryos descend to the uterus in 4–5 days, and at the morula stage, and implantation occurs in 12–13th days. The number of offspring varies depending on the breed, age, and number of ovulations that will occur in parallel with the number of mating. The gestation period varies depending on some factors such as breed and number of offspring, but it is approximately 62– 67 days (Pope, 2000).

2. In Vitro Technologies

a. Collection and Transport of Ovaries: The ovaries collected after routine ovariohysterectomy are used for in vitro studies of cats. The ovarian transport solution type, temperature, and duration are critical factors in the quality of oocytes to be recovered. Ovaries are usually transported to the laboratory within a few hours in isotonic solutions such as PBS or 0.9 % NaCl are generally preferred as transport solutions (Evecen et al., 2009; Evecen et al., 2003b; Johnston et al., 1989). Recently, a study was conducted with oocytes obtained from ovaries stored in ET-Kyoto solution (ET-K), which is used for the transportation of human organs. It is revealed that in vitro fertilized cat oocytes were more successful than the control group, in terms of cleavage and further embryo development in vitro (Yoshhida et al., 2022). The temperature of the transport solution was preferred by some researchers as warm (22–38 ^{o}C) (Karja et al., 2002; Merlo et al., 2005; Güriş and Birler, 2011; Evecen et al., 2016), and by some researchers as cold (5 ^{o}C) (Johnston et al., 1989; Wolfe and Wildt, 1996; Evecen et al., 2002). The ability of oocytes obtained after storing cat ovaries in cold environments for a long time to mature and be fertilized in vitro provides significant contributions to the protection of wildlife and endangered species (Wolfe and Wildt, 1996; Evecen et al., 2010; Arıcı et al., 2022). We demonstrated in our previous study that the in vitro maturation rates obtained after keeping cat ovaries at 5 ^{o}C for 24 hours, gave similar results (50.7 and 48.2%) to the control group (Evecen et al., 2010). Adding some antioxidants such as Superoxide dismutase (SOD), Catalase (Cocchia et al., 2015). Resveratrol, Melatonin, and Lycopene, in the transport solution (Swelum et al., 2022) help prevent oxidation that may occur during transport, improving oocyte yield and viability. It can make positive contributions to the quality of the embryos to be produced.

b. Harvesting of Oocytes: Oocytes from both domestic and wild felines can either obtained by stimulation of follicles with exogenous hormones or by collection from ovaries obtained after neutering (Pope 2000).
Stimulation of Ovaries: Oocytes are collected according to the principle of starting hormone therapy when female cats are in their interestrus period. The estrus period of a female cat is easily determined by vaginal cytology. To stimulate the ovaries, either a single dose of PMSG (50-100 IU) or FSH (1.5–4 mg) is applied for 4 days. For the maturation of follicles and oocytes, a single dose of hCG (100-150 IU) or 3 IU LH is administered 80-84 hours after PMSG. Then, matured oocytes are collected by aspiration of preovulatory follicles with the help of a laparoscope or laparotomy Hormone doses are calculated and applied in the same way for wild felines, calculated on a live weight basis (Pope 2000).
Slicing of Ovaries: If the ovaries have been transported at a cold temperature, they are kept at room temperature for 30–60 minutes before the processing begins. After cutting sections on the surface of the ovaries with the help of a scalpel (slicing), they are washed and rinsed with a suitable washing medium (Oocyte washing medium, M2 medium, TCM–199 with Hepes buffer) at 32–38 °C (Uchikura et al., 2011; Evecen et al., 2003a; Evecen et al., 2004).

c. Selection of Oocytes: The recovered oocytes are rinsed and evaluated under a stereo-microscope and only oocytes with Grade A quality are selected for in vitro maturation. The selection of Grade A oocytes to be matured in vitro is made according to the following criteria: A complete and intact zona pellucida, surrounded by at least four cumulus oophorous/ corona radiata cell layers, having large, homogeneous, and darkly pigmented vitellus structure filling the inside of the zona pellucida. Approximately 5–20 Grade A oocytes canbe obtained from each ovary, depending on the cat's age, weight, nutritional status, season, and estrus cycle stage (Evecen et al., 2003a; Evecen et al., 2004).

d. In Vitro Maturation: In the cat, the oocyte ovulates in the metaphase II stage (MII) and is ready for fertilization. However, the oocytes obtained by slicing the ovaries are still in the primary oocyte stage and are not yet mature. Thus, they must be matured in vitro

is generally carried out between 32–48 hours (Evecen et al., 2009; Karja et al., 2002; Evecen et al., 2002; Evecen et al., 201et al., 2009; Evecen et al., 2003a). In vitro maturation period 0; Pichardo et al., 2021). In vitro, maturation of oocytes is one of the most critical points of the in vitro embryo production process. Therefore, several researchers reported very different (2–82%) in vitro maturation results in cat (Pope 2000). Becausethe process can be affected by many variables such as season, nutritional status, and cyclic period of donor cats, the selected oocyte quality, the type of medium used, different substances added to the medium (proteins, hormones antioxidants, and growth factors), the gas ratios, the temperature and humidity of the incubator (Evecen et al., 2004).

The Season and The Cyclic Period of Donor Cats: Different studies on this subject have obtained either supporting or contradictory results in cats (Pope 2000). Some researchers declare that they did not find a difference between the in vitro maturation rates of oocytes obtained in different estrus cycle stages or seasons (Karja et al., 2002; Uchikura et al., 2011; Pichardo et al., 2021). In our previous study, we found that although not statistically significant, there were differences in in vitro maturation rates between different estrus cycle stages of queens and that the follicular stage was superior to other stages (Evecen et al., 2004).

In Vitro Maturation Chamber: The ambient temperature is regulated according to the body temperature of the animal species, and the gas composition varies depending on the type of environment used. Cat oocytes are matured in an incubator with a temperature of 38°C, containing 5% CO2 or a gas mixture (5% CO_2, 5% O_2, and 90% N_2) and approximately 100% humidified atmosphere (Pope 2000; Evecen et al., 2003a).

In Vitro Maturation Media: Because there is no medium specifically designed for the in vitro maturation of cat oocytes, researchers use media that is used for other mammalian oocytes. The most commonly used media are; TCM 199 (Karja et al., 2002; Evecen et al., 2016; Evecen et al., 2002; Evecen et al., 2003a), Ham's F-10 (Evecen et al., 2016; Evecen et al., 2003a). Synthetic Oviduct Fluid (Evecen et al., 2010; Evecen et al., 2009; Evecen et al., 2004), and Eagle's Minimal Essential Medium (Wolfe and Wildt, 1996). Several scientists used different media for in vitro maturation of cat oocytes and different results have been obtained (2–82 %) (Pope, 2000).

Supplements to In Vitro Maturation Media: In vitro maturation of immature oocytes is an attempt to mimic the in vivo environment (Pope et al., 2006). For this purpose, the temperature, humidity, and gas components of the incubator, there are also other important supplements such as Hormones, Proteins, Growth factors, and sometimes Antioxidants that need to be added to the medium (Pope 2000).

Hormones: Gonadotropic hormones such as FSH and LH are essential to support the maturation of cat oocytes in vitro (Pope et al., 2006). Additionally, in studies where steroids were used, it was reported that better in vitro maturation (Wolfe and Wildt, 1996; Wood et al., 1995; Pope et al., 2006) and in vitro fertilization (IVF) results were obtained compared to control groups (Wood et al., 1995).

Most researchers add FSH and LH to the maturation medium at 1-10 µg/ml (Evecen et al., 2009; Evecen et al., 2016; Wolfe and Wildt, 1996; Evecen et al., 2002; Pope et al., 2006) and estradiol-17b at 1 µg/ml (Wolfe and Wildt, 1996; Uchikura et al., 2011).

Protein Sources: Proteins are crucial molecules for the integrity of the zona pellucida, survival, and development of oocytes/embryos in vitro. Complex biological macromolecules with different structures are used as protein sources in many in vitro culture systems (Pope et al., 2006). Different homologous and heterologous protein sources such as Bovine Serum albumin (BSA), Fetal Calf Serum (FCS), Estrus Cat Serum (ECS), and Polyvinyl Alcohol (PVA) are generally used in various doses in cat studies in vitro and various results obtained (Pope et al., 2006). Researchers generally used BSA 3–6% mg/ml, FBS 5-10%, Polyvinyl Alcohol 1–3%, and OCS 5% in culture medium (Karja et al., 2002; Güriş and Birler, 2011; Wood et al., 1995; Pope et al., 2006; Nestle et al., 2012). Although some researchers claimed that the protein sources do not affect the quality of blastocysts produced during in vitro in cats (Nestle et al., 2012), many others have reported different results (Karja et al., 2002; Güriş and Birler, 2011; Wood et al., 1995; Pope et al., 2006; Nestle et al., 2012). It has been reported that the use of BSA was superior to Fetal Calf Serum (FCS) for oocytes reaching the M II stage (16,5 vs 5.4%) in cat oocytes in vitro (Güriş and Birler, 2011). It has been reported that BSA supports in vitro maturation rates more than FCS, whereas FCS is more successful in in vitro fertilization in cats (Wood et al., 1995). It has been also reported that FCS is more successful than BSA in terms of both reaching the embryo stage and the number of embryonic cells (Karja et al., 2002).

Growth Factors: Epidermal growth factor, which is thought to play an important role in folliculogenesis in cats, has been reported to be present in the theca interna cells of cats, smaller cells of the ovarian cortex, and corpus luteum (Göritz et al., 1996). EGF is known

to promote nuclear and cytoplasmic maturation in human, bovine, and porcine oocytes. Additionally, beneficial effects of EGF on oocyte maturation were found in rats, rabbits, buffalo, sheep, and horses (Merlo et al., 2005). It is reported that the addition of epidermal growth factor (EGF) to in vitro maturation of domestic cat oocytes in 10 ng/mL, enhances fertilization frequency and blastocyst development in vitro (Gomez et al., 2001). Another study comparing 10, 25, and 50 ng/ml EGF doses in cats, found that EGF did not contribute to in vitro maturation rates but at 25 ng/ml better supported in vitro fertilization and blastocyst rates (Merlo et al., 2005).

Antioxidants: Aerobic metabolism is associated with the production of pro-oxidant molecules called free radicals or reactive oxygen species (ROS). A state of oxidative stress begins when there is an imbalance between pro-oxidants and antioxidants. When free radicals begin to increase in the environment, it negatively affects gametes. Oxidative stress affects both embryonic implantation and early embryo development by interacting with cytokines and hormones (Agarwal et al., 2006). To protect oocytes and embryos from oxidative stress in the in vitro culture environment, some researchers sometimes prefer to add antioxidant substances. In studies conducted in different animal species, various antioxidants such as cysteamine cysteine, taurine and hypotaurine, βmercaptoethanol, vitamins E and C, superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT) have been used to reduce ROS and supports developing embryos in vitro (Cocchia et al., 2015; Agarwal et al., 2006). It reported that superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase (CAT) added to the medium provided significant benefits and while supplementing IVM media with SOD and CAT did not improve the oocyte maturation rate but it accelerated progression to the blastocyst stage on in vitro maturation and in vitro fertilization of cat oocytes (Cocchia et al., 2015). Another recent study showed that SOD and taurine supplementation promoted blastocyst development in low-quality cat oocytes (Ochota et al., 2016).

e. In Vitro Fertilization and Embryo Development: In vitro fertilization can be performed either by using in vivo matured (MII stage) oocytes (collected from female ovaries by aspiration) or in vitro matured oocytes. Thereafter, the mature oocytes and capacitated sperm are placed in the same environment, or sperm cells can be injected into the oocyte's cytoplasm (intracytoplasmic sperm injection: ICSI) (Johnstonet al.,1989). Various IVF protocols have also been developed for domestic cats and have been adapted to the wild cat species. However, in most studies the proportions of offspring surviving after ET have been highly irregular. Due to this inconsistency, IVF/ET is not currently used as a genetic management tool for the reliable production of offspring in any cat breed, but studies on this subject are ongoing (Pelican et al., 2006).

Sperm Capacitation: Ejaculated or epididymal collected cat semen can be used. Semen is diluted 1:1 with capacitation medium (Hams F-10, Synthetic Oviduct Fluid, TCM-199, Brackett Oliphant Medium, or others) including heparin, which improves the capacitation process and centrifuged at 300 g for 8 minutes. Then the supernatant is discarded, the remaining pellet is added to 100 µl of a medium, and the sample is kept at room temperature (swim up) for 1 hour. Later, about 50 µl of the upper layer containing active spermatozoa swimming upwards is taken and evaluated in terms of sperm motility, motility, and concentration (Evecen et al., 2003a).

In Vitro Fertilization (IVF) and Embryo Culture: The selected motile sperm concentration was adjusted to 2 –4x10^6/ml for fertilization. The prepared semen sample was left next to groups of 10–20 oocytes in fertilization medium drops under mineral oil. The gametes co-incubated for 20–24 hours, then transferred to the embryo culture medium. The embryo formation and development were checked every two days and eliminated those oocytes that had not divided. Also on the third and fifth days, the culture medium renewed, and in vitro culture continued for up to seven days (Johnstonet al.,1989; Karja et al., 2002; Evecen et al., 2003a). Although much lower rates were initially achieved (2–10 %), with the improvement of in vitro culture systems in recent years approximately 30 - 50 % of cat embryos produced in IVM/IVF developed into blastocysts on Day 7 of IVC (Pope 2000; Evecen et al., 2003a).

f. Embryo Cryopreservation and Transfer: Although both vitrification and slow freezing methods can be used to freeze cat embryos, the slow freezing method was found more successful (Pope 2000). Since the birth of live kittens from the transfer of in vitro derived and frozen embryos in 1994 (Pope et al., 1994), several pregnancies and births have occurred in both domestic and wild cat species (Pope 2000; Pope et al., 2006). In the first successful transfer of fresh cat embryos, 47 embryos from nine females were transferred to the uteri of nine recipients, and four live kittens were born (Pope et al., 2006). The first kittens after transfer of IVM/IVF-derived embryos were born in 1997 and three litters were produced (Pope 2000).

Embryos are transferred via laparotomy depending on the day of development, into the cornu uteri or oviduct of recipient females in domestic or wild cats whose estrus cycles are similarly synchronized (at varying doses) by exogenous gonadotropic hormones. After the first domestic kittens were born after transferring cryopreserved embryos derived from in vitro matured oocytes, several additional pregnancies and births have been reported by using the slow cryopreservation method in both domestic and non-domestic cats (African wildcat (Felis silvestris lybica), Ocelot (Leopardus), Pardalis, and Caracal (Caracal caracal) (Pope et al., 2006). Pregnancy success in both domestic and wild felines ranges from 0-50% (Pelican et al., 2006).

g. Semen Storage and Cryopreservation: The main purpose of storing and cryopreserving cats of domestic cat sperm is to preserve the gamet for future use and thus to apply the freezing techniques to wild felines that are in danger of extinction and vulnerable. Semen from domestic cats can be collected by the following methods: Artificial vagina (AV), Electroejaculation (EE), sperm collection from the epididymis or testicles, and a newly developed technique named "urethral catheterization after pharmacologically induced sedation". The cat embryo development rates in vitro, after IVF using ejaculated spermatozoa that had been stored in Tes-Tris egg yolk extender at cold temperature (4–8 ^{o}C) to 21 days were found similar to that obtained when fresh spermatozoa were used (Harris et al., 2002). Cat semen canbe successfully frozenby the paiette or pellet method in Tris (hydroxymethyl-aminomethane), TesT (N Trishydroxymethyl-methyl-2-aminomethane-sulfonic acid + Tris), and Tris-Fructose-Citric acid extenders, which contain 3–8% glycerol (Axner and Linde-Forsberg, 2002). There is no diluent specifically developed for cryopreservation of cat sperm. Therefore, diluents formulated for other species are used. Although many extenders such as; Skim milk-glucose-taurine (SMGT), egg yolk sodium citrate (EYC), and lactose egg yolk-based extenders have achieved similar success in freezing cat semen, Tris-egg yolk-based (TEY) extender, which contains glucose and was developed to freeze dog sperm, is the most commonly used extender in the cryopreservation of cat sperm. Besides, successful results are also obtained in studies in which lactose and fructose are added to the same diluent instead of glucose. If cat semen is cryopreserved as a pellet, a warm Tris-buffered solution that does not contain glycerol and egg yolk is generally used. However, if the semen has been cryopreserved in straws, it can be thawed by placing the straws in warm water for 30 sec. (37–38 ^{o}C) (Buranaamnuay 2017). In our previous study, we found that the post-thaw motility and morphologic defect rates in 3% and 4% glycerol-containing Tris extenders (including fructose instead of glucose) were similar and cat semen could be frozen with both glycerol levels successfully (Baran et al., 2010).

h. Artificial Insemination: Artificial insemination in domestic cats does not have widespread clinical application as it does in dogs. Many factors limit AI in cats. The main ones are; aggression, difficulty in semen collection techniques, very low semen volume, insemination method, ovulation induction, and sedation. Although intravaginal AI has been achieved in domestic cats and tigers, very high concentrations of sperm (107–108) were required to achieve pregnancy (Chagas e Silva et al., 2000). Although artificial insemination studies in cats have been tried for fifty years, the results are still very different and not at the expected level (Buranaamnuay 2017). However, recently some researchers announced that two healthy kittens were born from artificial insemination of domestic cats with fresh semen (Daşkın et al., 2022). It is known that the anesthesia applied during this procedure has a negative effect on both sperm transport and ovulation in domestic cats through mechanisms that reduce uterine contractions (Howard 1992). Freezing procedures reduce the potential fertility of semen (Buranaamnuay 2017). This necessitated the development of intrauterine AI procedures. While AI with fresh spermatozoa is applied successfully in domestic cats and cheetahs, this success rate is much less in other wild felines, mostly for unknown reasons (Pelican et al., 2006).

3. Somatic Cell Nuclear Transfer (Cloning)

Animal breeding through Somatic Cell Nuclear Transfer (SCNT) is a valuable tool for the conservation of vulnerable and endangered species and the production of transgenic animals. In recent years, great advances have been made in assisted reproductive technologies for the protection of endangered felines, as in other animal species. SCNT also known as cloning, is a very valuable technology in preserving genetic diversity. Although Nuclear transfer (NT) technology is used for producing identical individuals, it is also an important tool for understanding the cellular and molecular aspects of nuclear reprogramming (Gomez et al., 2004). Following the birth of the first cloned domestic cat kittens in 2002 (Shin et al., 2022), the first wild cat (African wildcat) was born in 2004 after SCNT and intra -species transfer (to the domestic cat) (Gomez et al.,

2004). Since endangered cat oocytes are very scarce, the ooplasm of a domestic cat oocyte can be used as a somatic cell nucleus recipient of an endangered cat. At the same time, collecting tissue samples (such as a piece of skin) from wild animals is easier, less costly and harmless than collecting gametes or embryos. The possibility of obtaining somatic resources from fetal cells offers the advantage of obtaining genetic material from animals even in cases of stillbirth and abortion. This provides great convenience in protecting wild felines with the help of domestic cats. Despite the common belief that cloned animals have the same cellular structure, telomere length in cats is independent of telomere length in donor cells (Imsoonthornruksa et al., 2012). Although offspring can be produced via SCNT in different mammalian species, success rates are still very low (1–11%). Different somatic cell types have been tried as sources of genetic material for SCNT. These; are fibroblasts, cumulus cells, and preadipocytes from adults and fetuses. However, fetal cells were generally found to be better than those obtained from adults. In our previous study, where we used cumulus cells as a source of somatic cells, we produced in vitro cat embryos with SCNT (Evecen et al., 2016). Howeveralthough cytoplasts were cleaved (15.75 %) and some of them (9.58%) reached the morula cell stage, none of them could reach the blastocyst stage. While some authors prefer to transfer early-stage embryos to the oviduct (Goodrowe et al., 1988), others have chosen to transfer more developed embryos (morula/ blastocyst) into the uterus (Pope et al., 2006). Both methods have resulted in pregnancy and the production of live kittens in both domestic and wild cats. The first cloned cat (a female named Copy Cat) reproduced naturally and gave birth to three healthy kittens (Shin et al., 2002). Thus, the view that cloned animals are normal is confirmed and that SCNT is therefore a valuable method to preserve endangered felines. Recently, the use of endoscopy for transcervical artificial insemination in cats has created excitement for the application of this method in embryo transfer (Zambelli et al., 2015).

4. Transgenesis

Studies in domestic cats have discovered that cats have 18 pairs of autosomal chromosomes and XY sex chromosome pairs. Gene mapping studies in cats have shown that the genome organization of cats is more similar to humans than that of dogs or mice (Muldoon et al., 1994). Recently it has been demonstrated that 90 % of the genes discovered in the cat carry homologues of human genes (Gomez et al., 2009). Sandhoff disease is a genetic disorder characterized by the deposition of GM2-gangliosides and other related asialoglycolipids in the brain and other tissues. Children with the infantile form of this disease usually die by the age of four. In 1985, researchers detected a disorder in Korat cats that is analogous to human type II GM2-gangliosidosis (Neuwelt 1985). Studies are being carried out to examine the mutations that cause diseases in cats to develop a cat model for similar diseases in humans. The Korat cat provides an animal model that may be suitable for testing gene replacement therapy in, Sandhoff disease in humans (Muldoon et al., 1994). Autosomal dominant polycystic kidney disease (ADPKD) is one of the most common genetic diseases in humans and affects approximately five million people in the world. Polycystic kidney disease is a disease seen in longhaired Persian cats and is similar to autosomal dominant polycystic kidney disease (ADPKD) in humans. Cats are also a valuable animal model for studies on the treatment of this disease in humans (Biller et al., 1996). Recently, different researchers have conducted successful transgenic studies in domestic cats, proving that green and red fluorescence genes are expressed in the born kittens (Gomez et al., 2009; Yin et al., 2008). Transgenic studies such as these offer great opportunities to create desired disease models and cure individuals with genetic diseases with gene therapy (Gomez et al., 2009).

Conclusion

Since the first successful embryo transfer, significant advances have been made in assisted reproductive technologies in cats. These advances have encouraged researchers to adapt these techniques to wild cat species, mostly in the last two decades. Thanks to these technologies, domestic cats are now successfully cloned commercially and provide great happiness to their owners whose pets have aged or died by giving them new ones. Thus it becomes much easier to protect endangered wild cat species, with domestic cats becoming good model animals for wild cats or serving as interspecies embryo carriers. Additionally, due to their genetic similarity cats are helpful as animal models in the study of many genetic diseases found in humans that are similar to their feline counterparts. ARTs also make great contributions to the development of future treatment methods by helping to investigate and understand the mechanisms of many genetic diseases that are similar to their counterparts in humans and cats.

References

Agarwal, A., Gupta, S., & Sikka, S. (2006). The role of free radicals and antioxidants in reproduction. *Current Opinion in Obstetrics and Gynecology, 18*(3), 325-332.

Arıcı, R., Ak, K., Pabuccuoğlu, S., Birler, S., Demir, K., Yağcıoğlu, S., Eser, A., Ersoy, N., Oruç, İ., Bakirer Öztürk, G., Kömürcü Bayrak, E., Özsait Selçuk, B., Kılıçkap, A., & Evecen, M. (2022). Effects of melatonin addition to the cold storage medium on cumulus oocyte complex apoptosis, viability and in vitro maturation rates of cat oocytes. *Kafkas University Veterinary Faculty Journal, 28* (2), 193-200.

Axner, E., & Linde-Forsberg, C. (2002). Semen collection and assessment, and artificial insemination in the cat. http:// www.ivis.org, Communications date: 15.09.2002.

Baran, A., Tek, Ç., Demir, K., Sabuncu, A., & Özdaş, Ö.B. (2010). Intrauterine insemination with cat semen frozen with various extenders. *Turkish Journal of Veterinary and Animal Sciences, 35*, 311-318.

Biller, D.S., DiBartola, S.P., Eaton, K.A., Pflueger, S., Wellman, M.L., & Radin, M.J. (1996). Inheritance of polycystic kidney disease in Persian cats. *Journal of Heredity, 87*(1), 1 -5.

Buranaamnuay, K. (2017). Protocols for sperm cryopreservation in the domestic cat: A review. *Animal Reproduction Science, 183*, 56-65.

Chagas e Silva, J.N., Leitao, R.M., Lapao, N.E., da Cunha, M.B., da Cunha, T.P., da Silva, J.P., & Paisana, F.C. (2000). Birth of Siberian tiger (Panthera tigris altaica) cubs after transvaginal artificial insemination. *Journal of Zoo and Wildlife Medicine, 31*(4), 566-569.

Cocchia, N., Corteggio, A., Altamura, G., Tafuri, S., Rea, S., Rosapane, I., Sica, A., Landolfi, F., & Ciani, F. (2015). The effects of superoxide dismutase addition to the transport medium on cumulus-oocyte complex apoptosis and IVF outcome in cats (Felis catus). *Reproductive Biology, 15*(1), 56-64.

Daşkın, A., Totan, E., & Olğaç, K.T. (2022). Artificial insemination in a cat: Report of first successful performance resulted with parturition in Turkey. *Ankara University Veterinary Faculty Journal, 69*(2), 225-227.

Evecen, M., Baran, A., & Tek, Ç. (2002). The effects of gonadotrophins on in vitro maturation of cat oocytes. *Turkish Journal of Veterinary and Animal Sciences, 26*(6), 1315-1320.

Evecen, M., Atalla, H., Birler, S., Baran, A., & Ak, K. (2003a). Studies on the in vitro maturation of cat oocytes. Turkish *Journal of Veterinary and Animal Sciences, 27*(6), 1409-1412.

Evecen, M., Şahin, E., Baran, A., Ak, K., & İleri, İ.K. (2003b). Kedi oositlerinin in vitro fertilizasyonu. Istanbul *University Veterinary Faculty Journal, 29*(2), 253-258.

Evecen, M., Özdaş, Ö.B., Pabuccuoğlu, S., Birler, S., Tek, Ç., & İleri, İ.K. (2004). The effects reproductive status of queens on in vitro maturation of cat oocytes. *Indian Veterinary Journal, 81*, 1005-1009.

Evecen, M., Cirit, U., Demir, K., Karaman, E., Hamzaoğlu, A.İ., & Bakirer, G. (2009). Developmental competence of domestic cat oocytes from ovaries stored at various durations at 4 degrees C temperature. *Animal Reproduction Science, 116*(1-2), 169-172.

Evecen, M., Cirit, U., Demir, K., Özdaş, Ö.B, Taş, M., Birler, S., & Pabuccuoğlu, S. (2010). Effects of estrous cycle stage and transport temperature of ovaries on in vitro maturation of canine oocytes. *Animal Reproduction Science, 117*(1-2), 160-165.

Evecen, M., Pabuccuoğlu, S., Demir, K., Yağcıoğlu, S., Can, A., Ertürk, E., Sandal, A.I., Arici, R., Öztürk, G., Ak, K., & Birler, S. (2016). Somatic cloning in cats using MI or MII oocytes. *Kafkas University Veterinary Faculty Journal, 22*(6), 923-928.

Gomez, M.C., Pope, C.E., Davis, A.M., Harris, R.F., & Dresser, B.L. (2001). Addition of epidermal growth factor (EGF) during in vitro maturation of domestic cat oocytes enhances fertilization frequency and blastocyst development in vitro. *Theriogenology, 55*, 472.

Gomez, M.C., Pope, C.E., Giraldo, A., Lyons, L.A., Harris, R.F., King, A.L., Cole, A., Godke, R.A., & Dresser, B.L. (2004). Birth of African wildcat cloned kittens born from domestic cats. *Cloning and Stem Cells, 6*(3), 247-258.

Gomez, M.C., Pope, C.E., Kutner, R.H., Ricks, D.M., Lyons, L.A., Ruhe, M.T., Dumas, C., Lyons, J., Dresser, B.L., & Reiser, J. (2009). Generation of domestic transgenic cloned kittens using lentivirus vectors. Cloning *and Stem Cells, 11*(1), 167-176.

Goodrowe, K.L., Wall, R.J., O'Brien, S.J., Schmidt, P.M., & Wildt, D.E. (1988). Developmental competence of domestic cat follicular oocytes after fertilization in vitro. *Biology of Reproduction, 39*(2), 355-372.

Göritz, F., Jewgenow, K., & Meyer, H.H. (1996). Epidermal growth factor and epidermal growth factor receptor in the ovary of the domestic cat (Felis catus). *Journal of Reproduction and Fertility, 106*(1), 117-124.

Güriş, A.E., & Birler, S. (2011). The effects of various protein supplementations on in vitro maturation of cat oocytes. *Kafkas University Veterinary Faculty Journal, 17*(1), 37-40.

Harris, R.F., Gomez, M.C., Leibo, S.P., & Pope, C.E. (2002). In vitro development of domestic cat embryos after in vitro fertilization of oocytes with spermatozoa stored for various intervals at 4°C. *Theriogenology, 57*, 365.

Howard, J.G., Barone, M.A., Donoghue, A.M., & Wildt, D.E. (1992). The effect of pre-ovulatory anaesthesia on ovulation in laparoscopically inseminated domestic cats. *Journal of Reproduction and Fertility, 96*(1), 175-186.

Imsoonthornruksa, S., Sangmalee, A., Srirattana, K., Parnpai, R., & Ketudat-Cairns, M. (2012). Development of intergeneric and intrageneric somatic cell nuclear transfer (SCNT) cat embryos and the determination of telomere length in cloned offspring. *Cell Reprogramming, 4*(1), 79-87.

Johnston, L.A., O'Brien, S.J., & Wildt, D.E. (1989). In vitro maturation and fertilization of domestic cat follicular oocytes. *Gamete Research, 24*(3), 343-356.

Karja, N.W., Otoi, T., Murakami, M., Yuge, M., Fahrudin, M., & Suzuki, T. (2002). Effect of protein supplementation on development to the hatching and hatched blastocyst stages of cat IVF embryos. Reproduction, Fertility *and Development, 14*(5-6), 291-296.

Merlo, B., Iacono, E., Zambelli, D., Prati, F., & Belluzzi, S. (2005). Effect of EGF on in vitro maturation of domestic cat oocytes. *Theriogenology, 63*(7), 2032-2039.

Muldoon, L.L., Neuwelt, E.A., Pagel, M.A., & Weiss, D.L. (1994). Characterization of the molecular defect in a feline model for type II GM2-gangliosidosis (Sandhoff disease). *American Journal of Pathology, 144*(5), 1109-1118.

Nestle, E., Graves-Herring, J., Keefer, C., & Comizzoli, P. (2012). Source of protein supplementation during in vitro culture does not affect the quality of resulting blastocysts in the domestic cat. *Reproduction in Domestic Animals, 47* (6), 152-155.

Neuwelt, E.A., Johnson, W.G., Blank, N.K., Pagel, M.A., Maslen-McClure, C., McClure, M.J., & Wu, P.M. (1985). Characterization of a new model of GM2-gangliosidosis (Sandhoff's disease) in Korat cats. *Journal of Clinical Investigation, 76*(2), 482-490.

Ochota, M., Pasieka, A., & Nizanski, W. (2015). Superoxide dismutase and taurine supplementation improves in vitro blastocyst yield from poor-quality feline oocytes. *Theriogenology, 85*(5), 922-927.

Pelican, K.M., Wildt, D.E., Pukazhenthi, B., & Howard, J. (2006). Ovarian control for assisted reproduction in the domestic cat and wild felids. *Theriogenology, 66*(1), 37-48.

Pichardo, J., Reyes, M., Kjelland, M., & Suastegui, J. (2021). Effect of different reproductive stages and culture times on domestic cat in vitro oocyte maturation. *Advances in Reproductive Sciences, 9*, 129-138.

Pope, C.E. (2000). Embryo technology in conservation efforts for endangered felids. Theriogenology, 53(1), 163-174.

Pope, C.E., Gómez, M.C., & Dresser, B.L. (2006). In vitro production and transfer of cat embryos in the 21st century. *Theriogenology, 66*(1), 59-71.

Pope, C.E., McRae, M.A., Plair, B.L., Keller, G.L., & Dresser, B.L. (1994). Successful in vitro and in vivo development of in vitro fertilized two- to four-cell cat embryos following cryopreservation, culture and transfer. *Theriogenology, 42*(3), 513-525.

Shin, T., Kraemer, D., Pryor, J., Liu, L., Rugila, J., Howe, L., Buck, S., Murphy, K., Lyons, L., & Westhusin, M. (2002). A cat cloned by nuclear transplantation. *Nature, 415*(6874), 859.

Swelum, A.A., Abdelnour, S.A., Sheiha, A.M., Hashem, N.M., Taha, A.E., Khafaga, A.F., Attia, Y., Al-Mutary, M.G., Abdel-Bar, M.M., Ohran, H., Nagadi, S.A., & Abdel-Hack, M.E. (2022). Enhancing in vitro oocyte maturation competence and embryo development in farm animals: roles of vitamin-based antioxidants – a review. *Annals of Animal Science, 22*(1), 3-19.

Uchikura, K., Nagano, M., & Hishinuma, M. (2011). The effect of ovarian status and follicular diameter on maturational ability of domestic cat oocytes. Journal *of Veterinary Medical Science, 73*(5), 561-566.

Wolfe, B.A., & Wildt, D.E. (1996). Development to blastocysts of domestic cat oocytes matured and fertilized in vitro after prolonged cold storage. *Journal of Reproduction and Fertility, 106*(1), 135-141.

Wood, T.C., Byers, A.P., Jennette, B.E., & Wildt, D.E. (1995). Influence of protein and hormone supplementation on in vitro maturation and fertilization of domestic cat eggs. *Journal of Reproduction and Fertility, 104*(2), 315-323.

Yin, X.J., Lee, H.S., Yu, X.F., Choi, E., Koo, B.C., Kwon, M.S., Lee, Y.S., Cho, S.J., Jin, G.Z., Kim, L.H., Shin, H.D., Kim, T., Kim, N.H., & Kong, I.K. (2008). Generation of cloned transgenic cats expressing red fluorescence protein. *Biology of Reproduction, 78*(3), 425-431.

Yoshida, T., Alam, M.E., Hanafusa, K., Tsujimoto, Y., Tsukamoto, M., Kanegi, R., Inaba, T., Sugiura, K., & Hatoya, S. (2022). Effects of the preservation medium and storage duration of domestic cat ovaries on the maturational and developmental competence of oocytes in vitro. *Journal of Reproduction and Development, 68*(2), 160-164.

Zambelli, D., Binii, C., Küster, D.G., Molari, V., & Cunto, M. (2015). First deliveries after estrus induction using deslorelin and endoscopic transcervical insemination in the queen. *Theriogenology, 84*(5), 773-778.

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Medical management of idiopathic chylothorax in a crossbreed cat with octreotide and rutin use: A case report

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ABSTRACT

This case report describes the diagnosis and treatment of idiopathic chylothorax in a 5-year-old female crossbreed cat who presented with respiratory distress, tachypnea, cyanosis, exercise intolerance and weight loss over a short period of time. Based on the clinical examination, blood results, radiological and echocardiographic findings, the patient was diagnosed with chylous effusion. Chylothorax was considered idiopathic because there was no underlying trauma or disease etiology. Effusion drainage was performed by thoracocentesis to reduce respiratory stress. After thoracocentesis, followed by using medical octreotide- a somatostatin analogue (Sandostatin™, 0.1 mg/ml ampoule, Novartis, USA) and rutin - a flavone benzo-γ-pyrone plant fruit extracted from the Brazilian plant Fava D'anta (Dimorphandra mollis) (Rutin - Plant-Based Bioflavonoid, 500 mg tablet, Solgar™, USA), were administered in addition to supportive treatment. Rutin and ocreotide have been used successfully in humans, dogs and cats to the treatment of pleural effusions as presented various studies. It is hoped that these drugs may also be useful for decreasing pleural effusion in cats with chylothorax. In this represented case; partial resolution of pleural effusion was observed after octreotide usage and complete resolution of pleural effusion was observed after rutin (plant-based bioflavonoid) usage. No recurrence was observed during 7 months of regular follow-up.It was determined that the use of octreotide and rutin after thoracocentesis gave successful results in the medical management of idiopathic chylothorax in cats.

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Introduction

Chylothorax is the effusion of lymph into the pleural cavity (Chinnock, 2003; Fossum, 2006). This is a complex condition and relatively rare in animals. However, it has been reported in horses, cattle, rats, dogs and cats. Chylothorax represents 2% to 3% of pleural effusions and can be classified into four different types: traumatic, malignant, idiopathic or miscellaneous (Gupta and Faith, 1977; Bichard et. al., 1995; Brink et. al., 1996; Litvin et al., 2023). Etiologies of chylothorax in cats include neoplasia (e.g. mediastinal lymphosarcoma), congenital anomalies, dirofilariasis, blastomycosis, pericardial diseases, cardiomyopathies (especially secondary to hyperthyroidism), cardiogenic disorders (e.g.

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paroxysmal atrioventricular block), thoracic duct rupture or leak, and fungal granulomas. Also rare cases reported secondary to diaphragmatic herni and lung lobe torsion. In the majority of affected cats, even after wide diagnostic examination, chylothorax is classified as idiopathic, when the underlying etiology cannot be determined. Although any condition causing increased venous pressure can cause chylothorax, a differential diagnosis should be made together with a full cardiac assessment. As the management of this condition depends on the underlying etiology and type of chylothorax, the presence of coexisting conditions should be eliminated before definitive diagnosis and treatment is administered (Fossum, 2006; Nikiphorou et al., 2016; Hambrook and Kudnig, 2012; Mclane and Buote, 2011).

Idiopatic chylothorax is a disease characterised by gradual deterioration of the cat and usually present with respiratory distress due to atelectasis of the lung caused by chylous fluid. These patients may also display symptoms such as exercise intolerance, lethargy, rapid breathing, coughing and weight loss. As the volume of the pleural fluid increases, the patient becomes more tachypneic, more dyspneic and less able to tolerate exercise (Kopko, 2005). The loss of chyle has several adverse effects; lipids, proteins, lymphocytes, electrolytes and fluids are lost. This is extremely harmful if the animal's thorax is drained regularly over weeks or months. If fluid and calorie intake is not increased, dehydration and loss of weight going to occur (Birchard and Fossum 1987; Stockdale et al., 2018).

The diagnosis of chylothorax is based on the patient's history, chest and lung sounds oscultation, and diagnostic procedure that thorax radiography, ultrasound and/or computed tomography (CT), cardiac echocardiography and Doppler imaging. Loss of cardiac silhouette, nonairated atelectatic lungs, indistinct interlobar margins, sometimes slightly dorsally elongated trachea confirm pleural effusion. Analysis of thoracocentesis fluid can be used to determine the nature of the fluid. In particular, the detection of high triglyceride levels and the identification of large numbers of lymphocytes, characteristic dencity and protein content indicate the presence of chyle (modified transudate). Its protein concentration is between 2.5-4 g/dL, with a cell count below 7000/µL and a specific gravity below 1.032. Further laboratuary test carried out to find the underlying cause (Beatty and Barrs, 2010; Spencer and Karen, 2012; Singh et al., 2012).

The management of idiopatic chylothorax in cats includes medical, surgical or both treatment modalities. Conventional medical management and palliative treatment (involving frequent thoracentesis and a low-fat diet), of idoipathic chylothorax in cats has been associated with a poor diagnosis. Therefore, limiting fluid accumulation is crucial to prevent the need for repeated thoracentesis, which can be finally lead to severe fibrosing pleuritis due to chyle - resulting in pleural thickening and impaired pulmonary expansion and increase the risk of mortality due to anesthesia-related complications post-fluid removal. Currently surgical options avaible for cat with idiopathic chylothorax, include thoracic duct ligation and mesenteric lymphangiography, post-cisterna chyli and thoracic duct glue embolization, passive and active pleuroperitoneal shunting, active pleurovenous shunting, subtotal pericardectomy, omentalization and pleurodesis. Surgical interventions such as ligation of the thoracic duct have been only limited success in relieving chylous effusions. Post-operative recurrence and death has also been reported in some of these cases (Fossum, 2001; Stockdale et al., 2018).

The primary goal of idiopathic chylothorax treatment in cats, is to achieve resolution of the chylous fluid without re-occurance. Recent studies have reported the use of octreotide (a somatostatin analogue) and rutin (a flavone benzo-γ-pyrone extracted from the Brazilian plant - Fava D'anta, Dimorphandra mollis) administration for this purpose. In medical treatment, the efficacy of octreotide has been reported in recent cases, with rutin (plant-based bioflavonoid) usage also showing effectiveness in previously reported cases (Thompson et al., 1999; Gould, 2004; Kopko, 2005). Octreotide mimics the naturally occurring hormone known as somatostatin in humans. Produces an increase in water absorption and intestinal transit and a decrease in pancreatic-duodenal secretion. More importantly, the resistance to splenic blood flow increases, and intestinal arteriolar flow decreases, in turn reducing lymphatic flow due to the inhibition of serotonin and other intestinal peptides (Esme,2019). Rutin (benzo-γ-pyrone), a bioflavonoid found in the fruit of the Brazilian fava d'Anta tree, is classified as nutraceutical and is available without a prescription. It has been used successfully to treat lymphedema in humans and it is hoped that this drug may be useful in reducing pleural effusion in cats with chylothorax (Gingirelli et al., 2016; Kopko, 2005; Thompson et al., 1999). However, the effectiveness of rutin (plant-based bioflavonoid) in reducing pleural effusion in chylothorax-afflicted cats remains uncertain. Some reports indicate complete resolution of pleural effusion in cats treated with rutin (50-100 mg/kg, po, q8h) after 2 months, although it is controversial whether this outcome is due to the drug or

spontaneous healing (Gould, 2004). In a separate study involving rutin treatment (benzo-γ-pyrone Dimorphandra mollis based bioflavonoid; 250mg or 500mg, per cat, q8h for 63 days), complete resolution was observed in a domestic cat (Kopko, 2005), and another study showed clinical improvement 3 out of 4 cats (Thompson et al., 1999). Recent findings have highlighted the efficacy of octreotide, which directly impacts lymphatic flow, in neoplastic and traumatic cases of chylothorax in humans (Esme, 2019). However, the response to octreotide treatment in cats with chylothorax varies. In one study (Yılmaz and Kocatürk, 2023), octreotide administration at 10 µg/kg subcutaneously, 3 times daily for 2-3 weeks, led to complete resolution in one case.

This case report aimed to investigate the efficacy of medical octreotide and rutin (benzo-γ-pyrone Dimorphandra mollis based bioflavonoid) usage in the treatment of idiopathic chylothorax in a cat. The researchers aim to achieve successful results with this treatment approach, reduce the necessity for surgical interventions and make significant contribution to the existing literature. This study on the treatment of this rare condition in cats, provides veterinarians with a scientifically grounded method to invastigate medical treatment options and implement healing interventions.

Case

The case was a 5 years old female crossbreed sterilised cat who was referred to the İstanbul University-Cerrahpaşa, Faculty of Veterinary Medicine, Surgery Department with complaints of respiratory difficulties, cyanosis of mucous membranes, intermittent cough, exercise intolerance and rapid weight loss in a short period of time (3.8kg to 3.2kg). During the clinical examination of the patient, it was in a sitting sternal position and moderately stressed. The patient noted dyspnea and tachypnea (44 bpm-beats per minute), along with a slight increase in body temperature (39° C). The mucosa was slightly cyanotic and the oxygen saturation (SpO2) was 92%. Heart rate was within the reference range for cats. Auscultation of the lungs revealed that breathing sounds were slightly muffled. After 30 minutes of observation in the oxygen cabinet, saturation levels increased to 95% according to the admissions. Laterolateral (LL) and ventrodorsal (VD) thorasic and abdominal radiographs of the patient were taken. Radiographs showed fluid accumulation in the interlobar cracks, significant thickening of the visceral pleura and widening of the mediastinum. Blunting and rounding of the costophrenic and lumbodiaphragmatic angles of the lung lobes was observed, along with border effacement with the cardiac silhouette and diaphragm. Interlobar fissure lines and cranial lung lobes atelectasis were noted, with dorsal elevation of caudal lung lobes. In addition to these findings, thoracic radiography (VD) showed the cardiac silhouette was almost completely absent, especially on the right side (Figure 1, Figure 2).

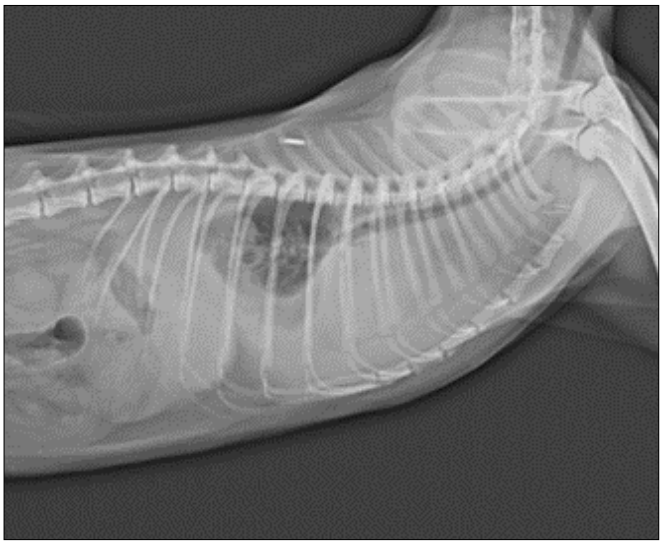

Figure 1. Thorax LL (laterolateral) radiography.

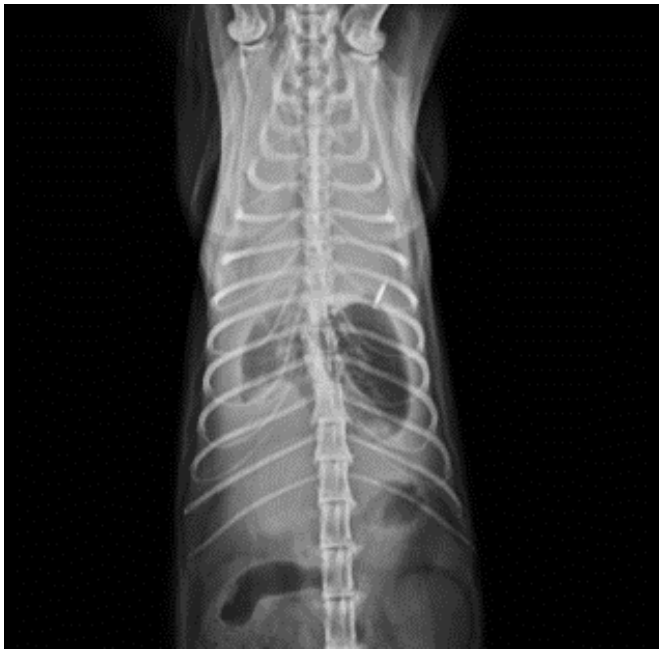

Figure 2. Thorax VD (ventrodorsal) radiography

Thoracic and abdominal ultrasound, examinations, along with color Doppler echocardiography (Mindray Vetus 8, Shenzhen Mindray Animal Medical Technology Co., LTD.; China), were performed. Pleural fluid was anechoic on thoracic ultrasonography (USG), with intact diaphragmatic integrity. Other ultrasound findings were unremarkable. In 2D, M-mode echocardiography the systolic and diastolic diameters of the left ventricular free wall (LVFW) and interventricular septum (IVS) were within normal

range (<6mm), along with normal left ventricular systolic function, indicated by fractional percentages (FS & EF), and a Sphericity index of 1.42 (normal: 1.43 ±0.12). No evidence of pericardial effusion or cardiac neoplasm was found. However, there was generalized bilateral pleural effusion. Sinus tachycardia (212 bpm) with normal QRS morphology on ECG was considered to be stress-related.

The drainage of pleural effusion was decided upon to regulate dyspneic respiration, reduce compression on the lungs, and determine the character of the effusion. For the purpose of prevent to triggering stress, mild sedation was administered with butorphanol (Butomidor, 10mg/ml, Richter Farma, Wels-Austria) at a dose of 0.2mg/kg intramuscularly (im), before 10 minutes of thoracosenthesis. During administration, to achieve stabilization, sedation depth was increased with propofol (Propofol-PF, 10 mg/ml, Polifarma, Turkey) at a dose of 1-4mg/kg intraveneously (iv) and local anesthesia was applied to the intercostal muscles and skin with lidocaine HCl (Jetokain Simplex, 20mg/2ml, ampoule, ADEKA Pharmaceutical Industry and Trade Inc., Turkey; im infiltration applied until desensitization is achieved) while the patient was conscious and in sternal position. Bilateral thoracentesis was performed using 23 gauge butterfly cannula and 3-way stopcock. The fluid obtained from thoracentesis was creamy in color, had an oily consistency, milk-like character and was odorless, costent with chylothorax (Figure 3).

No alteration in the color of the fluid was observed post-centrifugation. The milky fluid had a specific gravity of 1.025 (reference range for chylous fluid, 1.010 to 1.050) and total protein content of 373g/dL (reference range 6.0-7.9g/dL) was very high. The Rivalta test was negative and rapid biogram (Speed Biogram, Virbac, England) yielded no microbial growth from chylous fluid.

Figure 3. Milky pleural aspirate.

Post- thoracentesis radiograhic imaging of thorax revealed consolidation of lung lobes with boundaries marked. Cranial lung lobes exhibited atelectasis, while aerated lung was present only in the caudal 1/3 of thorax. Further investigations through thoracic and abdominal computed tomography (SOMATOM go.Now, Simens, Germany) revealed findings

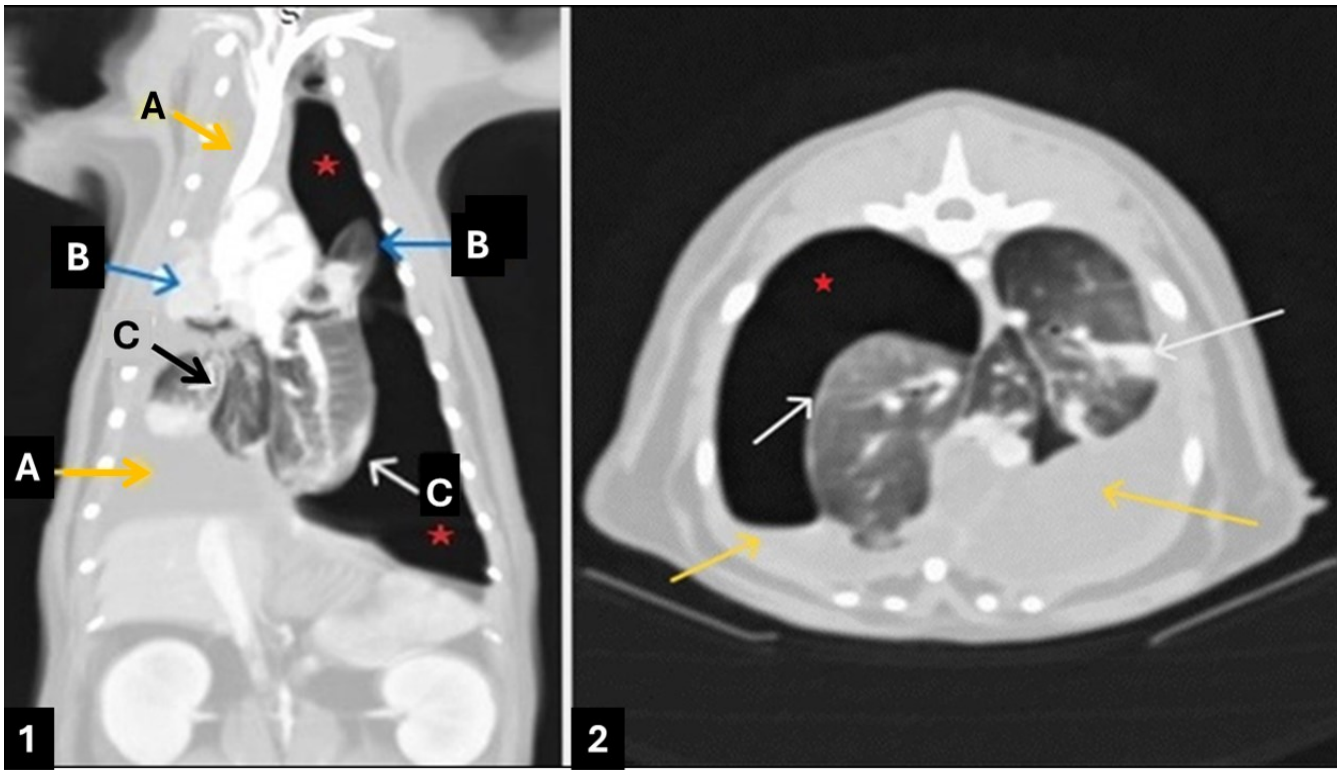

Figure 4. Computed Tomography Dorsal Plan (1) and Transversal Plan(2) Thorax Images. Pleural effusion - yellow arrows (A), lung athelactesis - blue arrows (B), visseral pleura dencity incraese - white arrows (C), left sided pneumothorax red stars represented.

pleural efusion reaching a thickness of 12 mm on the right and 38 mm on the left; was observed. Increased soft tissue density in a thick band-like pattern on the right lung suggests compression atelectasis. Retrosternal localization lymph nodes were enhanced in the cranial mediastinum (Figure 4).

The biochemical profile (Fuji DRI-CHEM NX600V, Fujifilm, Japan) was normal (alkaline phosphatase - ALP, gamma glutamyl transferase- GGT, glucose, total bilirubin - TBIL, creatinine - CRE, blood urea nitrogen - BUN, albumine - ALB, phosphor, total cholesterol, triglyceride, calcium) except alanine aminotransferase - ALT 151 u/L (reference ranges 22-84 u/L) total protein - TP 8.4 g/dL (reference ranges 5.7-7.8 g/dL), ammonia - NH_3 93 µg/L (23-78 µg/L) which were increased. Hemogram (BC60R Vet, Mindray China) analysis abnormalities noted were mild lymphopenia (0.298 x10^9/L, referance range 0.7-7.4 x10^9/L) and low PCT (0.25ml/L, reference range 0.9-7 ml/L). Feline NT-ProBNP <50pmol/L (referece range <100pmol/L:normal; 100pmol/L and >100pmol/L: abnormal,V-Check, Bionote Inc., Korea) ve feline troponin I, 0.12ng/ml (referece ranges, <0.14 ng/ml:normal; 0.14-0.22ng/ml equivo and > 0.22ng/ml abnormal, V-Check, Bionote Inc., Korea) founded. Feline immunodeficiency virus (FIV Ab), feline leukemia virus (FeLV Ag) and feline heartworm (FHW Ab) tests performed on the patient (Anigen Rapid FIV Ab/FeLV Ag Test Kit and Anigen FHW Ab; Anigen Animal Genetics Inc., Korea) also gave negative results.

Based on laboratory results, clinical, radiological and echocardiographic findings, the patient was diagnosed with chylous effusion. Since there was no underlying trauma or disease etiology, diagnosis was considered idiopathic chylothorax.

Octreotide (Sandostatin®, 0.1 mg/ml ampoule, Novartis, USA; 10 µg/kg, q8h, sc, 21 days) was used for the resolution of chylous effusion. Salbutamole (Ventolin® Nebules®, 2,5 mg/2,5 ml nebule, GlaxoSmithKline, Australia Pty. Ltd., Australia; 0.5mg per cat, q12h, nebule) and fluticasone propionate (Flixair 500 µg /2 ml, aerosol, VEM Pharmaceutical Ind. and Trade Inc, Ankara; 110 µg per cat, q12h, nebule) were added to the treatment for the first 3 days as supportive therapy. After 21 days, the chylous fluid had slightly decreased but not completely resolved. Subsequently of these finding, a second thoracentesis was performed. Following this procedure, rutin (Rutin - Plant-Based Bioflavonoid, 500 mg tablet, Solgar®, USA; 250mg/per cat, q8h, 63 days) was initiated. Two weeks later respiratory findings had normalised and the pleural effusion had significantly decreased. Haematological parameters were found to non-specific. Biochemical analysis showed that ALT returned to normal reference range whereas glucose and TP were slightly increased. The patient, who was noted to have a normal mental status and appetite, was noted to have gained weight (4.3 kg) after treatment. No clinical signs were observed at the 4th, 8th and 12th week examinations. It was observed that atelectatic cranial lobes stayed non-aerated and did not return to normal localization on radiography (Figure 5). Rounded lung lobes and findings consistent with pleural fibrosis were observed on radiography. It was determined that the chylous effusion was completely resolved.

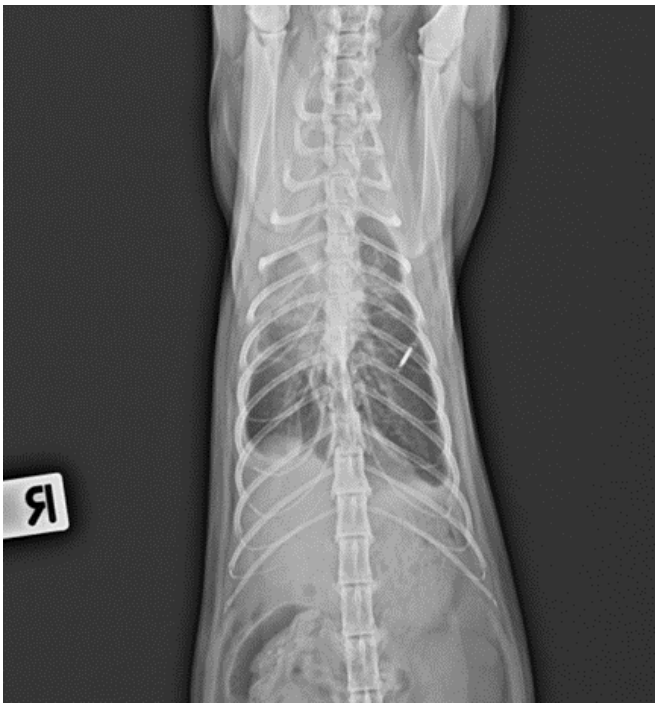

Figure 5. Thorax VD radiography – after two weeks of rutin (plant-based bioflavonoid) treatment.

Disscussion

Chylothorax is a rare type of pleural effusion in animals and has been reported in many different species. In cats, it is expressed that it is mostly seen in pure breeds such as Siamese and mostly affects aged cats (Fossum, 1991; Beatty and Barrs 2010). In this case, the patient was a crossbreed and a 5-year-old middle aged cat. The study presented (Gould, 2004) is supported by the case being neither purebred nor aged. Various factors such as neoplasia, cardiogenic disorders, diaphragmatic hernias and trauma play a role in the formation of chylothorax in cats (Fossum 2006; Nikiphorou et al., 2016; Hambrook and Kudnig,2012; Mclane and Buote, 2011). Clinical examination, radiological and echocardiographic evaluation of this case revealed no findings of any disease that could lead to chylothorax formation. Thoracic and abdominal ultrasound imaging along with color Doppler echocardiography represented that the

masses (Gould, 2004) and computed tomography findings consist with that findings (McGrath, 2011). As there was no underlying trauma or disease etiology (Fossum 2006), based on the radiographic findings of pleural effusion as well as the appearance and analysis of pleural fluid, the diagnosis was considered to be idiopathic chylothorax. The milky apperiance, protein concentration and density of the chylous fluid also found to confirm the diagnosis. (Beatty and Barrs, 2010; Spencer & Karen, 2012; Singh et al., 2012).

Idiopathic chylothorax is a disease characterised by gradual progressive detorioration of the cat's clinic condition which usually present with respiratory difficulty, rapid breathing and coughing due to atelectatic lung caused by chylous fluid (Kopko, 2005). In this presented case, significant dyspnea, increased respiratory rate and intermittent cough were observed. In addition to respiratory symptoms, a poor appetite and weight loss (Gould, 2004; Beatty and Bars, 2010) have been observed to accompany the clinical presentation. The fast decrease in body weight in this patient history, was presumed to originate from the energy deficit occurring during respiratory effort and the loss of chyle fluid as reported in the literatures (Kopko 2005; Bitchard at all., 1998).

Also dsypnea and coughing findings (Gould, 2004; Yılmaz and Kocatürk, 2023) consistent with radiological findings (USG, thoracic radiography and CT) which presented of a bilateral diffuse pleural effusion and a large area of atelectasis. The presence of pneumothorax on CT images was considered as an air-filled area remaining from the unventilated atelectic lungs remaining from the chylous fluid formed after thoracocentesis. The cardiac silhouette in the VD radiograph is indistinct compared to that in the LL radiograph which was thought to be due to the displacement of the chylous fluid in the mediastinal apertures according to the patient position (Beatty and Barrs, 2010).

There was no spesific medical treatment of idiopathic chylothorax as known. The management of idiopathic chylothorax in cats poses a challenge, with the primary goal being the resolution of chylous fluid accumulation without recurrence (Thompshon, 1999). Recent studies have explored various treatment modalities, including the use of octreotide and rutin. Octreotide has garnered attention for its potential effectiveness in preventing chyle formation, as evidenced by recent cases (Ghiringhelli, 2016; Yılmaz & Kocatürk, 2023; Bichard, 2012; Esme,2019). However, the response to octreotide treatment in cats with chylothorax appears to variable; some cases achieving complete resolution with prolonged administration (Yılmaz & Kocatürk, 2023), while others may require additional interventions (Fossum 1991; Singh at all,2012; Reeves et al., 2020).

In our case, octreotide administration post-thoracentesis resulted in a partial reduction in chyle fluid volume by the 21st day of treatment initiation, although complete resolution was not achieved. Subsequently, rutin (plat-based bioflavonoid) was introduced, which has been reported to aid in the dissolution of chyle (Gould, 2004; Kopko, 2005; Thompson et al., 1999). After two weeks of rutin (plant -based bioflavonoid) administration, complete resolution of chyle fluid was observed during the initial follow-up. This outcome prompts discussion regarding the mechanisms underlying treatment success. While some literature suggests that the resolution may be attributed to time-dependent healing processes, others argue for the efficacy of medication. Despite octreotide usage not achieved complete response within 21 days; after two week rutin (plant-based bioflavonoid) usage, the resolution of chyle observed. This indicated the potential effects of rutin, which contribute to the resolution of chyle accumulation in idiopathic chylothorax.

The findings from the 4th, 8th, and 12th-week examinations indicated that the patient was able to breathe comfortably without respiratory distress, exhibited normal appetite, and even gained weight compared to admission. However, the cranial atelectatic lung lobes remained non-aerated bilaterally, and radiographic evidence consistent with pleural fibrosis manifested as rounded, consolidated lung lobes and accentuated interlobar pleural lines (McGrath 2011, Beatty and Barrs 2012, Sack et al., 2022) was observed. Nevertheless, there was no recurrence of chylous fluid.

Surgical treatment is considered in cases where medical treatment cannot be performed or unsuccessful (Fossum 2001). However, no further surgical intervention was required in this case diagnosed as idiopathic chylothorax because the patient responded positively to medical octreotide and a specially rutin (plant based bioflavonoid) treatment.

This synthesis highlights the ongoing discussions about the optimal management of idiopathic chylothorax in cats and underlines the need for further research to explore the mechanisms of its possible effects and to optimise treatment strategies (Reeves et al., 2020).

In conclusion, our study demonstrated that administration of octreotide and rutin (plant-based bioflavonoid) treatment following thoracocentesis yields favorable results in the medical management of idiopathic chylothorax in cats. The utilization of these treatments before considering invasive surgical interventions may offer a beneficial therapeutic approach.

References

Beatty, J., & Barrs, V. (2010). Pleural effusion in the cat: a practical approach to determining aetiology. *Journal of Feline Medicine and Surgery, 12*(9), 693-707.

Birchard, S. J., & Fossum, T. W. (1987). Chylothorax in the dog and cat. *Veterinary Clinics of North America: Small Animal Practice, 17*(2), 271-283.

Birchard, S. J., McLoughlin, M. A., & Smeak, D. D. (1995). Chylothorax in the dog and cat: a review. *Lymphology, 28* (2), 64-72.

Brink, P., Wilkins, L. P., & Spano, J. S. (1996). Chylothorax due to primary thoracic haemangiosarcoma in a horse. *Equine Veterinary Journal, 28*(3), 241-244.

Chinnock, B. F. (2003). Chylothorax: case report and review of the literature. *The Journal of Emergency Medicine, 24* (3), 259-262.

Esme, H. (2019). The use of octreotide in the treatment of chylothorax. *Journal of Contemporary Medicine, 9*(4), 432-435.

Fossum, T. W., Forrester, S. D., Swenson, C. L., et al. (1991). Chylothorax in cats: 37 cases (1969–1989). *Journal of the American Veterinary Medical Association, 198*, 672-678.

Fossum, T. W. (2001). Chylothorax in cats: is there a role for surgery?. *Journal of Feline Medicine and Surgery, 3*, 73-79.

Fossum, T. W. (2006). Chylothorax: surgery is effective!.

Ghiringhelli, M., Brizzola, S., Barella, G., Lodi, M., Faverzani, S., & Acocella, F. (2016). Octreotide as medical therapy of idiopathic chylothorax in 3 cats after surgery. *Conference: BSAVA Congress* 7-10 April 2016 Birmingham UK.

Gould, L. (2004). The medical management of idiopathic chylothorax in a domestic long-haired cat. The *Canadian Veterinary Journal, 45*(1), 51.

Gupta, B. N., & Faith, R. E. (1977). Chylothorax in a rat. *Journal of the American Veterinary Medical Association, 17*, 973-974.

Hambrook, L. E., & Kudnig, S. T. (2012). Lung lobe torsion in association with a chronic diaphragmatic hernia and haemorrhagic pleural effusion in a cat. *Journal of Feline Medicine and Surgery, 14*(3), 219-223.

Kato, Y., Asano, K., Namiki, M., Kutara, K., Sasaki, Y., Teshima, K., Edamura, K., & Tanaka, S. (2005). Use of rutin for successful management of feline idiopathic chylothorax. *Journal of the Japan Veterinary Medical Association*, *58* (1), 54-57.

Kopko, S. H. (2005). The use of rutin in a cat with idiopathic chylothorax. *The Canadian Veterinary Journal, 46*(8), 729.

Litvin, R., Razok, A., Dasgupta, M., Trevino, E. M., Eldin, M. S., & Fakhran, S. (2023). Nontraumatic chylothorax: a case series from a safety-net hospital. *Annals of Internal Medicine: Clinical Cases, 2*(9), e230119.

Mclane, M. J., & Buote, N. J. (2011). Lung lobe torsion associated with chylothorax in a cat. Journal *of Feline Medicine and Surgery, 13*(2), 135-138.

Nikiphorou, X., Chioti, R., Patsikas, M. N., & Papazoglou, L. G. (2016). Peritoneopericardial diaphragmatic hernia in the dog and cat. *Journal of the Hellenic Veterinary Medical Society, 67(3), 189-194.*

Sack, D., Hyndman, P., Milligan, M., & Spector, D. (2022). Decortication, thoracic omentalization, and pericardiectomy for treatment of severe fibrosing pleuritis in a cat. *Journal of the American Veterinary Medical Association, 260*(3), 335-340*.*

Sicard, G. K., Walker, K. R., & McAnulty, J. F. (2005). The effect of cisterna chyli ablation combined with thoracic duct ligation on abdominal lymphatic drainage. *Veterinary Surgery, 34,* 64-70.

Singh, A., Brisson, B., & Nykamp, S. (2012). Idiopathic chylothorax: pathophysiology, diagnosis, and thoracic duct imaging. Compendium: *Continuing Education for Veterinarians, 34*(8), E2.

Spencer, A. J., & Karen, M. T. (2012). *Thoracic cavity: veterinary surgery small animal volume two*. Elsevier Saunders, Missouri, pp: 1787-1812.

Stockdale, S. L., Gazzola, K. M., Strouse, J. B., Stanley, B. J., Hauptman, J. G., & Mison, M. B. (2018). Comparison of thoracic duct ligation plus subphrenic pericardiectomy with or without cisterna chyli ablation for treatment of idiopathic chylothorax in cats. *Journal of the American Veterinary Medical Association, 252(8), 976-981.*

Thompson, M. S., Cohn, L. A., & Jordan, R. C. (1999). Use of rutin for medical management of idiopathic chylothorax in four cats. *Journal of the American Veterinary Medical Association, 215(3),* 345-348.

Yılmaz, Z., & Kocatürk, M. (2023). The effect of octreotide in medical treatment of idiopathic chylothorax in a cat. *Türk Veteriner Cerrahi Dergisi, 1*(2), 19-22.

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Treatment of upper eyelid agenesis in a Scottish Fold cat using cauterization and modified holtz- celsus surgical technique

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ABSTRACT

A six-month-old, female Scottish fold cat was presented to our Surgery Department of the Faculty of Veterinary Medicine, University of Istanbul-Cerrahpasa due to complaints of blepharospasm, keratitis and epiphora in right eyes present since birth. In the ocular examination, lacking a part of the palpebra in the right eye and secondary trichiasis were observed. Palpebral defects and secondary trichiasis were repaired using an epilation, cauterization and modified Holtz-Celsus surgical technique. This technique provided a good cosmetic appearance and functional outcome to the patient.

Keywords: agenesis, cat, coloboma, eyelid, trichiasis

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Introduction

Chylot Eyelids are mobile skin folds that move thanks to muscles and protect the eye against external dangers by covering the eyeball (Page et al., 2016). Feline eyelid agenesis describes a congenital condition in which patients are born with a deformed eyelid. In other words; eyelid agenesis describes colobomas on the eyelids (Esson & Calvarese, 2022). The term coloboma is most commonly used for ophthalmic conditions resulting from the congenital absence of any ocular tissue which have been reported in humans, peregrine falcons, cheetahs, snow leopards, sheep, goats and cats (Warren et al., 2020). Eyelid coloboma may be unilateral or bilateral, symmetrical or asymmetrical. It may be associated with other ocular anomalies such as microophthalmia, persistent pupillary membrane (PPM), choroidal and optic nerve colobomas, retinal dysplasia, and dermoids (Etemadi et al., 2013). Lateral involvement of the upper eyelids is typical in cats (Warren et al., 2020). Although its etiology is unknown, conditions such as recessive genetic disorders and teratogenic effects have been reported (Demir & Karagözoğlu, 2019). Eyelid coloboma causes clinical findings such as blepharospasm, epiphora, and irregular corneal surface caused by trichiasis (Etemadi et al., 2013). Medical treatments often include treatment of inflammatory or ulcerative lesions, as well as the application of a topical lubricant to protect the corneal surfaces (Esson & Calvarese, 2022). Surgical treatments include; the Mustardé technique, lip-to-lid technique, Roberts-Bistner technique, bucket handle technique and cryo-epilation technique (Beel, 2015).
This report, describes the use of epilation cauterization and modified Holtz-Celsus surgical technique to treatment of unilateral congenital eyelid coloboma in a cat. This technique has simply achieved both functional and cosmetic success.

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Case description

A six-month-old female Scottish fold cat with blepharospasm, keratitis, epiphora and photophobia was referred to the Surgery Department of the Faculty of Veterinary Medicine, University of Istanbul-Cerrahpaşa. In the ophthalmic examination, it was observed that a part of the right upper palpebrae and its edges were unilaterally absent, and the skin hairs were in contact with the corneas, causing keratitis and blepharospasm (Figure 1). The palpebral fissures were not completely closed during blinking and causing epiphora.There is also congenital microphthalmia and mucopurulent discharge was observed in the left eye. Using of appropriate surgical procedures was planned to correct to all these ocular malformations and situations.

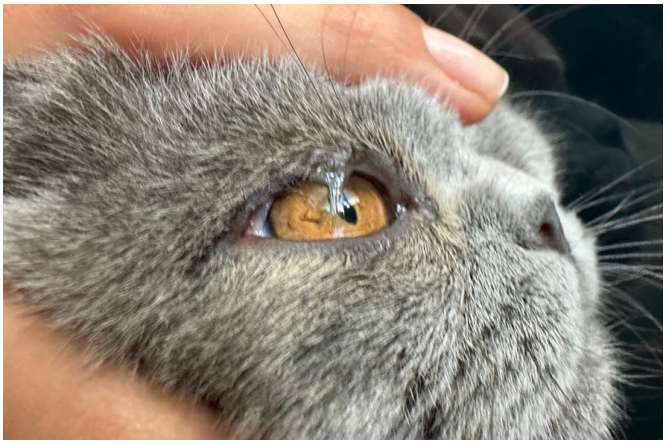

Figure 1. Lateral part of the upper palpebrae and its edges absent and trichiasis

General anaesthesia was carried out with medetomidin hydrochloride (0.08 ml/kg, IV, Domitor, Zoetis, Turkey) and ketamine hydrochloride (5mg/kg IV, Ketalar®, Pfizer, Turkey) followed by 2% isoflurone (Forane®, Abbott, Turkey) in oxygen. Analgesia was provided by meloxicam (0.2mg/kg, SC, Melox, Nobel, Turkey) 30 minutes before surgery. Amoxicillin clavulanic acid (12.5 mg/kg SC, Synulox, Zoetis, Turkey) was administered during to surgery.

Initially, the hairs in contact with the cornea were removed. The skin area which we extracted the hair follicles were cauterized to a depth of 3 mm (Figure 2). The patient's left eye was extirpated in the same operation. An Elizabethan collar was worn to the patient and discharged. Antibiotic eye drop and artificial tear drops were prescribed for ten days. The patient returned to our clinic with the same complaints in the right eye one month after the operation. The left eye extirpation area was healed uneventfully.

During the ocular examination, the same symptoms were observed again, and using a different

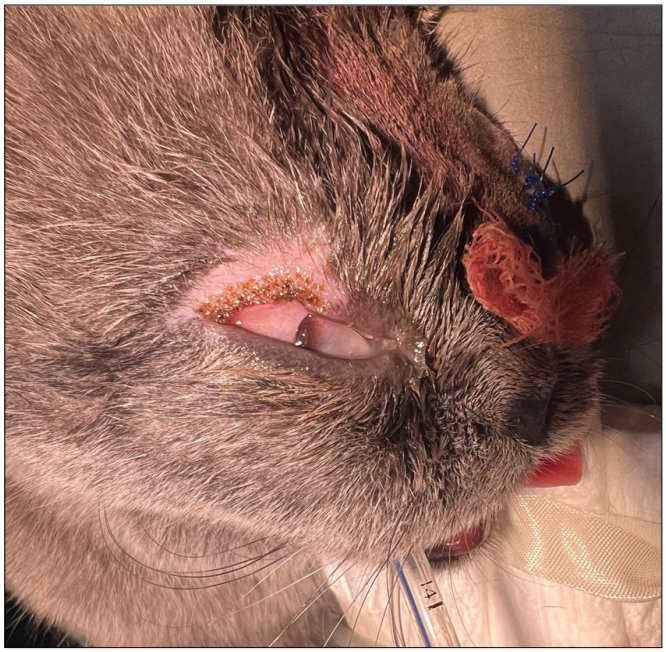

Figure 2. The skin area which we extracted the hair follicles were cauterized.

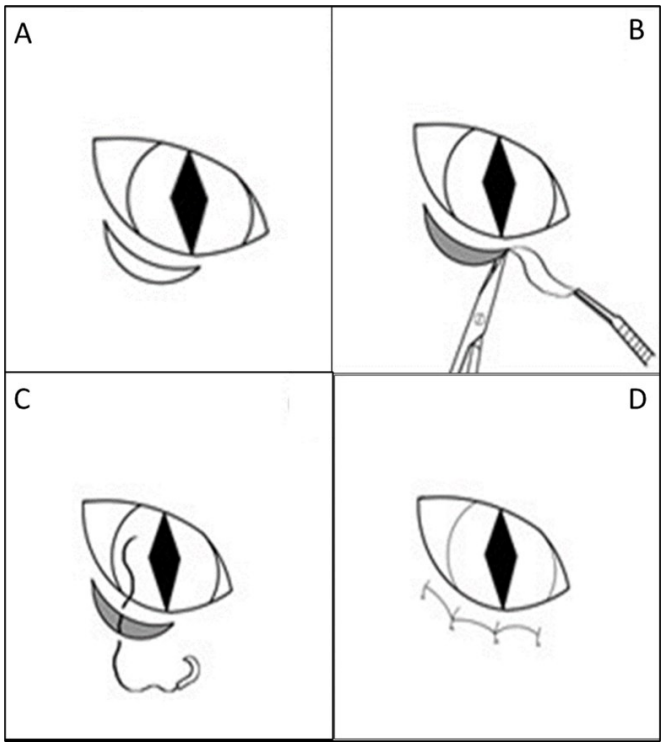

Figure 3. Modified Holtz-Celsus surgical technique (Diaz & Grundon, 2015). A) An initial incision is made with a scalpel blade 2mm from, but parallel to, the lid margin, extending along the area plus 2mm to 3mm either side. B) A second curve incision is applied ventral of the first incision, at a varying distance depending on the size of the damage, and the intervention tissue is resected. C-D) The wound is closed with simple interrupted sutures.

surgical technique was decided. In this operation; epilation, cauterization and modified Holtz-Celsus surgical technique (Figure 3) were applied together. A small piece of skin was removed, and the operation

line was stitched using a simple separate stitch technique with 4/0 PGA. When the stitches were removed at 10th post-operative day, ocular examination findings were normal. Antibiotic eye drop use was ceased but the artificial tear drops use were continued. No recurrence was observed in the patient's right eye in postoperative 1st, 3rd and 6th month follow-ups (Figure 4).

Figure 4. Patient's right eye in postoperative 1st, 3rd and 6th month follow-ups.

Discussion

Congenital eye anomalies are less common than other organ anomalies. Eyelid agenesis is one of the important anomalies of the eye and the most common area in cats is the upper eyelid, especially the lateral part (Demir & Karagözoğlu, 2019). In this case, agenesis was diagnosed in the lateral 1/3 of the right upper eyelid.

There is no breed predisposition. Eyelid agenesis can occur alone or with other ocular disorders such as microphthalmia, dermoids, PPM, lacrimal gland aplasia, retinal dysplasia and anophthalmia (Etemadi et al., 2013). In our case, while there is agenesis of the upper eyelid in the right eye, microphthalmia was present in the left eye. Various medical treatment options are available for eyelid agenesis in cats (Woerdt, 2004). Patients with mild eyelid defects may only need to apply artificial tear lubricating ointment or gel (Warren et al., 2020). However, since it is difficult to get a response to medical treatment in eyelid anomalies, surgical methods are generally preferred (Demir & Karagözoğlu, 2019). Trichiasis can be resolved with various epilation procedures (Warren et al., 2020).

If the defective area is smaller than 1/3 of the eyelid width, primary closure after wedge-shaped excision is the ideal treatment, whereas complex techniques are required for larger defects (Whittaker et al., 2010). These are the applicable operating techniques in severe cases; direct surgical closure or partial-thickness eyelid repair for small defects, Roberts- Bistner technique, bucket handle technique and cryo-epilation, lip commissure to eyelid transposition, sliding skin graft, switch flap reconstruction, modified Mustarde technique and combined with Stades subdermal collagen injection (Warren et al., 2020).

Since our patient had agenesis in the lateral 1/3 of the upper eyelid and the case was considered mild, simple surgical procedures were preferred. The direction of the hair on the eyelid was towards the cornea, so at first epilation and cauterization were applied to the parts close to the eye, but it was unsuccessful. Because of these to slightly tighten the palpebral skin but not prevent the eye from closing, a small piece was removed using the modified Hotz-Celsus technique in the second operation, which is also used in enteropion patients. Considering the patient's condition and these operation techniques applied together; it has been observed that possible complications are minimized due to the small incision line.

In conclusion, a large number of upper eyelid reconstruction options are available. When evaluating them and choosing the appropriate one, it is necessary to select the method that gives the best result and causes the least damage.

References

Beel, J. (2015, November 23). Bilateral upper eyelid coloboma in a cat: surgical treatment. Retrieved from https://www.vettimes.co.uk/article/bilateral-upper-eyelid-coloboma-in-a-cat-surgical-treatment/

Demir, A., & Karagözoğlu, G. S. (2019). Reconstruction of bilateral upper eyelid coloboma in a domestic shorthaired cat using roberts and bistner technique. *Kocatepe Veterinary Journal, 12*(2), 213-220.

Diaz, J., & Grundon, R. (2015, July 13). Diagnosis and treatment of entropion in felines. Retrieved from https://www.vettimes.co.uk/app/uploads/wp-post-to-pdf-enhanced-cache/1/diagnosis-and-treatment-of-entropion-in-felines.pdf

Esson, D. W., & Calvarese S. (2022). *Eyelid agenesis. In D.W. Esson, S.Calverese (ed) Clinical atlas of canine and feline ophtalmic disease 2th ed*. (pp. 62). California, USA: Wiley- Blackwell.

Etemadi, F., Fard, R. M. N., Tamimi, N., Rezaei, M., Vahedi, S. M., Golshahi, H. & Seyed, A. J. (2013). Coincidence of upper eyelid coloboma with posterior synechia in a cat: a case report. *Journal of Veterinary Science &Technology, 4*(4), 139.

Page, L., Gruaz, M., & Praag, E. (2016, May). Newborn rabbit with a congenital upper eyelid agenesis and a cephalocele. Retrieved from http:// www.medirabbit.com/EN/Eye_diseases/Congenital/ Colobome_bilat_en.pdf

Warren, C., Grodzanic, S., & Reinstein, S. (2020). Use of free oral mucosal graft for treatment of feline eyelid agenesis in seven patients. *Veterinary Ophthalmology, 23*(4), 659-667.

Whittaker, C. J. G., Wilkie, D. A., Simpson, D. J., Deykin, A., Smith, J. S., & Robinson, C. L. (2010). Lip commissure to eyelid tansposition for repair of feline eyelid agenesis. *Veterinary Ophthalmology,* 13(3), 173-178.

Woerdt, A. (2004). Adnexal surgery in dogs and cats. *Veterinary Ophthalmology, 7*(5), 284-290.

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Herbal compounds used in canine cognitive dysfunction

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ABSTRACT

The prevalence of chronic diseases in dogs has been increasing due to their longer life spans. One of the diseases developing with age is Canine Cognitive Dysfunction (CCD) and it is a neurodegenerative disease that affects geriatric dogs. In dogs with cognitive dysfunction, behavioral changes such as anxiety, alterations in sleep patterns, and house soiling can be observed. The treatment protocols used for CCD focus on alleviating the symptoms of the disease. Since this dysfunction cannot be cured, in addition to medications, lifestyle changes and dietary interventions are used to manage the symptoms. Herbal compounds frequently used in CCD have been the topic of recent studies. This review article presents the herbal compounds that can be used in dogs with CCD and summarizes the findings from studies on these supplements.

Keywords: dog, canine cognitive dysfunction, herbal compounds, polyphenol

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Introduction

It has been reported that the average life span of dogs, regardless of breed, has increased over the years and reached 13.6 years (Brace, 1981). The increase in the average lifespan of dogs can be attributed to advancements in veterinary medicine and balanced nutrition. It has been reported that there has been a 50% increase in the average life expectancy of dogs in Japan since 1980 (Inoue et al., 2018), and an increase in the average life expectancy in the USA between 2013-2018 (Montaya et al., 2023). The prevalence of chronic diseases in dogs has been increasing due to their increasing life span. The period after 7 years of age is considered geriatric for dogs (Harvey, 2021). Aging in dogs is a natural process and occurs over time in all living creatures, rather than a pathological process. The normal aging process does not have a significant impact on the dog's daily life but altered by cognitive disorders and chronic diseases with aging (Salvin et al., 2011).

Canine Cognitive Dysfunction (CCD) is a neurodegenerative disease that develops progressively in geriatric dogs. In order to diagnose canine cognitive dysfunction, it is necessary to rule out chronic diseases causing pain and can lead to behavioural changes, as well as conditions such as hearing and visual dysfunction. (Bellows et al., 2015). CCD is considered an under-diagnosed disease. A study conducted in 2006 reported that 14.2% of dogs exhibited behavioral changes associated with cognitive dysfunction. However, only 1.9% of these dogs were diagnosed with cognitive dysfunction in veterinary clinics (Salvin et al., 2010). Behavioral changes are observed along with cognitive dysfunctions in dogs. Seperation anxiety, new phobias, changes in sleeping cycle, house soiling, disorientation, excessive vocalization are the most common changes observed in dogs with CCD (Colle et al., 2000; Osella et al., 2007; Salvin et al., 2011a, 2011b; Fast et al., 2013). CCD is associated with

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age-related oxidative damage (Ames et al., 1993; Liu and Mori, 1999; Head, 2002, 2009), reduction in brain volume (Tapp et al., 2004), accumulation of amyloid beta (Aβ) plaques (Cummings et al., 1996; Head et al., 2000; Tapp et al., 2004;), lipofuscin accumulation (Borràs et al., 1999), changes in neurotransmitters, along with an increase in monoamine oxidase B (Landberg and Araujo, 2005), increase in ventricular volume, and cortical atrophy (Su et al., 1998). Cognitive dysfunction in dogs is a similar pathology to Alzheimer's disease in humans (Head et al., 2010). With aging, Aβ plaques accumulate in the brains of dogs, similar to those seen in humans (Cummings et al., 1996a, 1996b). Amyloid deposition started in the prefrontal region at the age of 9 years and in the occipital, parietal and entorhinal cortex at the age of 14 years in a research dogs involved between 2 and 18 years of age (Head et al., 2000). Aβ proteins, polyglucosan and lipofuscin deposits increased with age (Borràs et al., 1999). In a study conducted on geriatric dogs aged 9-15 years, MRI scans were used to examine the brain tissue, revealing an increase in ventricular volume and cortical atrophy (Su et al., 1998).

Herbal compounds and canine cognitive dysfunction

Increase in oxidative damage with age is considered one of the causes of Canine Cognitive Dysfunction (Head et al., 2002; Skoumalova et al., 2003). Normal activities in the brain produce oxidants or reactive oxygen species (ROS). ROS are products of mitochondrial oxygenated respiration and can contain any atom or electron. ROS are unstable and these unstable oxygen free radicals cause cell damage and loss of function. Excessive production of ROS can damage proteins, lipids and nucleoids (Ames et al., 1993). These damages lead to neuronal damage and death of neurons in the brain (Liu and Mori,1999; Floyd et al., 2001). Reducing the production of ROS may be useful for slowing down the aging process. Six weeks usage of antioxidant-enriched supplements in geriatric dogs, dogs' errors in cognitive function tests decreased, their learning abilities increased, and after 2 years of use, their memory improved and the formation of Aβ plaques decreased (Head, 2009). In a study examining seven dogs diagnosed with cognitive dysfunction, administration of supplements containing Ginkgo biloba, phosphatidylserine, pyridoxine, alpha tocopherol, and resveratrol led to a reduction in symptoms associated with cognitive dysfunction (Osella et al., 2007). Cognitive dysfunction is a progressive disease that can significantly impact a dog's quality of life. Studies suggest that early dietary supplementation with herbal compounds may help reduce symptoms and improve behavioural changes associated with cognitive dysfunction in dogs (Cotman et al., 2002; Head, 2009a, 2009b).

Polyphenol

Polyphenols are chemical compounds found in fruits and vegetables. Polyphenols are classified as flavonoids, stilbenes, lignans and phenolic acids (Naomi et al., 2023). Studies suggest that their use may be beneficial in cognitive dysfunctions due to their antioxidant effects and neuroprotective properties (Reichling et al., 2006; Fragua et al., 2017; Lee et al., 2022; Naomi et al., 2023). A study was conducted to investigate the impact of antioxidants on the cognitive function of 35 dogs aged between 8 and 14.5 years. The dogs were divided into two groups; the treatment group received supplements derived from grapes and blueberries rich in polyphenols, and the control group, consisting of 11 dogs that did not receive supplements. Within the treatment group, 12 dogs were supplemented at a dose of 240 ppm, while another 12 dogs at a dose of 480 ppm. The genes linked to oxidative stress in dogs were analyzed using the Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) method, and a Delayed Nonmatching to Position (DNMP) test was performed. The results of the DNMP test showed that cognitive function improved in dogs received supplements, regardless of the dose. Dogs receiving high doses of supplements had higher levels of nuclear factor erythroid 2-related factor 2 (Nrf2), while dogs receiving low doses had higher levels of superoxide dismutase compared to other groups (Fragua et al., 2017). In the nucleus, Nrf2 binds to the gene region associated with the expression of the antioxidant response element (ARE). Through this mechanism, Nrf2 is responsible for the synthesis of antioxidants such as superoxide dismutase (Francisqueti-Ferron et al., 2019). The effects of flavonoids on cognitive function in dogs were studied using supplementation (Lee et al., 2022). Nine dogs, older than 7 years of age, were fed with a food containing honey nuts, which had cyanidin-3-O-glucoside as the active ingredient. Cognitive function tests were conducted before and after the 12-week feeding period. The dogs were received food containing 10.5 mg cyanidin-3-O-glucoside per 100 grams once a day. Weekly phone calls were made, and blood tests and weight measurements were taken at least 4 times. At the end of 12 weeks, the physical examination findings of the animals remained unchanged. However, there was a significant decrease in Aβ oligomer markers in serum. In the test for the detection of cognitive dysfunction, a significant decrease in symptoms was observed at the end of 90

days, except for one dog. At the end of 90 days, inflammation markers, including Tumor Necrosis Factor Alpha (Tnf-α), Interleukin-6 (IL-6), C-reactive protein (CRP), and Interleukin-1 Beta (IL-1β), decreased. Additionally, antioxidant levels, such as L-carnitine and glutathione reductase, increased.

Plant extracts

In a study examining the effects of antioxidant supplements on oxidative stress in geriatric dogs, a supplement containing various carotenoids and polyphenols was used. The supplement included extracts from Grifola frondosa, Curcuma longa, Carica papaya, Punica granatum, Aloe vera, Polygonum cuspidatum, Solanum lycopersicum, Vitis vinifera, and Rosmarinus officinalis. The study was conducted over a period of 6 months to evaluate the effects of supplements on reactive oxygen metabolites, biological antioxidant properties, oxidative stress, and brain-derived neurotrophic factor (BDNF) in dogs. Blood analyses were performed before and after the diets were introduced. The results showed a decrease in reactive oxygen metabolites in the two groups of dogs that received supplements, while no change was observed in the other two groups that did not receive supplements. According to the report, brain-derived neurotrophic factor (BDNF) increased in two groups of dogs that received supplements, while it remained unchanged in the other two groups (Sechi et al., 2015). Ginkgo biloba is one of the oldest living trees. Extracts from its leaves are used in the treatment of Alzheimer's disease in humans (Amieva et al., 2013). The leaves of Ginkgo biloba contain flavonoids and terpenes as active ingredients (Gold et al., 2002). Terpenes act as antagonists of platelet activating factor and suppress platelet accumulation (Koltai et al., 1991). The extract obtained from the leaves of Ginkgo biloba contains flavonoids, which have been demonstrated to possess antioxidant properties (Oyama et al., 1994). The use of Gingko biloba supplementation in cognitive dysfunction in animals is being studied. Studies on mice have investigated the use of Gingko biloba supplementation in cognitive dysfunctions, and have observed positive effects on cognitive function that deteriorates with age (Stackman et al., 2003) and improves memory (Stoll et al., 1996; Tadano et al., 1998). In a study, 42 geriatric dogs with an average age of 11.2 years and age-related cognitive dysfunction were given 40 mg of Gingko biloba extract per 10 kg body weight for 8 weeks. Positive changes were observed in the dogs' behavior within the first 4 weeks. At the end of 8 weeks, a significant improvement in the dogs' general condition was reported, and clinical symptoms such as disorientation, changes in sleep and activity, and behavioral changes disappeared in 36% of the dogs (Reichling et al., 2006).

Phosphatidylserine

Phosphatidylserine is a phospholipid found in the structure of cell membranes. It is abundant in the nervous system and plays a role in maintaining the normal function of the cell membranes of the nervous system (Ramesh et al., 2019). Soybeans are one of the natural sources of phosphatidylserine (Kid, 1996; Ye et al., 2020). According to recent studies (Crook et al., 1992; Zhang et al., 2015; Ye et al., 2020), phosphatidylserine supplements may be used in the treatment of cognitive dysfunction. Several studies have investigated the relationship between acetylcholinesterase and Alzheimer's disease (Talesa, 2001; Herholz, 2008; Singh et al., 2013), and it has been observed that acetylcholinesterase activity may be associated with the accumulation of amyloid plaques (Talesa, 2001). One of the drugs used in human Alzheimer's disease is an acetylcholinesterase inhibitor (Moreta et al., 2021). Low concentrations of phosphatidylserine stimulated ATPase and acetylcholinesterase, while high levels of phosphatidylserine inhibited these enzymes in dogs (Tsakiris and Deliconstantinos, 1984). A study was conducted on rats with Alzheimer's disease. One group of rats was designated as the control group, while two groups with Alzheimer's disease were given phosphatidylserine supplementation at doses of 30mg/kg and 15mg/kg, respectively. The study examined the effects of phosphatidylserine supplementation on cholinesterase, hydroxyl radicals, and superoxide dismutase. When comparing the supplemented group to the control group, the rats given phosphatidylserine had increased levels of hydroxyl radical inhibition and superoxide dismutase, and decreased levels of acetylcholinesterase (Zhang et al., 2015). One study showed that supplementing phosphatidylserine for 12 weeks improved memory in aged mice (Zanotti et al., 1989).

A study was conducted to investigate the effects of supplements on the memory of dogs. Five female Beagle dogs, aged between 7 and 12.7 years, were given capsules containing 25 mg of phosphatidylserine, 24 mg of Ginkgo biloba extract (24%), 20.5 mg of pyridoxine, and 33.5 mg of d-alpha-tocopherol at a rate of 1 capsule per 5 kilograms BW for 70 days. DNMP tests were used to assess the short-term visual memory of the dogs and found that the memory of the dogs in the supplemented group improved .Additionally, the effects on memory were reported to continue for 70 days after the

supplements were stopped. The conclusion of the study was that the use of dietary supplements before the onset of pathological changes in the brain may contribute to the maintenance of cognitive function (Araujo et al., 2008).

Lycopene

Lycopene is a carotenoid pigment found in fruits and vegetables, such as tomatoes, papaya, and watermelon. It is known for its antioxidant and anti-inflammatory effects, which play a neuroprotective role in the nervous system and support cognitive function (Zhao et al., 2018; Chen et al., 2019 Lycopene reduced Aβ accumulation and oxidative stress and improved memory in aged mice (Zhao et al., 2018). In diabetic rats, an increase in acetylcholinesterase levels, a decrease in superoxide dismutase, and an increase in TNF-α have been observed in the brain. There was a prevention of the increase in acetylcholinesterase levels, a decrease in TNF-α levels, an increase in superoxide dismutase levels, and positive developments in cognitive tests in lycopene supplemented diabetic rats (Kuhad et al., 2008). In a study of geriatric dogs , an antioxidant-rich supplement containing papaya and tomato was used and results showed decreased reactive oxygen metabolites and increased neurotrophic factor levels (Sechi et al., 2015).

Alpha lipoic acid

Alpha-lipoic acid plays a role in mitochondrial energy metabolism and has antioxidant, anti-inflammatory properties, and effective in maintaining cognitive function (Shay et al., 2009). Twenty-four beagles between the ages of 8.05 and 12.35 years were administered tocopherol, alpha-lipoic acid, L-carnitine, vitamin C supplements and spinach, carrot pieces, orange, grape, and citrus pulp. The dogs also underwent behavioral enrichment treatment, which included socializing with other dogs, playing with toys, and walking activities. The study aimed to analyze the effects of antioxidant use, behavioral enrichment, or both on cognitive function by examining Aβ deposits in the brain. The study showed that the decrease in the Aβ plaque accumulation was more pronounced in the dogs that received both antioxidant and behavioral enrichment treatments than in the dogs that received behavioral enrichment alone (Pop et al., 2010). A later study analyzed reactive oxygen species in 24 beagle dogs taking the same supplements and found that ROS levels were higher in the brains of older dogs, while mitochondrial ROS levels decreased in dogs taking antioxidant supplements (Head et al., 2009). In a study involving dogs aged between 7.6 and 8.8 years, supplementation with alpha lipoic acid and L-carnitine resulted in improved cognitive function test scores. Result suggests that these supplements may slow down mitochondrial deterioration and could potentially be used to treat cognitive dysfunctions (Milgram et al., 2007). A study investigated the effects of alpha lipoic acid, L-carnitine, and antioxidants on cognitive function in dogs. The results showed that dogs given L-carnitine and alpha lipoic acid improved on cognitive function tests (Snigdha et al., 2016).

Curcumin

Curcumin is a polyphenolic compound derived from the plant Curcuma longa, commonly known as turmeric (Farooqui, 2016). It is known for its antioxidant properties and neuroprotective effects through its interaction with amyloid oligomers (Farooqui, 2016; Akinyemi et al., 2017). Research has demonstrated that curcumin can reduce neuroinflammation and may be effective in treating cognitive dysfunctions. A study investigated the effect of curcumin on neuroinflammation in mice injected with lipopolysaccharide, a known cause of neuroinflammation. The results demonstrated that curcumin reduced neuroinflammation (Sorrenti et al., 2018). Curcumin inhibits acetylcholinesterase and provides protection against cognitive impairment (Akinyemi et al., 2017). In mice treated with curcumin, brain-derived neurotrophic factor (BDNF) increased and Aβ accumulation decreased (Okuda et al., 2019). Curcumin has been reported to interact with Aβ oligomers and reduce their toxicity (Rao et al.,2015; Thapa et al., 2016). A study conducted on individuals aged between 50 and 80 years found that supplements containing curcumin resulted in improved memory and reduced stress levels (Cox et al., 2020). These studies suggest that curcumin may slow the pathologies caused by oxidative damage and Aβ plaque accumulation, which are associated with cognitive dysfunction. Ten dogs aged nine years or older diagnosed with cognitive dysfunction were evaluated based on specific behavioral factors (disorientation, sleep, socialization, house soiling, anxiety, activity, excessive vocalization, etc.). The dogs were administered supplements containing SAMe, phosphatidylserine, curcumin, coenzyme Q10, vitamin E, and zinc for a period of two months. At the end of the first and second months, the cognitive function test was repeated based on the initial scoring to evaluate the dogs' cognitive functions. The results showed that all dogs had improved cognitive function scores (Dewey et al., 2023). The study indicates that reducing oxidative damage may be beneficial for cognitive dysfunction. In an another study, dogs with

an average age of 9 years were given a herbal mixture rich in antioxidants, including curcumin (Sechi et al., 2015). The researchers evaluated reactive oxygen metabolites and BDNF levels in dogs given antioxidant-rich supplements. The study reported a decrease in reactive oxygen metabolites and an increase in BDNF levels in dogs received antioxidant-rich supplements. The data suggests that a diet enriched with antioxidants may be beneficial in the management of cognitive dysfunction in dogs.

Conclusion

Nowadays, there is a continued search for alternatives to improving the quality of life, both in animals and in humans, in addition to the use of traditional treatment methods. Recent studies have demonstrated that herbal compounds are effective in slowing the progression of pathologies that cause cognitive impairment. Polyphenols, which are well known for their antioxidant properties, are becoming increasingly prominent in the research literature. The results of the studies showed that oxidative damage in geriatric dogs can be reduced by supplements containing these herbal compounds. Several studies have shown that herbal supplements can improve memory and cognitive function in dogs. Based on those findings, adding herbal compounds to a dog's diet before the onset of impairment may be more effective in reducing symptoms of cognitive dysfunctions in dogs. To obtain more conclusive results, further studies should be conducted on a larger scale. Given the limited number of studies in this area, further research is needed to provide more definitive results regarding the use of herbal compounds for the treatment of cognitive dysfunction in dogs.

References

Akinyemi, A. J., Okonkwo, P. K., Faboya, O. A., Onikanni, S. A., Fadaka, A., Olayide, I., Akinyemi, E. O., & Oboh, G. (2017). Curcumin improves episodic memory in cadmium induced memory impairment through inhibition of acetylcholinesterase and adenosine deaminase activities in a rat model. *Metabolic Brain Disease, 32*(1), 87–95.

Ames, B. N., Shigenaga, M. K., & Hagen, T. M. (1993). Oxidants, antioxidants, and the degenerative diseases of aging. *Proceedings of the National Academy of Sciences of the United States of America, 90*(17), 7915–7922.

Amieva, H., Meillon, C., Helmer, C., Barberger-Gateau, P., & Dartigues, J. F. (2013). Ginkgo biloba extract and long-term cognitive decline: a 20-year follow-up population-based study. *PloS One, 8*(1), e52755.

Araujo, J. A., Landsberg, G. M., Milgram, N. W., & Miolo, A. (2008). Improvement of short-term memory performance in aged beagles by a nutraceutical supplement containing phosphatidylserine, Ginkgo biloba, vitamin E, and pyridoxine. *The Canadian Veterinary Journal, 49*(4), 379–385.

Bellows, J., Colitz, C. M., Daristotle, L., Ingram, D. K., Lepine, A., Marks, S. L., Sanderson, S. L., Tomlinson, J., & Zhang, J. (2015). Defining healthy aging in older dogs and differentiating healthy aging from disease. *Journal of the American Veterinary Medical Association, 246*(1), 77–89.

Borràs, D., Ferrer, I., & Pumarola, M. (1999). Age-related changes in the brain of the dog. *Veterinary Pathology, 36* (3), 202–211.

Brace J. J. (1981). Theories of aging: an overview. *The Veterinary Clinics of North America. Small Animal Practice, 11*(4), 811–814.

Chen, D., Huang, C., & Chen, Z. (2019). A review for the pharmacological effect of lycopene in central nervous system disorders. *Biomedicine & Pharmacotherapy, 111*, 791–801.

Colle M-A, Hauw J-J, Crespeau, F., Uchihara, T., Akiyama, H., Checler, F., Pageat, P., & Duykaerts, C. (2000). Vascular and parenchymal abeta deposition in the aging dog: correlation with behavior. *Neurobiology of Aging, 21*(5), 695–704.

Cotman, C. W., Head, E., Muggenburg, B. A., Zicker, S., & Milgram, N. W. (2002). Brain aging in the canine: a diet enriched in antioxidants reduces cognitive dysfunction. *Neurobiology of Aging, 23*(5), 809–818.

Cox, K. H. M., White, D. J., Pipingas, A., Poorun, K., & Scholey, A. (2020). Further evidence of benefits to mood and working memory from lipidated curcumin in healthy older people: A 12-week, double-blind, placebo-controlled, partial replication study. *Nutrients, 12*(6), 1678.

Crook, T., Petrie, W., Wells, C., & Massari, D. C. (1992). Effects of phosphatidylserine in Alzheimer's disease. *Psychopharmacology Bulletin, 28*(1), 61–66.

Cummings, B. J., Head, E., Afagh, A. J., Milgram, N. W., & Cotman, C. W. (1996). Beta-amyloid accumulation correlates with cognitive dysfunction in the aged canine. *Neurobiology of Learning and Memory, 66*(1), 11–23.

Cummings, B. J., Head, E., Ruehl, W., Milgram, N. W., & Cotman, C. W. (1996). The canine as an animal model of human aging and dementia. *Neurobiology of Aging, 17*(2), 259–268.

Dewey, C. W., Rishniw, M., Sakovitch, K., & Hollenbeck, J. (2023). Oral administration of an integrative supplement (CogniCaps®) improves cognitive scores in aging dogs with canine cognitive dysfunction for at least two months: An open-label investigation in 10 dogs. *Open Veterinary Journal, 13*(2), 188–192.

Fragua, V., Lepoudère, A., Leray, V., Baron, C., Araujo, J. A., Nguyen, P., & Milgram, N. W. (2017). Effects of dietary supplementation with a mixed blueberry and grape extract on working memory in aged beagle dogs. *Journal of Nutritional Science, 6*, e35.

Fast, R., Schütt, T., Toft, N., Møller, A., & Berendt, M. (2013). An observational study with long-term follow-up of canine cognitive dysfunction: clinical characteristics, survival, and risk factors. *Journal of Veterinary Internal Medicine, 27*(4), 822–829.

Floyd, R. A., West, M., & Hensley, K. (2001). Oxidative biochemical markers; clues to understanding aging in long -lived species. *Experimental Gerontology, 36*(4-6), 619–640.

Fragua, V., Lepoudère, A., Leray, V., Baron, C., Araujo, J. A., Nguyen, P., & Milgram, N. W. (2017). Effects of dietary supplementation with a mixed blueberry and grape extract on working memory in aged beagle dogs. *Journal of Nutritional Science, 6*, e35.

Francisqueti-Ferron, F. V., Ferron, A. J. T., Garcia, J. L., Silva, C. C. V. A., Costa, M. R., Gregolin, C. S., Moreto, F., Ferreira, A. L. A., Minatel, I. O., & Correa, C. R. (2019). Basic concepts on the role of nuclear factor erythroid-derived 2-like 2 (Nrf2) in age-related diseases. *International Journal of Molecular Sciences, 20* (13), 3208.

Gold, P. E., Cahill, L., & Wenk, G. L. (2002). Ginkgo biloba: A Cognitive Enhancer?. Psychological science in the public interest : *The Journal of the American Psychological Society, 3*(1), 2–11.

Harvey N. D. (2021). How old is my dog? Identification of rational age groupings in pet dogs based upon normative age-linked processes. *Frontiers in Veterinary Science, 8*, 643085.

Head E. (2009). Oxidative damage and cognitive dysfunction: antioxidant treatments to promote healthy brain aging. *Neurochemical Research, 34*(4), 670–678.

Head, E., Liu, J., Hagen, T. M., Muggenburg, B. A., Milgram, N. W., Ames, B. N., & Cotman, C. W. (2002). Oxidative damage increases with age in a canine model of human brain aging. *Journal of Neurochemistry, 82*(2), 375–381.

Head, E., McCleary, R., Hahn, F. F., Milgram, N. W., & Cotman, C. W. (2000). Region-specific age at onset of beta -amyloid in dogs. *Neurobiology of Aging, 21*(1), 89–96.

Head, E., Nukala, V. N., Fenoglio, K. A., Muggenburg, B. A., Cotman, C. W., & Sullivan, P. G. (2009). Effects of age, dietary, and behavioral enrichment on brain mitochondria in a canine model of human aging. Experimental *Neurology, 220*(1), 171–176.

Head, E., Pop, V., Sarsoza, F., Kayed, R., Beckett, T. L., Studzinski, C. M., Tomic, J. L., Glabe, C. G., & Murphy, M. P. (2010). Amyloid-beta peptide and oligomers in the brain and cerebrospinal fluid of aged canines. *Journal of Alzheimer's Disease, 20*(2), 637–646.

Herholz K. (2008). Acetylcholine esterase activity in mild cognitive impairment and Alzheimer's disease. *European Fournal of Nuclear Medicine and Molecular Imaging, 35* Suppl 1, S25–S29.

Inoue, M., Kwan, N. C. L., & Sugiura, K. (2018). Estimating the life expectancy of companion dogs in Japan using pet cemetery data. *The Journal of Veterinary Medical Science, 80*(7), 1153–1158.

Kidd, P. M. (1996). Phosphatidylserine; Membrane nutrient for memory. A clinical and mechanistic assessment. *Alternative Medicine Review, 1*(2), 70-84.

Koltai, M., Hosford, D., Guinot, P., Esanu, A., & Braquet, P. (1991). Platelet activating factor (PAF). A review of its effects, antagonists and possible future clinical implications (Part I). *Drugs, 42*(1), 9–29.

Kuhad, A., Sethi, R., & Chopra, K. (2008). Lycopene attenuates diabetes-associated cognitive decline in rats. *Life Sciences, 83*(3-4), 128–134.

Landsberg, G., & Araujo, J. A. (2005). Behavior problems in geriatric pets. *The Veterinary clinics of North America. Small Animal Practice, 35*(3), 675–698.

Lee, Mi-Jin, Miey Park, and Hae-Jeung Lee. (2022). "Dietary Supplemented Anthocyanin Reduced Serum Amyloid Beta Oligomers and Improved Cognitive Dysfunction Scores in Elderly Dogs" *Applied Sciences 12*, no. 23: 12130.

Liu, J., & Mori, A. (1999). Stress, aging, and brain oxidative damage. *Neurochemical research, 24*(11), 1479–1497.

Milgram, N. W., Araujo, J. A., Hagen, T. M., Treadwell, B. V., & Ames, B. N. (2007). Acetyl-L-carnitine and alpha-lipoic acid supplementation of aged beagle dogs improves learning in two landmark discrimination tests. *FASEB Journal, 21*(13), 3756–3762.

Montoya, M., Morrison, J. A., Arrignon, F., Spofford, N., Charles, H., Hours, M. A., & Biourge, V. (2023). Life expectancy tables for dogs and cats derived from clinical data. *Frontiers in Veterinary Science, 10*, 1082102.

Moreta, M. P., Burgos-Alonso, N., Torrecilla, M., Marco-Contelles, J., & Bruzos-Cidón, C. (2021). Efficacy of Acetylcholinesterase Inhibitors on Cognitive Function in Alzheimer's Disease. Review of Reviews. *Biomedicines, 9* (11), 1689.

Naomi, R., Yazid, M. D., Teoh, S. H., Balan, S. S., Shariff, H., Kumar, J., Bahari, H., & Embong, H. (2023). Dietary Polyphenols as a Protection against Cognitive Decline: Evidence from Animal Experiments; Mechanisms and Limitations. *Antioxidants (Basel, Switzerland), 12*(5), 1054.

Okuda, M., Fujita, Y., & Sugimoto, H. (2019). The additive effects of low dose intake of ferulic acid, phosphatidylserine and curcumin, not alone, improve cognitive function in APPswe/PS1dE9 transgenic mice. *Biological & Pharmaceutical Bulletin, 42*(10), 1694–1706.

Osella, M.C., Re, G., Odore, R., Girardi, C., Badino, P., Barbero, R., & Bergamasco, L. (2007). Canine cognitive dysfunction syndrome: Prevalence, clinical signs and treatment with a neuroprotective nutraceutical. *Applied Animal Behaviour Science, 105*, 297-310.

Oyama, Y., Fuchs, P. A., Katayama, N., & Noda, K. (1994). Myricetin and quercetin, the flavonoid constituents of Ginkgo biloba extract, greatly reduce oxidative metabolism in both resting and Ca(2+)-loaded brain neurons. *Brain Research, 635*(1-2), 125–129.

Pop, V., Head, E., Hill, M. A., Gillen, D., Berchtold, N. C., Muggenburg, B. A., Milgram, N. W., Murphy, M. P., & Cotman, C. W. (2010). Synergistic effects of long-term antioxidant diet and behavioral enrichment on beta-amyloid load and non-amyloidogenic processing in aged canines. *The Journal of Neuroscience, 30*(29), 9831–9839.

Ramesh C. Gupta, Ajay Srivastava, Rajiv Lall (2019). *Nutraceuticals for Cognitive Dysfunction. In Ramesh et al., (Ed).* Nutraceuticals in Veterinary Medicine. (pp:404). Cham, Switzerland: Springer Cham.

Rao, P. P., Mohamed, T., Teckwani, K., & Tin, G. (2015). Curcumin binding to beta amyloid: A computational study. *Chemical Biology & Drug Design, 86*(4), 813–820.

Reichling, J., Frater-Schröder, M., Herzog, K., Bucher, S., & Saller, R. (2006). Reduction of behavioural disturbances in elderly dogs supplemented with a standardised Ginkgo leaf extract. *Schweizer Archiv fur Tierheilkunde, 148*(5), 257–263.

Salvin, H. E., McGreevy, P. D., Sachdev, P. S., & Valenzuela, M. J. (2011). Growing old gracefully—Behavioral changes associated with "successful aging" in the dog, Canis familiaris. *Journal of Veterinary Behavior: Clinical Applications and Research, 6*(6), 313–320.

Salvin, H. E., McGreevy, P. D., Sachdev, P. S., & Valenzuela, M. J. (2010). Under diagnosis of canine cognitive dysfunction: a cross-sectional survey of older companion dogs. *Veterinary Journal (London, England : 1997),* 184(3), 277–281.

Salvin, H. E., McGreevy, P. D., Sachdev, P. S., & Valenzuela, M. J. (2011). The canine cognitive dysfunction rating scale (CCDR): a data-driven and ecologically relevant assessment tool. *Veterinary Journal (London, England : 1997), 188*(3), 331–336.

Sechi, S., Chiavolelli, F., Spissu, N., Di Cerbo, A., Canello, S., Guidetti, G., Fiore, F., & Cocco, R. (2015). An antioxidant dietary supplement improves brain-derived neurotrophic factor levels in serum of aged dogs: Preliminary results. *Journal of Veterinary Medicine, 2015,* 412501.

Shay, K. P., Moreau, R. F., Smith, E. J., Smith, A. R., & Hagen, T. M. (2009). Alpha-lipoic acid as a dietary supplement: molecular mechanisms and therapeutic potential. *Biochimica et Biophysica Acta, 1790*(10), 1149–1160.

Singh, M., Kaur, M., Kukreja, H., Chugh, R., Silakari, O., & Singh, D. (2013). Acetylcholinesterase inhibitors as Alzheimer therapy: from nerve toxins to neuroprotection. *European Journal of Medicinal Chemistry, 70*, 165–188.

Skoumalova, A., Rofina, J., Schwippelova, Z., Gruys, E., & Wilhelm, J. (2003). The role of free radicals in canine counterpart of senile dementia of the Alzheimer type. *Experimental Gerontology, 38(*6), 711–719.

Snigdha, S., de Rivera, C., Milgram, N. W., & Cotman, C. W. (2016). Effect of mitochondrial cofactors and antioxidants supplementation on cognition in the aged canine. *Neurobiology of Aging, 37*, 171–178.

Sorrenti, V., Contarini, G., Sut, S., Dall'Acqua, S., Confortin, F., Pagetta, A., Giusti, P., & Zusso, M. (2018). Curcumin prevents acute neuroinflammation and long-term memory impairment induced by systemic lipopolysaccharide in mice. *Frontiers in Pharmacology, 9*, 183.

Stackman, R. W., Eckenstein, F., Frei, B., Kulhanek, D., Nowlin, J., & Quinn, J. F. (2003). Prevention of age-related spatial memory deficits in a transgenic mouse model of Alzheimer's disease by chronic Ginkgo biloba treatment. *Experimental Neurology, 184*(1), 510–520.

Stoll, S., Scheuer, K., Pohl, O., & Müller, W. E. (1996). Ginkgo biloba extract (EGb 761) independently improves changes in passive avoidance learning and brain membrane fluidity in the aging mouse. *Pharmacopsychiatry, 29*(4), 144–149.

Su, M. Y., Head, E., Brooks, W. M., Wang, Z., Muggenburg, B. A., Adam, G. E., Sutherland, R., Cotman, C. W., & Nalcioglu, O. (1998). Magnetic resonance imaging of anatomic and vascular characteristics in a canine model of human aging. *Neurobiology of Aging, 19*(5), 479–485.

Tadano, T., Nakagawasai, O., Tan-no, K., Morikawa, Y., Takahashi, N., & Kisara, K. (1998). Effects of ginkgo biloba extract on impairment of learning induced by cerebral ischemia in mice. The *American journal of Chinese Medicine, 26*(2), 127–132.

Talesa V. N. (2001). Acetylcholinesterase in Alzheimer's disease. *Mechanisms of Ageing and Development, 122* (16), 1961–1969.

Tapp, P. D., Siwak, C. T., Gao, F. Q., Chiou, J. Y., Black, S. E., Head, E., Muggenburg, B. A., Cotman, C. W., Milgram, N. W., & Su, M. Y. (2004). Frontal lobe volume, function, and beta-amyloid pathology in a canine model of aging. The *Journal of Neuroscience, 24*(38), 8205–8213.

Thapa, A., Jett, S. D., & Chi, E. Y. (2016). Curcumin attenuates amyloid-β aggregate toxicity and modulates amyloid-β aggregation pathway. *ACS Chemical Neuroscience, 7*(1), 56–68.

Tsakiris, S., & Deliconstantinos, G. (1984). Influence of phosphatidylserine on (Na+ + K+)-stimulated ATPase and acetylcholinesterase activities of dog brain synaptosomal plasma membranes. *The Biochemical Journal, 220*(1), 301 –307.

Ye, M., Han, B. H., Kim, J. S., Kim, K., & Shim, I. (2020). Neuroprotective effect of bean phosphatidylserine on TMT-induced memory deficits in a rat model. *International Journal of Molecular Sciences, 21* (14), 4901.

Zanotti, A., Valzelli, L., & Toffano, G. (1989). Chronic phosphatidylserine treatment improves spatial memory and passive avoidance in aged rats. *Psychopharmacology, 99*(3), 316–321.

Zhang, Y. Y., Yang, L. Q., & Guo, L. M. (2015). Effect of phosphatidylserine on memory in patients and rats with Alzheimer's disease. *Genetics and Molecular Research, 14* (3), 9325–9333.

Zhao, B., Liu, H., Wang, J., Liu, P., Tan, X., Ren, B., Liu, Z., & Liu, X. (2018). Lycopene supplementation attenuates oxidative stress, neuroinflammation, and cognitive impairment in aged CD-1 mice. Journal *of Agricultural and Food Chemistry, 66*(12), 3127–3136.

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Preliminary studies on the detection and presence of lymphocystis disease virus (LCDV) in sea breams (*Sparus aurata*) raised in the Aegean Sea

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ABSTRACT

Lymphocystis disease (LCD) is the most frequently reported viral infection in sea bream farms in the South Atlantic and Mediterranean regions. Therefore, in this study, the presence of lymphocystis disease virus (LCVD) which is the causative agent of LCD was investigated in sea bream (*Sparus aurata*) farm in the Aegean region. The 78 fish samples, 40 of them showing fin/skin lesions characteristic to LCD and 38 fishes without skin lesions were collected. Samples from skin lesions and spleen and livers were taken from the fishes without skin lesions. The samples pooled were analyzed for the presence of LCDV by SYBR-Green real time PCR. All samples were found to be positive by real time PCR, but an amplification was seen only in 1 sample by conventional PCR. Sequence analysis has indicated that nucleotide sequences were belong to capsid gene of LCDV. In conclusion, this study shows that LCDV is present in Türkiye and causes serious health problems in sea bream in Izmir, Türkiye. Screening of fishes for LCDV by real time PCR is very crucial especially in fishes without skin lesions. Sequence analysis helps to determine circulating strains and variants of the virus in Türkiye.

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Introduction

Increase in World population and demand for animal proteins have the pressure on production of animals including fishery in the World as well as in Türkiye. Therefore, there is need to produce more but healthy food for human and animal consumption with minimized bacterial and viral infections in the populations. For these hygienic facilities and preventive measurements are necessary like vaccination and monitoring for infectious agents in aquaculture (Benkaroun et al., 2022).

There are two production methods in fishery industry; one of them is hunting and the other one is breeding which is called Aquaculture. Aquaculture is a fast-growing industry which has increased almost 12-fold in the last 30 years, with an average annual increase of 8.8 % worldwide (Ozrenk, 2023). Turkish aquaculture is rapidly growing in recent years, ranking among major producers in the World and the largest producer among the non-EU and EU member countries, together with Norway, UK, and Russia. In 2021, sea bream aquaculture has an important place in the income from fisheries in Türkiye with a 31 %

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share and a contribution of $ 637,187 to a total finance. Sea basses and sea breams are the most widely farmed species in marine waters in Türkiye (Yiğit et al., 2024).

Gilthead sea bream (*Sparus aurata*), a member of the Sparidae family, is naturally found in the Mediterranean region and from the British Isles to south Senegal. Türkiye, Greece and Spain are the main producers, accounting for more than 70 % of the sea bream production in the Mediterranean. (Borrego et al., 2017b). Sea bream production reached 152 thousand tons in 2022. Muğla and İzmir are at the top of the list in marine farming and sea bream production in Türkiye (WWF, 2021). Among other Sparidae species, Gilthead Sea bream is the most important fish species in Mediterranean aquaculture, providing good growth performances and regular production increases. However, health management remains one of the most important problems in sea bream culture, as diseases particularly viral infections can cause major losses in commercial production (Borrego et al., 2017a).

There are number of infectious agents which affect sea bream health and production. Lymphocystis disease (LCD) is the most frequently reported viral infection in sea bream farms in the South Atlantic and Mediterranean regions (Valverde et al., 2017). Lymphocystis disease virus (LCDV) a member of the Iridoviridae family, is the causative agent of lymphocystis disease (LCD). The aetiological agent is LCDV, a double-stranded DNA virus of cytoplasmic replication with complex icosahedral particles ranging from 130 to 300 nm in diameter, belonging to the genus Lymphocystivirus. (Chinchar et al., 2011).

At present, complete genome sequences are only available for 3 distinct isolates, LCDV-1, isolated from European flounder Platichthys flesus (Tidona & Darai 1997), LCDV-China, collected from Japanese flounder Paralichthys olivaceus (Zhang et al., 2004), and LCDV-Sa, from gilthead seabream *Sparus aurata* (López-Bueno et al., 2016). According to the sequences of the conserved viral major capsid protein (MCP), 9 different genotypes have been proposed, with clustering related to host species rather than geographic location (Kitamura et al., 2006; Hossain et al., 2008; Cano et al., 2010; Palmer et al., 2012; Labella et al., 2019)

LCD is a self-limiting disease causing hypertrophy of fibroblastic cells in the connective tissue of fishes characterised by the occurrence of whitish, reddish, or grayish nodules of hypertrophic fibroblastic cells in the dermis and sometimes in the viscera. These hypertrophied cells, referred to as lymphocysts or lymphocystis cells, are usually observed in the skin and fins, but they have also been described in several internal organs (such as the stomach, spleen, liver, kidney, and heart). In sea bream, LCD-associated lesions have been observed only in the skin and fins of affected fish and usually disappear after 20–45 days, depending on water temperature (Colorni & Diamant, 1995).

Diagnosis of LCDV is generally made by histopathology of skin lesions and detection of virus in clinical or subclinical samples. Several studies have shown that viral antigens can be detected in a number of organs and skin lesions of infected fish (Valverde et al., 2017). Molecular methods such as real time PCR and PCR frequently have been used. LCDV infects more than 150 marine and freshwater fish species belonging to 42 families causing great economical losses worldwide (López-Bueno et al., 2016). Lymphocystis disease was first described in gilthead seabream in Israel in 1982 (Paperna et al., 1982), and since then it has been frequently reported in several countries from the same geographic area (Labella et al., 2019). Outbreaks of LCD have been reported worldwide, but little is known about the spread and frequency of the virus in Türkiye (Pekmez et al., 2022). Therefore, this study was performed to investigate the presence of LCDV by PCR on clinical and subclinical samples reported in a sea bream farm in İzmir province, Türkiye.

Materials and Methods

Fish farm and sampling

A sea bream production farm located in Aegean sea having suspected cases of LCD was visited. The fishes showing clinical signs of LCD mainly skin lesions were examined. History and clinical signs of the fishes were recorded. LCDV-suspected fishes (n=78) were collected from the sea-cages of a commercial sea bream farm. They were then transported to the laboratory, Department of Virology, Veterinary Faculty, Istanbul University-Cerrahpaşa in a cold chain (4-8 °C). The samples were either processed directly or stored at -20 °C until required.

The samples were pooled and analysed for initial screening of LCDV by SYBR-Green real time PCR. Pool samples were prepared for DNA extraction. For pooling, the samples were taken from the fin/skin lesions (n = 40) and from the liver and spleen of the fishes (n = 38) without fin/skin lesions after necropsy. A total of 12 pools were formed.

DNA Extraction

All pooled samples were first homogenised separately using the tissue disrupter (Bullet Blender, Next

Table 1. Primers, reaction mixtures and PCR conditions for PCR analyses of the LCDV

Test	Target Genes	Primers (5'-3')	Product size	Reaction mixture	PCR Conditions	References
Real-Time PCR	MCP	qPCR-F1 AATGAAATAAGATTAACGTTTCA	151	MM:12,5 µl	95 °C- 10m	Ciulli et al., 2015
				Primer F: 1 µl		
		qPCR-R2 CGTTAGTGATCCATACTTG		Primer R: 1 µl	45 cycles of 95 °C- 15s 50 °C-30s 72 °C-30s	
				SYBR Green : 0,5 µl		
				Water: 8 µl		
				DNA: 2 µl		
Conventional PCR	Capsit Gene	LF7-F CGCGCTGCCTTATAATGA	789	MM:12,5 µl	95 °C- 3m	Ciulli et al., 2015
				Primer F: 1 µl		
		LC1-R GGAAAACCCATTGATCCGT		Primer R: 1 µl	35 cycles of 94 °C- 1m 55 °C-2m 72 °C-1m	
				Water: 7,5 µl		
				DNA: 3 µl		
					Last ext. 72 °C-3m	

Advance). Viral DNA was extracted from the homogenised tissue by using a commercial DNA extraction kit (The PureLink™ Genomic DNA Mini Kit, Invitrogen™, Cat No: K1820-02, Carlsbad, CA 92008) as described by the manufacturer. The amount of DNA in the extracts was measured by nanodrop device (NanoDrop, Thermo Scientific, Waltham, USA). They were then stored at -20 °C until required.

SYBR-Green real time PCR

SYBR Green based real time PCR and primers were used to detect LCDV in samples as described previously (Table 1) (Ciulli et al., 2015). In an optimized PCR reaction, a 25 ml PCR mixture composed of 2 ml template DNA, 12.5 ml mastermix (Maxima hotstart mastermix, Thermoscientific, USA), 1 ml forward and reverse primers, 0.5 ml of SYBR-Green and 8 ml nuclease-free water (Table 1). The PCR reaction was placed in a thermal cycler (StepOnePlus™ Real Time PCR System, Applied Biosystems™) using the cycling conditions as follows: after 10 minutes of initial incubation at 95 °C, 45 cycles of denaturation steps at 95 °C for 15 seconds, annealing at 50 °C degrees for 30 seconds, and extension at 72 °C for 30 seconds (Table 1). For all PCR reactions, negative and positive controls were always used. Positive controls were from the samples that were previously found to be positive in the Department of Virology, Veterinary Faculty of Istanbul University-Cerrahpasa. As negative control, nuclease free water was added in place of template DNA.

Results

Clinical signs and necropsy

It was observed that all the fishes analysed in this study were clinically ill. At necropsy, amongst 78 fishes, 40 showed significant skin and fin lesions (Figures 1 and 2). Among the fishes (38) that did not show lesions at necropsy examination, there were no obvious gross lesions in spleen and liver samples (Figure 3).

Figure 1. Fin/skin lesions on the fishes on clinical observation.

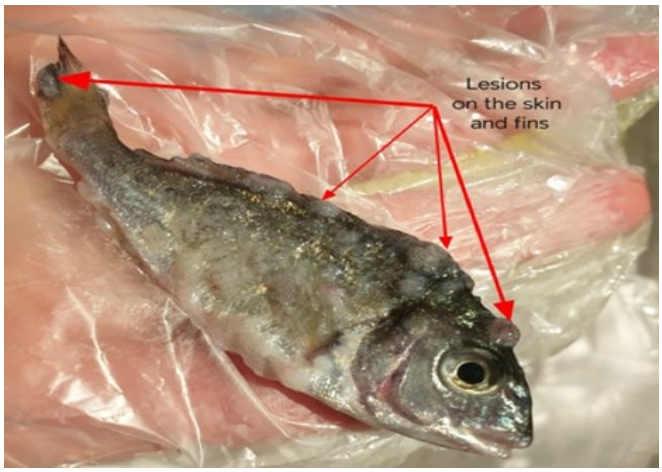

Figure 2. Skin and fin lesions on fish found to be positive for LCDV by both real time PCR and conventional PCR. Arrows indicate fin and skin lesions seen in PCR positive fishes.

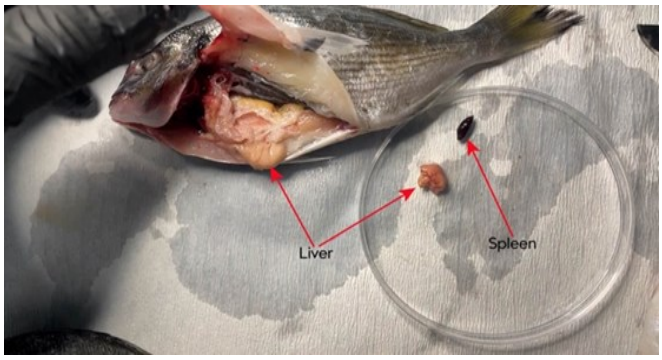

Figure 3. Spleen and liver samples taken from fish without skin and fin lesions.

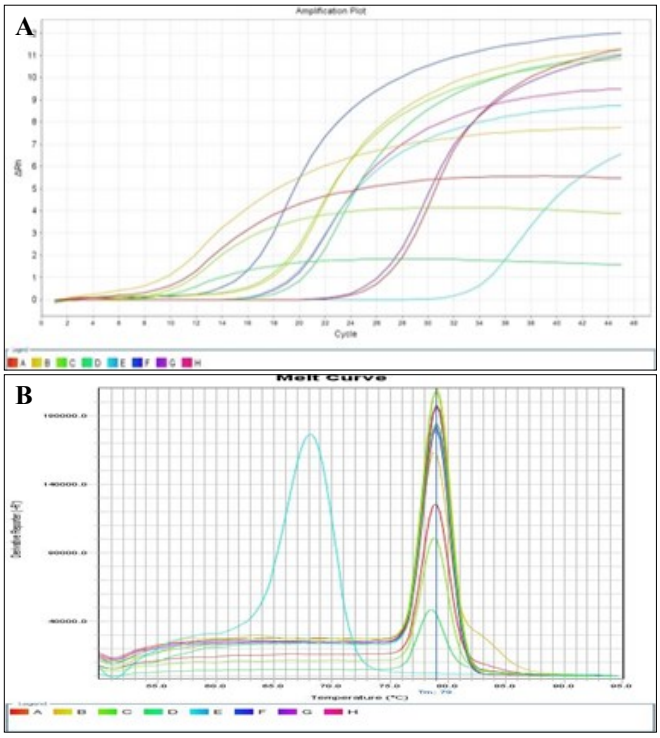

Figure 4. Ct values (A) and melting curves (B) of samples and controls analysed by SYBR-Green real time PCR

SYBR-Green real time PCR

Amplification was seen in all pool samples (n = 12) and positive control analysed by SYBR-Green real time PCR. Ct values were detected between 5.87-25.08 (Figure 4-A). No Ct was detected in negative control. Melting curves of positive control and positive samples were determined between 78.55-79 °C (Figure 4-B). Whereas melting curve of negative control was 68 °C (Figure 4-B).

Conventional PCR

When real time PCR positive samples were analysed by conventional PCR, an amplification product (789 bp) was seen on agarose gel electrophoresis. Sequencing data has indicated that the nucleotide sequences (data not shown) were part of capsid gene of LCDV.

Discussion

Lymphocystis disease (LCD) is a well-known iridoviral infection that affects both wild and cultured fish species living in freshwater and marine life worldwide. The clinical signs of the disease are characterized by the development of small pearl-like macroscopic nodules (0.3–2.0 mm) located mostly on the fins and skin, although internal organs may also be affected. The disease occurs in a wide range of salinities and water temperatures, and it has been reported in at least 150 fish species from 42 families. LCD has been reported in many countries but the knowledge about LCD in Türkiye is limited (Palmer et al., 2012; Ciulli et al., 2015; Labella et al., 2019; Pekmez et al., 2022). Therefore, this study was performed to investigate presence of LCDV in sea breams in Izmir, Turkey.

LCDV has caused significant economic losses in aquaculture in many parts of the world, especially in the South Atlantic and Aegean sea. Therefore, this study was performed in Izmir located in Agean sea. Our neighbours, especially Greece, have reported serious health problems in sea breams due to LCD (Colorni et al., 2011). Apart from Greece in the Aegean sea, LCDV has also been reported in countries such as Italy, Spain and Portugal, as well as in our neighbour Iran (Labella et al., 2019; Pekmez et al., 2022; Rahmati-Holasoo et al., 2023). It has been proposed that LCDV was spread through the Atlantic coasts of Europe and the Mediterranean along with the international trade of gilthead seabream (Chinchar et al., 2017). LCDV has an incidence rate as high as 70 % meaning it causes significant economic losses in the aquaculture sector, as external lesions appear and samples with disease symptoms are difficult to commercialize (Masoero et al., 1986).

Diagnosis of LCD is mainly based on pathological and molecular techniques. Presence of small pearl-like

macroscopic nodules on the fins and skin of the fish is characteristic appearance of LCD as reported in previous studies (Samalecos, 1986). In this study, serious pearl-like nodular lesions were observed in 40 of 78 sea bream fish produced in a seafood farm in Agean sea in Izmir. LCD was detected in all samples taken from these lesions. However, the problem in diagnosis of LCD is the cases without fin or skin lesions. Therefore, in the present study, liver and spleen samples were taken from the fishes without skin lesions and LCDV DNA was detected. These results and the results of other study (Ciulli et al., 2015) indicate that visceral organs can be used to investigate presence of LCDV in fishes without skin lesions. For this, the primers targeting to MCP gene of LCDV is highly conserved region of iridoviruses and were used in the present study and they are capable of detecting all LCDV strains as indicated previously (Tidona et al., 1998; Ciulli et al., 2015). Similarly, the primers and real time PCR detected LCDV in all pooled samples analysed in this study.

Incidence of LCD is very high and can be up to 70 % as reported previously (Masoero et al., 1986). In the surveillance studies carried out in Spain, the incidence in juvenile seabream samples from asymptomatic farms was 87.5-100 %, whereas it was detected in 30-100 % of symptomatic farms (Valverde et al., 2017) In this study, LCDV was detected by real-time PCR in samples taken from sea breams in 100 % of pooled samples with lesions and in 100 % of pooled samples formed from organs of fishes without lesions .

Conclusion

This study shows that LCDV is present in Türkiye and causes serious health problems in Izmir, Turkey. Screening of fishes for LCDV by real time PCR is very crucial especially in fishes without skin lesions. Conventional PCR helps for sequencing the virus to determine circulating strains and variants of the virus. This will input data in vaccine preparation and vaccination strategies.

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References

Benkaroun, J., Bergmann, S. M., Römer-Oberdörfer, A., Demircan, M. D., Tamer, C., Kachh, G. R., & Weidmann, M. (2022). New insights into lymphocystis disease virus genome diversity. *Viruses, 14*(12), 2741.

Borrego, J. J., Labella, A. M., Castro, D., Ortiz-Delgado, J. B., Sarasquete, C. (2017a) Updated of the pathologies affecting cultured gilthead seabream, *Sparus aurata*. *Annals of Aquaculture Research, 4*(2), 1033.

Borrego, J. J., Valverde, E. J., Labella, A. M. & Castro, D. (2017b). Lymphocystis disease virus: Its importance in aquaculture. *Reviews in Aquaculture, 9*, 179-193.

Cano, I., Valverde, E. J., Lopez-Jimena, B., Alonso, M. D., García-Rosado, E., Sarasquete, C., Borrego, J. J., & Castro, D. (2010). A new genotype of Lymphocystivirus isolated from cultured gilthead seabream, *Sparus aurata* L., and Senegalese sole, Solea senegalensis (Kaup). *Journal of Fish Diseases, 33*(8), 695-700 .

Chinchar, V. G., Yu, K. H., & Jancovich, J. K. (2011). The molecular biology of frog virus 3 and other iridoviruses infecting cold-blooded vertebrates. *Viruses, 3*(10), 1959-1985.

Chinchar, V. G., Hick, P., Ince, I. A., Jancovich, J. K., Marschang, R., Qin, Q., Subramaniam, K., Waltzek, T. B., Whittington, R., Williams, T., Zhang, Q. Y., & Ictv Report Consortium (2017). ICTV Virus taxonomy profile: iridoviridae. *The Journal of General Virology, 98*(5), 890-891.

Ciulli, S., Pinheiroa, A. C. A. S, Volpe, E., Moscato, M., Jung T. S., Galeotti, M., Stellino, S., Farnetie, R. & Prosperi, S. (2015). Development and application of a real-time PCR assay for the detection and quantitation of lymphocystis disease virus. *Journal of Virological Methods, 213*, 164-173.

Colorni, A. & Diamant, A. (1995), Splenic and cardiac lymphocystis in the red drum, *Sciaenops ocellatus* (L.). *Journal of Fish Diseases, 18*, 467-471.

Colorni, A., Padrós, F., Pavlidis, M. A., & Mylonas, C. C. (2011). Sparidae: biology and aquaculture of gilthead sea bream and other species. John Wiley & Sons.

Hossain, M., Song, J. Y., Kitamura, S. I., Jung, S. J., & Oh, M. J. (2008). Phylogenetic analysis of lymphocystis disease virus from tropical ornamental fish species based on a major capsid protein gene. *Journal of Fish Diseases, 31*(6), 473-479.

Kitamura, S., Jung, S. J., & Oh, M. J. (2006). Differentiation of lymphocystis disease virus genotype by multiplex PCR. *Journal of microbiology (Seoul, Korea), 44*(2), 248-253.

Labella, A. M., Leiva-Rebollo, R., Alejo, A., Castro, D., & Borrego, J. J. (2019). Lymphocystis disease virus (LCDV-Sa), polyomavirus 1 (SaPyV1) and papillomavirus 1 (SaPV1) in samples of Mediterranean gilthead seabream. *Diseases of Aquatic Organisms, 132*(2), 151-156.

López-Bueno, A., Mavian, C., Labella, A. M., Castro, D., Borrego, J. J., Alcami, A., & Alejo, A. (2016). Concurrence of iridovirus, polyomavirus, and a unique member of a new group of fish papillomaviruses in lymphocystis disease-affected gilthead sea bream. *Journal of Virology, 90(*19), 8768-8779.

Masoero, L., Ercolini, C., Caggiano, M., & Rossa, A. (1986). Osservazioni preliminary sulla linfocisti in una maricoltura intensive italiana. *Rivista Italiana di Piscicoltura e Ittiopatologia, 21*, 70-74.

Ozrenk K. (2023). International research and reviews in agriculture, *Forestry and Aquaculture Sciences.* Seruven Publishing.

Palmer, L. J., Hogan, N. S., & van den Heuvel, M. R. (2012). Phylogenetic analysis and molecular methods for the detection of lymphocystis disease virus from yellow perch, Perca flavescens (Mitchell). *Journal of Fish Dseases, 35*(9), 661-670.

Paperna, I., Sabnai, I. & Colorni, A. (1982) An outbreak of lymphocystis in *Sparus aurata* L. In the Gulf of Aqaba, Red Sea. *Journal of Fish Diseases,5*, 433-437.

Pekmez, K., M. Kaplan, A. A. Çağırgan, F. Arslan, B. Kafa, & G. Kalaycı. (2022). Molecular characterisation of lymphocystis disease virus detected from sea bream in Turkey. *Bulletin of the European Association of Fish Pathologists 42*(2), 46-58.

Rahmati-Holasoo, H., Ghalyanchilangeroudi A., Kafi Z. Z., Marandi, A., Shokrpoor, S., Imantalab, B. & Mousavi, H. E. (2023). Detection of lymphocystis disease virus (LCDV) from yellowbar angelfish (Pomacanthus maculosus Forsskål, 1775) in Iran: Histopathological andphylogenetic analysis. *Aquaculture, 562*, 738862.

Samalecos, C. P. (1986). Analysis of the structure of fish lymphocystis disease virions from skin tumours of pleuronectes. *Archives of Virology, 91*(1-2), 1-10.

Tidona, C. A. & Darai, G. (1997). The complete DNA sequence of lymphocystis disease virus. *Virology, 230,* 207-216.

Tidona, C. A., Schnitzler, P., Kehm, R., & Darai, G. (1998). Is the major capsid protein of iridoviruses a suitable target for the study of viral evolution? *Virus Genes, 16*(1), 59-66.

Valverde, E. J., Borrego, J. J., Sarasquete, M. C., Ortiz-Delgado, J. B., & Castro, D. (2017). Target organs for lymphocystis disease virus replication in gilthead seabream (*Sparus aurata*). *Veterinary Research, 48*(1), 21.

WWF, (2021). Sea bass and sea bream supply chain study: from Turkey to Europe. Fish forward project: Responsible seafood consumption for the benefit of people, oceans and climate. WWF-UK.

Yiğit, M., Ergün, S., Yıldız, M., (2024), Developments of the Turkish aquaculture industry, Yıldız, M., Karataş, S., present-day *Turkish Aquaculture and Trends in International Research,* (pp 3-4). Istanbul University Press E-ISBN 978-605-07-1593-4,

Zhang, Q. Y., Xiao F., Xie, J., Li, Z. Q. & Gui, J. F. (2004). Complete genome sequence of lymphocystis disease virus isolated from China. *Journal of Virology, 78(13), 6982-6994.*

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The effects of KISS1, GDF9 and BMP15 genes on reproductive traits in goats: A review

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Review Article

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ABSTRACT

This review article presents a comprehensive analysis of the genetic factors determining reproductive performance in goats. Reproductive capacity is a critical parameter that directly affects economic efficiency in the livestock industry. Genetic studies have enabled the identification of various genes and genetic mechanisms influencing reproductive performance in goats, including key genes such as KISS1, GDF9 and BMP15. It is essential to examine the reproductive conditions and traits in farm animals, particularly in indigenous goat breeds historically, culturally, and economically significant in our country. This review examines KISS1, GDF9 and BMP15 genes associated with reproduction, their functions, and their impacts on reproductive biology in light of current literature. The importance of modern genetic techniques and biotechnological applications in enhancing reproductive efficiency in goat populations is also discussed. This review provides data for the optimization of gene selection strategies and the integration of current genetic knowledge to improve reproductive performance in goat breeding.

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Introduction

After dogs, goats (*Capra hircus*), as the second domesticated animal, have provided significant benefits to human agricultural communities by facilitating the transition to settled life through the production of products such as meat, milk, and wool (Porter, 1996; Pringle, 1998; Zeder and Hesse, 2000). Goats are found on every continent except Antarctica, and surprisingly, 93.4% of these animals are located in Asia and Africa. Their presence is crucial in various roles, from livelihood sustenance to agricultural production, spanning from sparsely populated and non-industrialized countries to industrialized ones. Africa raises approximately 35.7% of the world's goat population, with 60% of these animals concentrated in the sub-Saharan region, including countries such as Chad and Ethiopia. (World Population Review, 2021). According to statistics, hair goats make up the largest proportion of the total goat population in Türkiye, accounting for approximately 93% of the total goat herd (Daşkıran et al., 2018). When goat farming is mentioned in Turkey, Hair goats generally come to mind. According to the 2020 Turkish Statistical Institute (TÜİK) data, the goat population is 12,350,811 heads. Hair goats and their crosses account for

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approximately 98% of the total goat population, and Angora goats account for 2%. The share of the goat population in our country's small ruminant population is approximately 22.43% (Turkish Statistical Institute, 2024). The suitability of Turkey's natural resources, mainly pastures, for breeding sheep and goat species, as well as factors such as consumption habits in rural areas, create a favorable environment for small ruminant breeding (Kaymakçı et al., 2006).

Primates and many ruminants typically release a single oocyte at each cycle whereas species such as mice and pigs have consistently high ovulation rates. In mammals the ovulation rate and the litter sizes are a result of well-regulated interactions of endocrine and paracrine mediators. How precisely the litter size is controlled remains a critical and essential question in reproductive biology (Polley et al., 2009).

Through genome scans, many genomic regions (Quantitative Trait Loci, QTL) that influence various productive traits have been identified, and candidate gene studies are being conducted to identify genes involved in the physiological regulation of nutrition, growth, and energy metabolism (Schrooten et al., 1996; Ashwell et al., 2004; Weikard et al., 2004). The basis of QTL mapping studies is to determine the relationship between specific genetic markers and phenotypes. In farm animals, marker-QTL linkage studies are generally conducted within populations and require the presence of polymorphic marker regions (Primrose et al., 2006; Weller 2009). Molecular markers in farm animals are extensively used to reveal genetic variation at the DNA level, associate gene regions with economically important traits, or identify single genes affecting a particular trait (Balcıoğlu et al., 2014). The data acquired herein is employed in selection investigations aimed at augmenting productivity within the domain of animal husbandry. The amelioration of reproductive attributes in livestock species has garnered escalating attention, notably in ovine and caprine species, where even marginal enhancements in litter size can yield substantial profitability increments.

Consequently, several studies have been conducted to identify genes and candidate genes associated with fertility traits in goats (Getaneh and Alemayehu, 2022).

In recent years, there has been significant progress in understanding the genetic factors that influence reproductive performance in goats. Among these factors, candidate genes such as KISS1 (Kisspeptin), GDF9 (Growth differentiation factor 9) and BMP15 (Bone morphogenetic protein 15) have garnered considerable attention due to their critical roles in regulating reproductive traits. This review examines these key genes in detail, exploring their functions, the mechanisms by which they impact reproductive biology, and their potential applications in genetic selection strategies. By integrating the latest findings from the current literature, this review will provide a comprehensive overview of how KISS1, GDF9 and BMP15 contribute to enhancing reproductive efficiency in goat populations.

Kisspeptin (KISS1)

The kisspeptin/GPR54 pathway is recognized as a key regulator of pubertal development and reproductive function. Several studies have been conducted on the KISS1 gene as a candidate gene for animal reproductive traits (West et al., 1998). KISS1 neurons in the hypothalamus contribute to crucial aspects of reproductive maturation and function, such as sexual differentiation at the brain level, the onset of puberty, and the neuroendocrine regulation of gonadotropin secretion and ovulation (Caraty et al., 2010). So far, the literature on KISS1 and goat reproduction is limited. Due to the importance of KISS1 as a regulator of puberty onset, it is likely that polymorphisms in this gene may be associated with some reproductive traits in goats, such as high productivity, precocious puberty, and year-round estrus phenotypes (Sharma et al., 2013).

According to genetic research, the KISS1 gene is a major fecundity gene in goats. Polymorphisms of the KISS1 gene were associated with higher litter size (Cao et al., 2010; An et al., 2013).

The KISS1 gene in goats is located in chromosome 16. KISS1 gene consists of two coding regions (exons) and one single non-coding region (intron), and the transcript length is 408 bp and encodes 135 amino acids. This gene reaches around 2.62 kilobases (Febriana et al., 2022). This gene encodes a family of neuropeptides called kisspeptins, which activate G protein-coupled receptor-54 (GPR54) (Ohtaki et al., 2001; Yeo and Colledge, 2018; Harter et al., 2018).

In a study conducted on 90 female goats, consisting of 30 individuals from each of the Kacang, Kejobong, and Senduro breeds, the sequence alignment analysis revealed one insertion/deletion and fourteen polymorphic sites in the intron 1 KISS1 gene on three Indonesian native goat breeds. SNP9 at intron 1 of the KISS1 gene was found to be in strong linkage with SNP8 and SNP10; and identified to have a significant association with litter size and parity. Furthermore, the does with H2 haplotype (CCATAGCGCAACGT) had higher litter size than those other haplotypes (Febriana et al., 2022).

In another study conducted using the PCR-RFLP method on 124 animals, comprising 62 Cyprus goats and 62 Iraqi black goats, three polymorphic sites (with transversion mutations) in exon 1 (893G>C, 973C>A, and 979T>G) were detected. The reference sequences of the gene (ID: JX047312.1 for Cyprus goat samples and KC989928.1 for Iraqi black goat samples) were used for comparison. The results of this study showed that polymorphisms of the KiSS1 gene cause an increase of the litter size in two goat breeds. The genetic diversity of polymorphism content in combination with genotypes of different KiSS1 gene loci was associated with litter size performed in both Cyprus and Iraqi black Doe at locus g. 893G>C. Significant ($P<0.05$) genetic diversity was determined at that locus in the HWE test in Cyprus and Iraqi black goats (Rahawy and Al-Mutar, 2021).

In a study that utilized 723 goats belonging to three breeds, including 306 Xinong Saanen, 221 Guanzhong, and 196 Boer, the SNPs of the KISS1 gene were genotyped by PCR-RFLP. Two SNPs (g.2124T>A and g.2270C>T) were genotyped in the three goat breeds. These two SNP loci were in Hardy-Weinberg disequilibrium in the SN and GZ breeds ($P < 0.05$), which showed that the genotypic frequencies had been affected by selection, mutation or migration (An et al., 2013).

In another study conducted on 89 Gaddi goats, the PCR-RFLP assay revealed polymorphism in the amplified product of intron 1 of the kisspeptin (KISS1) gene in the migratory Gaddi goat population. DNA sequencing confirmed one nucleotide mutation (T125A) in the intron 1 region of the KISS1 gene with the allelic frequency of alleles A and B as 0.43 and 0.57, respectively. A significant association with T allele for litter size was observed in screened migratory Gaddi goats (Sankhyan et al., 2020).

Growth differentiation factor 9 (GDF9)

The growth differentiation factor 9 (GDF9) gene is one of the most important fecundity genes that widely studied in goats belonging to the transforming growth factor β (TGFβ) superfamily. The protein is one of the important fecundity genes which plays a critical role during early folliculogenesis as a growth and differentiation factor secreted by oocytes in mammals (Elvin et al,. 1999).

Mammalian oocytes secrete GDF9 and is a key regulator of follicular proliferation, ovulation and fertilization, and also improves the developmental competence of oocytes in females (Wang et al., 2019). The secretion of GnRH stimulates the release of gonadotropins, luteinizing hormone (LH) and follicle-stimulating hormone (FSH) from the pituitary gland, ultimately controlling gonadal function (Jih and Wu, 1995; Fulghesu et al., 1997).

The GDF9 gene is located on caprine Chromosome 7 and has a coding region of 4720 base pairs and two exons. Many single nucleotide polymorphisms (SNPs) that have been identified in the gene, and the SNPs have been reported influence on litter size in goats. The most common SNP in the caprine GDF9 gene is V397I or c.1198G>A located on exon (Mahmoudi et al., 2019).

Literature has shown that the V397I SNP was polymorphic in many goat populations while non-polymorphic in other goat breeds (Polley et al., 2009). A study investigated the polymorphisms of GDF9 genes in 641 goats of three breeds: Xinong Saanen, Guanzhong and Boer. The biochemical and physiological functions, together with the results obtained in the investigation, suggest that the GDF9 genes could serve as genetic markers for litter size in goat breeding (An et al., 2012).

Feng et al (2011) concluded that the C allele at locus 959 of the GDF9 gene was associated with high litter size in Jining Grey goats ($P < 0.01$).

In a study, 48 female Pote goats from smallholder farms were utilised, with a range of permanent incisive (0, 1, 2, 3, 4). A survey method was used to collect data on the litter size of Pote goats and the PCR-RFLP method was used for laboratory observation to detect GDF9 gene polymorphism. This study shows that GDF9 gene polymorphism is associated with litter size of Pote goats. Based on these results, it means that the GDF9 gene mutation is significantly associated with the litter size trait in Pote goats, and the genetic variant of the GDF9 gene is considered as a genetic marker for increased proliferation in Pote goats (Imaniah et al., 2023).

In another study, the investigation was conducted to identify point mutations in two goat breeds from Indonesia, namely Kosta and Lakor, native livestock from Banten and Southwest Maluku regency, respectively. This investigation specifically targeted the analysis of exon region 1 of the GDF9 about litter size ability in both breeds. The multiple alignment results of exon-1 from the GDF9 gene revealed the presence of 2 nucleotides with mutations. However, it was observed that these mutations did not result in the encoding of different amino acids. This suggests that the mutation is associated with the litter size of Lakor and Kosta goat populations (Rumanta et al., 2023).

In a study conducted on 15 local Iraqi Bucks, direct sequencing was used to screen potential SNP loci in the goat GDF9 exon one. As a result, one SNP locus site, 2006 CTC < CTA (leu < leu), was positively

identified. This study is the first to show a significant association of GDF9 in the initiation or maintenance of spermatogenesis in male goats (Jassim and Al-Azzawi, 2022).

In another large-scale study, 45 SNP loci were gathered and sorted from the goat GDF9 gene. The main focus of the analysis and discussion revolved around the relationship between a subset of potentially "true" SNPs and the reproductive traits of goats. Among these mutations, three non-synonymous mutations A240V, Q320P, and V397I and three synonymous mutations L61L, N121N, and L141L were found to have a high mutant frequency in many fecundity goat breeds. Particularly, the mutations Q320P, V397I, L61L, and N121N exhibited high frequencies, ranging from 0.5 to 0.7. According to the summary and analytic results of the current SNP loci within the goat GDF9 gene in this study, it was found that A240V, Q320P, V397I, L61L, N121N, and L141L are six effective SNPs associated with the litter size trait. In most goat breeds worldwide, the V397I and L61L mutations showed a negative relationship with strong goat fertility, and the other four SNPs exhibited a positive effect (Wang et al., 2019).

Bone morphogenetic protein 15 (BMP15)

The critical role of BMP15 in early follicle growth is species-specific and revealed to be related to mono-polyovulatory animals (Moore and Shimasaki, 2005).

BMP15 regulates granulosa cell proliferation and differentiation by promoting granulosa cell mitosis, suppressing follicle-stimulating hormone receptor expression, and stimulating kit ligand expression. These functions play a pivotal roles in female fertility in mammals (Juengel et al., 2002).

There is a high influence of BMP15 and GDF9 on fecundity. These genes are produced by the ovary and influence its function. In addition to increasing the ovulation rate in goats, they also affect follicle growth and development at all stages of follicular genesis in females (Getaneh and Alemayehu, 2022).

Bone morphogenetic protein 15 (BMP15) is a member of the TGFβsuperfamily that is especially expressed in oocytes. The goat BMP15 gene maps to the X chromosome (Farhadi et al., 2013).

In a study of the association of the bone morphogenetic protein 15 (BMP15) gene with the prolific characteristics of Surti goats managed under farm and field conditions, it is claimed that a mutation in the Exon-2 region of the BMP15 gene, with a base size of 575 bp, increases litter size. In total, 100 Surti goats were involved in the study, revealing the presence of two polymorphic sites. One site was identified at 500 base pairs, while the other was found at 400 base pairs. Out of the 100 Surti goats examined, 58 were identified with the AA genotype, while 29 had the AB genotype, and 13 had the AC genotype. A polymorphic region at 500 bp (AB genotype) plays a highly significant role in the higher prolificacy of Surti goats as compared to base size 575 bp (AA genotype) and polymorphic site 400 bp (AC genotype). Polymorphic region AB may be used as a marker genotype for early age selection of female Surti goat. It is claimed that the polymorphic region AB may be used as a marker genotype for early age selection of female Surti goats (Dangar et al., 2022).

In addition, the association of BMP15 gene with prolificacy/litter size was investigated in Jamunapari and crossbred goats (Shaha et al., 2022), in Haimen, Boer, and Huanghui goat breeds of China (He et al., 2010), and Markhoz goats of Iran (Ghoreishi et al., 2019; Getaneh and Alemayehu, 2022).

Polymorphisms of BMP15 gene exon 2 and its relationship with the prolificacy of goats were detected by PCR-SSCP and DNA sequencing methods in two Chinese local goat breeds (Wang et al., 2011).

In a study, 200 adult female Markhoz goats were examined with the PCR-RFLP method for the FecXH, FecXI, FecXG and FecXB mutations. The results showed no polymorphism in the tested Markhoz goats (Shokrollahi, 2015). However, in another study, the mutation of BMP15 genes associated with the goat fecundity has been confirmed in Markhoz goats (Paulini and de Oliveira Melo 2011).

In contrast, homozygous mutant animals showed higher numbers of kids in Beetal goats (Islam et al., 2019).

Conclusion

This review underscores the critical roles of the KISS1, GDF9, and BMP15 genes in managing reproductive traits in goats. Each of these genes uniquely contributes to reproductive efficiency, affecting crucial aspects such as the onset of puberty, follicle development, ovulation, and litter size.

The KISS1 gene is essential for starting puberty and regulating reproductive functions through the neuroendocrine system. Variations in this gene are linked to better reproductive outcomes, including larger litter sizes. The GDF9 gene is vital for early follicle development, with several variations associated with increased fertility, making it a valuable marker for breeding programs. The BMP15 gene also plays a key role in the growth and differentiation of granulosa cells, with its variations linked to higher fertility in different goat breeds.

Incorporating genetic knowledge about KISS1, GDF9, and BMP15 into breeding strategies offers significant potential for improving goat reproductive performance. Advances in genetic technologies and biotechnological applications allow for precise selection and breeding decisions, boosting productivity and economic efficiency in the livestock sector. This review highlights the need for ongoing research into these genes to develop optimized genetic selection strategies, enhancing goat breeding programs by leveraging current genetic insights.

References

An, X. P., Hou, J. X., Zhao, H. B., Li, G., Bai, L., Peng, J. Y., Yan, Q. M., Song, Y. X., Wang, J. G., & Cao, B. Y. (2012). Polymorphism identification in goat GnRH1 and GDF9 genes and their association analysis with litter size. *Animal Genetics, 44*, 234-238.

An, X., Ma, T., Hou, J., Fang, F., Han, P., Yan, Y., Zhao, H., Song, Y., Wang, J., & Cao, B. (2013). Association analysis between variants in KISS1 gene and litter size in goats. *BMC Genetics, 14*(63), 1-6.

An, X., Han, P., Hou, J., Zhao, H. B., Yan, Y., Ma, T., Fang, F., Meng, F., Song, Y. X., Wang, J. G., & Cao, B. (2013). Molecular cloning and characterization of KISS1 promoter and effect of KISS1 gene mutations on litter size in the goat. *Genetics and Molecular Research: GMR, 12(*4), 4308-4316.

Ashwell, M. S., Hayen, D. W., Sonstegard, T. S., Van Tanssell, C. P., Da, Y., VanRaden, P. M., Ron, M., Weller, J. I. & Lewin, H. A. (2004). Detection of quantitative trait loci affecting milk production, health and reproductive traits in Holstein cattle. *Journal of Dairy Science, 87*, 468-475.

Balcıoğlu, M. S., Karslı, T., Şahin, E., Ulutaş, Z. & Aksoy, Y. (2014). Türkiye'de yetiştirilen bazı yerli koyun ırklarında kalpastatin (CAST) geni polimorfizminin PCR-RFLP yöntemiyle belirlenmesi. *Journal of Agricultural Sciences, 20*, 427-433.

Caraty, A., Franceschini, I., & Hoffman, G. E. (2010). Kisspeptin and the preovulatory gonadotrophin-releasing hormone/luteinising hormone surge in the ewe, basic aspects and potential applications in the control of ovulation. *Journal of Neuroendocrinol, 22*, 710–715.

Cao, G., Chu, M., Fang, L. J., Di, R., Feng, T., & Li, N. (2010). Analysis on DNA sequence of KiSS-1 gene and its association with litter size in goats. *Molecular Biology Reports, 37*, 3921-3929.

Dangar, N. S., Pandya, G. M., Ramani, U. V., Kharadi, V. B., & Brahmkshtri, B. P. (2022). Association study of fecundity gene BMP 15 with prolificacy in surti goats under farm and field condition of South Gujarat Region. *Indian Journal of Veterinary Sciencesand Biotechnology, 18,* 100-104.

Daşkıran, İ., Savaş, T., Koyuncu, M., Koluman, N., Keskin, M., Esenbuğa, N., Konyalı, A., Cemal, İ., Gül, S., Elmaz, Ö., Koşum, N., Dellal, G., & Bİngöl, M. (2018). Goat production systems of Turkey: Nomadic to industrial. *Small Ruminant Research, 163*, 15-20.

Elvin, J. A., Clark, A. T., Wang, P., Wolfman, N. M., & Matzuk, M. M. (1999). Paracrine actions of growth differentiation factor-9 in the mammalian ovary. *Molecular Endocrinology, 13*(6), 1035-1048.

Farhadi, A., Genualdo, V., Perucatti, A., Hafezian, S.H., Rahimi -Mianji, G., De Lorenzi, L., Parma, P., Iannuzzi, L., & Iannuzzi, A. (2013). Comparative FISH mapping of BMPR1B, BMP15 and GDF9 fecundity genes on cattle, river buffalo, sheep and goat chromosomes. *Journal of Genetics, 92*(3), 595-597.

Febriana, A., Sutopo, S., Kurnianto, E., & Widiyanto, W. (2022). A Novel SNPs of KISS1 gene strongly associated with litter size in indonesian goat breeds. *Tropical Animal Science Journal*. 45(3), 255-269.

Feng, T., Geng, C. X., Lang, X. Z., Chu, M. X., Cao, G. L., Di, R., Fang, L., Chen, H. Q., Liu, X. L., & Li, N. (2011). Polymorphisms of caprine GDF9 gene and their association with litter size in Jining Grey goats. *Molecular Biology Reports, 38*, 5189-5197.

Fulghesu, A. M., Lanzone, A., Apa, R., Guido, M., Ciampelli, M., Cucinelli, F., Caruso, A., & Mancuso, S. (1997). The hypothalamic-pituitary-luteal axis in women: effects of long-term orally active opioid antagonist (naltrexone) administration. *Journal of Endocrinological Investigation, 20*, 368-373.

Getaneh, M., & Alemayehu, K. (2022). Candidate genes associated with economically important traits in dairy goats. *Cogent Food & Agriculture, 8*(1), 2149131, 1-39.

Ghoreishi, H., Fathi-Yosefabad, S., Shayegh, J., & Barzegari, A. (2019). Identification of mutations in BMP15 and GDF9 genes associated with prolificacy of Markhoz goats. *Archives Animal Breeding, 62*(2), 565-570.

Harter, C. J., Kavanagh, G. S., & Smith, J. (2018). The role of kisspeptin neurons in reproduction and metabolism. The *Journal of endocrinology, 238,* 3, R173-R183.

He, Y., Ma, X., Liu, X., Zhang, C., & Li, J. (2010). Candidate genes polymorphism and its association to prolificacy in Chinese goats. *Journal of Agricultural Science, 2*(1), 88-92.

Imaniah, N. H., Susilorini, T. E., Kuswati, K., & Wahyuni, R. D. (2023). GDF9 gene polymorphism and its relation to litter size in East Java Pote goat germplasm. *BIO Web of Conferences, The 4th International Conference on Environmentally Sustainable Animal Industry (ICESAI 2023)*, 81, 7. https://doi.org/10.1051/bioconf/20238100007

Islam, M., Basheer, A., Javed, K., Anjum, A., & Zahoor, I. (2019). PCR-RFLP-based identification of polymorphisms in BMPR1B, GDF9, and BMP15 genes associated with litter size in Beetal and Teddy goats. South *African Journal of Animal Science, 49*, 697.

Jassim, A., & Al-Azzawi, S. H. (2022). Estimation of genetic variance based on the growth differentiation factor 9 (GDF9) gene and its association with semen quality in local Iraqi goats. *Diyala Agricultural Sciences Journal*, 14, 14-20.

Jih, M. H., & Wu, T. C. J. (1995). Altered regulation of pituitary luteinizing hormone secretion by GnRH and inhibin in the aged persistent-estrous female rat. *Mechanisms of Ageing and Development, 84*, 15–27.

Juengel, J. L., Hudson, N. L., Heath, D. A., Smith, P., Reader, K. L., Lawrence, S. B., O'Connell, A. R., Laitinen, M. P., Cranfield, M., Groome, N. P., Ritvos, O., & McNatty, K. P. (2002). Growth differentiation factor 9 and bone morphogenetic protein 15 are essential for ovarian follicular developmentin sheep. *Biology of Reproduction, 67*(6), 1777-1789.

Kaymakçı, M. (2006). Keçi Yetiştiriciliği. *İzmir ili damızlık koyun-keçi yetiştiricileri birliği yayınları, No:2*. Bornova-İzmir, Türkiye.

Kaymakçı, M., Koşum, N., Taşkın, T. & Akbaş, Y. (2006). Menemen koyunlarında kimi verim özelliklerinin belirlenmesi üzerine bir araştırma. *Ege Üniversitesi Ziraat Fakültesi Dergisi 43*, 63-74.

Mahmoudi, P., Rashidi, A., Rostamzadeh, J., & Razmkabir, M. (2019). Association between c.1189G>A. *Reproduction Science, 209*, 106140.

Moore, R.K., & Shimasaki, S. (2005). Molecular biology and physiological role of the oocyte factor, BMP-15. *Molecular Cell Endocrinology, 234*(1-2), 67-73.

Ohtaki, T., Shintani, Y., Honda, S., Matsumoto, H., Hori, A., Kanehashi, K., Terao, Y., Kumano, S., Takatsu, Y., Masuda, Y., Ishibashi, Y., Watanabe, T., Asada, M., Yamada, T., Suenaga, M., Kitada, C., Usuki, S., Kurokawa, T., Onda, H., Nishimura, O., & Fujino, M. (2001). Metastasis suppressor gene KiSS-1 encodes peptide ligand of a G-protein-coupled receptor. *Nature, 411*, 613-617.

Paulini, F., & de Oliveira Melo, E. (2011). The role of oocyte-secreted factors GDF9 and BMP15 in follicular development and oogenesis. *Reproduction in domestic animals = Zuchthygiene, 46*(2), 354-361.

Primrose, S. B. & Twyman, R. M. (2006). Principles of gene manipulation and genomics. 7th edition. Malden-Blackwell, United States of America.

Pringle, H. (1998). Neolithic agriculture:reading the signs of ancient animal domestication. *Sciences, 282*, 1448.

Polley, S., De, S., Batabyal, S., Kaushik, R., Yadav, P., Arora, J. S., et al. (2009). Polymorphism of fecundity genes (BMPR1B, BMP15 and GDF9) in the Indian prolific Black Bengal goat. *Small Ruminant Research, 85*(2-3), 122-129.

Porter, V. (1996). Goats of the world. Farming pres. Ipswich, United Kingdom.

Rahawy, M. A., & Al-Mutar, H. A. (2021). Association of the KiSS1 gene with litter size in Cyprus and Iraqi black goats. *Veterinary World, 14*, 1995-2001.

Rumanta, M., Kunda, R. M., Volkandari, S. D., & Munir, I. M., (2023). Impact of Environmental Geographic toward Point Mutations in Exon 1 of Growth Differentiation Factor (GDF9) Gene in Kosta and Lakor Goat Breeds. *Jurnal Penelitian Pendidikan IPA, 9*(6), 4813-4819

Sankhyan, V., Thakur, Y. P., & Dogra, P. K. (2020). Association of kisspeptin gene (KISS1) with litter size in migratory Gaddi goats in western Himalayan state of Himachal Pradesh. *The Indian Journal of Animal Sciences. 89*(12), 1352-1355.

Schrooten, C., Bovenhuis, H., Coppieters, W., & Van Arendonk, J. A. M. (1999). Whole genome scan to detect quantitative trait loci for conformation and functional traits in dairy cattle. *Journal of Dairy Science. 83*, 795-806.

Shaha, M., Miah, G., Lima, A., Miazi, O. F., Gupta, M. D., & Das, A. (2022). Identification of polymorphisms in GDF9 and BMP15 genes in Jamunapari and crossbred goats in Bangladesh. *Tropical Animal Health and Production, 54*(6), 350.

Sharma, A., Dutt, G., Jayakumar, S., Saroha, V., & Verma, N. K., 2013. Genetic structuring of nine Indian domestic goat breeds based on SNPs identified in IGF-1 gene. *Animal Biotechnology, 24*, 148-157.

Shokrollahi, B. (2015). Investigation of BMP15 gene polymorphisms associated with twining in Markhoz goat. *Biharean Biologist, 9*, 1-4.

Turkish Statistical Institute. (2024). Homepage. Turkish Statistical Institute. Retrieved May 2024, from http:// www.tuik.gov.tr/Start.do

Wang, X., Yang, Q., Wang, K., Yan, H., Pan, C., Chen, H., et al. (2019). Two strongly linked single nucleotide polymorphisms (Q320P and V397I) in GDF9 gene are associated with litter size in cashmere goats. *Theriogenology, 125*, 115–121.

Wang, X., Yang, Q., Zhang, S., Zhang, X., Pan, C., Chen, H., Zhu, H., & Lan, X. (2019). Genetic effects of single nucleotide polymorphisms in the goat GDF9 gene on prolificacy: True or false positive? *Animals (Basel), 9*(11), 886.

Wang, Y.Q., Li, Y.X., Zhang, N., Wang, Z.B., & Bai, J.Y. (2011). Polymorphism of exon 2 of BMP15 gene and its relationship with litter size of two Chinese goats. *Asian-Australasian Journal of Animal Sciences, 24*(7), 905-911.

Weikard, R., Kühn, C., Goldammer, T., Freyer, G. & Schwerin, M. (2004). The bovine PPARGC1A gene: molecular charaterization and association of an SNP with variation of milk fat synthesis. *Physiological Genomics. 21*, 1-13.

Weller, J. I. (2009). *Quantitative trait loci analysis in animals. Modular Texts.* Cambridge, United States of America.

West, A., Vojta, P. J., Welch, D. R., & Weissman, B. E. (1998). Chromosome localization and genomic structure of the KiSS1 metastasis suppressor gene (KiSS1). *Genomics, 54*, 145-148.

World Population Review. (2021). Goat Population by Country. World Population. Retrieved May 2024 from https://worldpopulationreview.com/country-rankings/ goat-population-by-country

Yeo, S. & College, W. H. (2018). The role of KiSS 1 neurons as integrators of endocrine, metabolic, and environmental factors in the hypothalamic–pituitary–gonadal axis. *Frontiers in Endocrinology, 9*(188), 1-13.

Zeder, M. A., & Hesse, B. (2000). The initial domestication of goats (Capra hircus) in the Zagros Mountain 10000 years ago. *Science. 287*, 2254-2257.

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Urinary incontinence due to estrogen deficiency in dogs

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ABSTRACT

Nowadays, various methods are used to control reproduction in domestic animals. The most commonly used method is ovariohysterectomy, in which the ovaries and uterus are removed together. In this review, an overview will be made of urinary incontinence due to estrogen deficiency, which is one of the complications that may occur after this operation, which is performed in almost every clinic, and which can negatively affect the life of the patient and the patient's relative. The exact mechanism involved with this condition is not yet fully understood but estrogen deficiency with a subsequent loss of urethral tonus is believed to trigger clinical signs. Also, information about the risk of urinary incontinence in animals that have undergone early ovariohysterectomy will be given.

Keywords: urinary incontinence, ovariohysterectomy

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Introduction

Definition

Urinary incontinence can be briefly defined as involuntary urination or incontinence. The condition, which is mostly observed as the animal wetting itself while sleeping, can also be observed at different times and positions such as running, jumping, coughing or barking, sitting position (Tektepe, 2019).

While the risk of urinary incontinence in unneutered dogs is less than 1%, in neutered dogs the risk varies between 5% and 20%. In some breeds, this rate can be as high as 60%. Urinary incontinence usually occurs on average 2-5 years after neutering. However, symptoms can also occur up to 10 years after neutering or immediately after the operation. (Reichler and Hubler, 2014).

The operation procedure, whether it was ovariectomy or ovariohysterectomy, had no effect on the incidence of urinary incontinence or the time interval between the occurrence of urinary incontinence after sterilization (Stöcklin-Gautschi et al., 2001).

As a result of the study (Tektepe, 2019) it was determined that decreases in urethral sphincter pressure were observed within a year following the operation and when this pressure fell below the critical level, urinary incontinence complication was observed. In the study conducted by Trusfield (1985), the link between sterilization and urinary incontinence was proven. It is suggested that it occurs as a result of neurological, vascular, and hormonal changes that occur after sterilization, rather than mechanical damage to the lower urinary tract during sterilization.
There are multiple factors involved in the etiology of urinary incontinence (Thrusfield, 1985). The probability of urinary incontinence in neutered dogs varies according to body weight. The probability of occurrence in neutered dogs with a body weight of less than twenty kilograms was 9.3%, while this rate was determined as 30.9% in dogs with a body weight of more than twenty kilograms. Breed predisposition should also be considered in urinary incontinence cases. While the rate of urinary incontinence in boxer

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dogs was 65%, this rate was found to be 10.6% in German shepherd dogs (Arnold et al., 1989). The probability of urinary incontinence was highest in female dogs spayed before the age of three months. According to the data collected in the study, the probability of urinary incontinence in the first six years of life was 12.9% in dogs spayed before the age of three months, while this rate was 5% in dogs spayed after three months of age (Spain et al., 2004). Although the advantages of neutering before the age of six months are defined as the ease of the operation, less postoperative trauma in young dogs, prevention of unwanted pregnancies, and decreased risk of mammary tumours, there is no significant association between early spaying and incontinence found in a study conducted by Bleser at al. (2011). Incontinent neutered bitches present lower levels of gonadotropins than continent neutered bitches, creating the hypothesis that a low endogen GnRH production is involved in incontinence. Although the mechanism is still unclear (Jesus et al., 2020). Estrogen receptors presence in the inferior urinary tract has been demonstrated, and such receptors can be detected in the vesical trigone, in the urethra and in the connective tissue around it (Batra and Iosif, 1983). Which justifies the deleterious effects on the urinary tract of bitches post-neutering with the reduction of circulating estrogen (Jesus et al., 2020). Since urinary incontinence is a condition that requires lifelong treatment and brings discomfort to the patient's relatives, all factors should be taken into consideration to choose the optimum period for sterilization (Bleser et al., 2011).

Some changes occur in the urinary bladder after sterilization. The changes are listed below.

1. Changes in the urinary bladder after neutering

1.1. Response to Muscarinic Stimulation: The response to muscarinic stimulation in the urinary bladder was lower in gonadectomized animals than in non-gonadectomized animals, irrespective of age, weight, and sex. The neurogenic response in the urinary bladder parallels the muscarinic response and also does not vary with age, weight and sex. It is significantly reduced in gonadectomized animals compared to non-gonadectomized animals (Coit et al., 2008).

1.2. The amount of collagen in the urinary bladder wall: The amount of collagen in the urinary bladder wall is also among the findings that change after gonadectomy. Collagen deposition increased significantly in the urinary bladder after ovariohysterectomy. Accumulated collagen accumulates between the muscle bundles, changes the structure of the bladder, and predisposes to urinary incontinence (Coit et al., 2008; Ponglowhapan et al., 2008). In the study conducted by Ponglowhapan (2008) gender differences were found in all four regions of the lower urinary tract in intact dogs but only in proximal urethra in gonadectomised dogs where spayed females had a higher proportion of collagen and less muscle. Excessive collagen deposits and less muscular volume may impair structural and functional integrity of the lower urinary tract which may associate with the development of post-neutering urinary incontinence in the dog (Ponglowhapan et al., 2008).

1.3. Urinary bladder glucosamine amount: It has been reported that the amount of glucosamine in the urinary bladder and urethra decreased in gonadectomized rodents due to gonadal hormone deficiency (Cabral et al., 2003). Similarly, a decrease in the amount of glucosamine in the tissues of the lower urinary tract has been reported in premenopausal and menopausal women (Bezerra et al., 2004). These observations in both species suggest that the glucosamine content in the lower urinary tract is regulated by the endocrine system and that altered glucosamine composition is associated with the development of urinary incontinence (Ponglowhapan et al., 2011). The study conducted by Ponglowhapan (2011) demonstrated the effect of gonadal status on the glycosamine profile in the lower urinary tract of dogs. The decrease in glycosamine composition in the lower urinary tract of gonadectomized dogs indicates the effect of gonadectomy on glucosamine metabolism.

Disorders in the urethral sphincter mechanism and their causes should also be considered in cases of urinary incontinence.

2. Causes of urethral sphincter mechanism incompetence (USMI)

Urethral incompetence is the most common cause of incontinence in adult female dogs (Adams, 2010). Adhesions at the bladder neck and vaginal-uterine adhesions, anatomical or neurologic damage, shortening of the urethra, caudal positioning of the bladder, hormonal changes after ovariohysterectomy, body size, race, presence of obesity, and tail docking can be listed as causes (Gregory, 1994). Holt and Thrusfield (1993) demonstrated a link between tail docking and urethral sphincter mechanism deficiency.

Since predisposition to urethral sphincter mechanism failure was observed in breeds with urinary incontinence after ovariohysterectomy and in breeds that routinely undergo tail docking, further studies are needed to explain the link between these conditions. (Bleser et al., 2011). Estrogen deficiency in spayed females is believed to lead to a type of urinary

incontinence known as "urethral sphincter mechanism incompetence" (USMI), which involves not only the urethra smooth muscle but submucosa vasculature and urothelium, culminating in urethra insufficient closure (Jesus et al., 2020). When the collagen content of periurethral tissues was measured after ovariohysterectomy, no significant change was observed in the amount of collagen in the periurethral tissues of unspayed and sterile dogs (Forsee et al., 2013).

3. Urinary incontinence treatment options

3.1. Using alpha-agonist agents: Phenylpropanolamine prevents involuntary contractions by acting on adrenergic receptors in the smooth muscles of the urethral sphincter with sympathomimetic effect. In this way, involuntary urine output is prevented. Blood pressure should be monitored at the beginning of treatment to eliminate the risk of high systolic blood pressure, one of the possible side effects of phenylpropanolamine. It should be used with caution or should not used at all in patients with heart problems after a general cardiac examination (Byron, 2018).

Blood pressure measurements should be performed regularly within 2-4 weeks after the start of treatment. Other side effects of phenylpropanolamine include restlessness, aggression, decreased appetite, and insomnia. These side effects disappear when the dose is reduced or treatment is discontinued. (Byron, 2018) Phenylpropanolamine dosage; 1-1.5 mg/kg 8h or 12h PO (Chew, 2011)

3.2. Estrogenic agents: Estrogenic agents such as estriol increase the sensitivity of alpha-adrenergic receptors in the urethral sphincter to catecholamines. Applying alpha-agonist treatment together with estrogen treatment increases the success rate with synergistic effect between the drugs. In estrogen therapy, estriol, which does not accumulate in the body and is excreted through urine without being metabolized in the liver, should be preferred (Chew, 2011). Estriol is a short-acting estrogen derivative. The side effects caused by estrogen (estrous symptoms and behavior, depression, pyometra) are not seen in estriol treatment. In a study conducted by Beceriklisoy et al. (2005), the treatment rate of urinary incontinence with estriol was reported to be 80% (Beceriklisoy, 2005). The recommended dose is reduced after improvement by 0.5 mg per week until the minimum effective dose is reached, which can be continued every other day. If no response to treatment is achieved after the first two weeks, it may be beneficial to continue dosing 2 mg/dog until clinical improvement is appreciated (Timmermans et al., 2019).

3.3 Combination therapy: If there is no response to phenylpropanolamine or estriol treatment or if relapse is observed, treatment with combined phenylpropanolamine and estriol is given. For dog's combination therapy, urodynamic evaluation is recommended. If urethral sphincter incompetence is diagnosed; injection of urethral bulking agents or surgical methods to increase urethral resistance applied (Adams, 2010).

3.4. Artificial urethral sphincter: Artificial urethral sphincter works by acting as a silicone cuff sphincter placed around the urethra. The cuff inside the artificial sphincter is adjusted by the operator to hold the urine during bladder filling, but to allow urine to pass out when a certain level of fluid pressure is reached. This treatment method can be used as an alternative in drug-insensitive patients, but its applicability is still in the framework of research as it requires a specialized operator (Rose et al., 2009).

3.5. Injection of urethral bulking agents: Bulking agents (glutaraldehyde cross-linked collagen) are applied to the proximal urethra as submucosal injection. In veterinary medicine, components containing bovine collagens are used for bulking. These agents, which lengthen the length of muscle fibers by acting as central padding, increase urethral bulging. Urethral bulging improves control of incontinence. The disadvantage of this application is that it requires repetition because of the agents used in the injection do not have a lifelong effect. The agents used are effective for 17-21 months. The fact that the application is easy and minimally invasive shows that it can be used as a treatment alternative in dogs resistant to medical treatment (Butty et al., 2018).

Conclusion

Although the etiologic origins of urinary incontinence in dogs are still unclear, the incidence of urinary incontinence increases after ovariohysterectomy. Also while the advantages of prepubertal ovariohysterectomy have been frequently discussed in the academic world recently, lifelong complications such as urinary incontinence should be mentioned and should be considered as a serious complication after sterilization. While treatment options for urinary incontinence vary, the most used techniques are the usage of alpha-agonist agents, the usage of estrogen or using them both as combined treatment. In recent years, with the development of veterinary surgical procedures and research on urogynecology, the rate of resolution of urinary incontinence cases has increased rapidly.

References

Adams, L. G. (2010, November 1), Treatment of refractory urinary incontinence (Proceedings), Retrieved from https://www.dvm360.com/view/treatment-refractory-urinary-incontinence-proceedings

Arnold, S., Arnold P., Hubler M., Casal, M., & Rüsch, P. (1989). Urinary incontinence in spayed female dogs: frequency and breed disposition. *Schweizer Archiv fur Tierheilkunde, 131*(5), 259-263.

Batra, S. C., & Iosif C. S. (1983). Female urethra: a target for estrogen action. *British Journal of Urology, 129*(2), 418-420.

Beceriklisoy, H. B., Kanca, H., Aksoy, O.A., Erünal-Maral, N., Fındık, M., Kaymaz, M., & Aslan, S. (2005). Effectiveness of different pharmacological approaches on post-ovariohysterectomy urinary incontinence in the bitch. *Ankara Universitesi Veteriner Fakültesi Dergisi, 52*, 157-160.

Bezerra, L. R. P. S., Feldner Jr, P. C., Kati, L. M., Girao, M. J. B. C., Sartori, M. G., Baracat, E. C., Rodrigues de Lima, G., Nader, H. B., & Dietrich, C. P. (2004). Sulfated glycosaminoglycans of the vagina and perineal skin in pre- and postmenopausal women, according to genital prolapse stage, International *Urogynecology Journal, 15*, 266–271.

Bleser, B., Brodbelt, D. C., Gregory, N. G., & Martinez, T. A. (2011). The association between acquired urinary sphincter mechanism incompetence in bitches and early spaying: A case-control study, *The Veterinary Journal, 187*(1), 42-47.

Butty, E., Vachon, C., & Dunn, M. (2018). Interventional therapies of the urinary tract. *Veterinary Clinics of North America, Small Animal Practice, 49*(2), 287-309.

Byron, J. K. (2018). Canine Urinary Incontinence. *American Veterinarian, 3*(5), 18-20.

Cabral, C. A. P., Sampaio, F. J. B., & Cardoso, L. E. M. (2003). Analysis of the modifications in the composition of bladder glycosaminoglycan and collagen as a consequence of changes in sex hormones associated with puberty or oophorectomy in female rats. *The Journal of Urology, 170*, 2512-2516.

Chew, D. J. (2011, October 1). Diagnosing and managing urinary incontinence in dogs (Proceedings). Retrieved from https://www.dvm360.com/view/diagnosing-and-managing-urinary-incontinence-dogs-proceedings

Coit, V. A., Gibson, I. F., Evans, N. P., & Dowell, F. J. (2008). Neutering affects urinary bladder function by different mechanisms in male and female dogs, *European Journal of Pharmacology, 584*(1), 153-158.

Forsee, K. M., Davis, G. J., Mouat, E. E., Salmeri, K. R., & Bastian, R. P. (2013). Evaluation of the prevalence of urinary incontinence in spayed female dogs:566 cases (2003-2008). *Journal of American Veterinary Medical Association, 242*(7), 959-962.

Gregory, S. P. (1994). Developments in the understanding of the pathophysiology of urethral sphincter mechanism incompetence in the bitch. *British Veterinary Journal, 150*(2), 135-150.

Jesus, L., Oliveira, M. C., & Pöppl, A.G. (2020). Post-neutering urethral sphincter mechanism incompetence in female dogs. *Veterinaria Noticias, 27*(1), 85-115.

Ponglowhapan, S., Church, D. B., & Khalid, M. (2008). Differences in the proportion of collagen and muscle in the canine lower urinary tract with regard to gonadal status and gender. *Theriogenology, 70*(9), 1516-1524.

Ponglowhapan, S., Church, D. B., & Khalid, M. (2011). Effect of the gonadal status and the gender on glycosaminoglycans profile in the lower urinary tract of dogs. *Theriogenology, 76*(7), 1284-1292.

Reichler, I. M., & Hubler, M. (2014). Urinary incontinence in the bitch: An Update. *Reproduction in Domestic Animals, 49*(2), 75-80.

Rose, S. A. Adin, C. A., Ellison, G. W., Sereda, C. W., & Archer L. L. (2009). Longterm efficacy of a percutaneously adjustable hydraulic urethral sphincter for treatment of urinary incontinence in four dogs. *Veterinary Surgery, 38*, 747-753.

Spain, V. C., Scarlett, J. M., & Houpt, K. A. (2004). Long-term risks and benefits of early-age gonadectomy in dogs, *Journal of American Veterinary Medical Association, 224*(3), 380-387.

Stöcklin-Gautschi, N. M., Hässig, M, Reichler, I. M., Hubler M., & Arnold S. (2001). The relationship of urinary incontinence to early spaying in bitches, *Journal of Reproduction and Fertility. Supplement, 57*, 233-236.

Tektepe, L. (2019). Complications seen after ovaryohysterectomy operation in cats and dogs, *Master thesis*, T.C. Kırıkkale University Institute of Health Sciences, Kırıkkale, Turkey.

Thrusfield, M. V. (1985). Association between urinary incontinence and spaying bitches. *Veterinary Record. 116*(26), 695.

Timmermans, J., Van Goethem, B., Rooster, H., & Paepe, D. (2019). Medical treatment of urinary incontinence in the bitch. *Vlaams Diergeneeskundig Tijdschrift, 88*(1), 3-8.

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Presence of *Clostridioides difficile* in poultry meat and meat products

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ABSTRACT

Clostridioides difficile, a Gram-positive spore-forming bacterium, has emerged as a significant cause of healthcare-associated infections (HAIs) on a global scale. While initial investigations predominantly linked *C. difficile* transmission to hospital settings, recent reports indicate a worrisome increase in community-acquired *C. difficile* infections (CDIs), irrespective of factors such as prior hospitalization or age. The CDC's 2021 Annual Report for *Clostridioides difficile* infection underscores this shift, revealing a slightly higher prevalence of CDIs in the community (55.9 cases/100.000 people) compared to healthcare settings (54.3 cases/100.000 people). These statistics highlight the substantial role of non-hospital sources in CDI transmission. Ongoing studies posits zoonotic pathways, particularly the consumption of contaminated food, as pivotal in community-acquired CDI transmission. Research findings indicate the detection of *C. difficile* in both raw and heat-treated meat, as well as meat products, raising significant concerns. Present investigations emphasize a noteworthy potential for the transmission of *C. difficile* to humans through the consumption of poultry meat. Although no traces of this bacterium have been identified in heat-treated poultry meat and products thus far, the risk of latent transmission through cooked poultry products should not be dismissed. Despite the absence of identified cases in processed poultry meat, the plausible transmission of *C. difficile* through these products underscores the exigency for further investigation in this field. This review provides an in-depth screening of studies on *C. difficile* contamination in poultry meat and its products worldwide. It also summarizes the risk factors associated with *C. difficile* infection through poultry meat consumption and outlines preventive measures to mitigate this risk.

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Introduction

Clostridioides difficile (previously known as *Clostridium difficile*) is a significant spore-forming enteropathogen that is associated with serious gastrointestinal disorders all over the world (Cohen et al., 2010). It is the primary agent responsible for nosocomial diarrhea and pseudomembranous colitis in individuals who have been subjected to antimicrobial treatment in the year 1978, *Clostridium difficile* infection (CDI) has been acknowledged as a hospital-acquired affliction (George et al., 1978; Hampikyan et al., 2018).

Initially, CDI was associated with hospitalized patients treated with antibiotics that are effective against a wide variety of bacteria. It has been held responsible for 20-30% of diarrhea cases caused by antimicrobial drugs (McFarland, 2007) and has been defined as a dangerous disease that can result in

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pseudomembranous colitis, toxic megacolon and even death. A notable shift in the epidemiology of CDI was observed during the initial years of the 21st century. The hyper-virulent fluoroquinolone-resistant strain of *C. difficile*, known as NAP1/BI/027, initially emerged in North America (He et al., 2013). Subsequently, there emerged accounts of CDI outbreaks attributed to the strain *C. difficile* RT 027 in both the United States and Europe, with cases exhibiting a twofold increase (Zilberberg et al., 2008; Jones et al., 2013). The incidence of CDI, which was previously rare and defined as community-acquired CDI (CA-CDI) and is independent of risk factors such as long-term antibiotic treatment, advanced age, a weakened immune system, gastric acid suppression, and previous hospitalization, has begun to increase significantly (Hampikyan et al., 2018; Song and Kim, 2019).

Especially, during the 2010s, there was a notable rise in the incidence of *C. difficile* contamination in both food and the surrounding environment (Rodriguez Diaz et al., 2018; Knight et al., 2015). Based on the findings presented in the CDC's Emerging Infections Program 2021 Annual Report for *Clostridioides difficile* Infection (CDC, 2023), it is observed that the incidence of community-associated cases slightly surpasses that of healthcare-associated cases. Specifically, the rate of community-associated cases stands at 55.9 cases/100.000 individuals, while healthcare-associated cases are reported at a rate of 54.3 cases/100.000 individuals. These statistics suggest that sources beyond the hospital environment play a substantial role in the transmission of CDI.

Ever since its initial discovery in birds and mammals during a scientific investigation carried out in Antarctica in the year 1960 (McBee, 1960), *C. difficile* has emerged as a causative agent for enteric disturbances and diarrheal episodes in numerous animal species, including poultry (Bingol et al., 2020). Numerous studies have substantiated the notion that animals possess the capacity to serve as vectors for the dissemination of the bacterium to human beings, either through direct contact or via indirect transmission facilitated by the consumption of raw sustenance or the ingestion of contaminated water sources (Songer and Anderson, 2006; Rupnik and Songer, 2010). The spread and resistance of *C. difficile* in poultry meat and their products, as well as the molecular linkages between strains isolated from poultry meat and humans, should be considered when evaluating the risk of *C. difficile* presence in poultry meat and investigating the possible threat it poses.

General features

Clostridioides difficile is classified as Gram-positive, toxigenic, and obligate anaerobic bacterium that possesses the ability to undergo spore formation (Akkaya and Hampikyan, 2019; Heise et al., 2021). It flourishes in an oxygen-deprived environment by metabolizing a diverse array of carbon and nitrogen sources, alongside simple nutrients such as trace elements (Rui et al., 2024). The bacterium's optimal growth occurs at temperatures ranging from 35 to 40 ° C; it is capable of fermenting amino acids to produce energy (adenosine triphosphate) while also metabolizing sugars (Gibbs, 2009). Nutrient deficiency, intercellular communication, and harsh environmental conditions activate the survival mechanisms of *C. difficile*. This triggers the activation of the vital Spo0A protein, which initiates endospore formation. These endospores gather around a dehydrated nucleus containing DNA, the bacterium's genetic material, transfer RNA required for protein synthesis, ribosomes, and essential enzymes for metabolic processes that initiate germination. Comparable to lifeboats, these endospores ensure the survival of the bacterium's core components, keeping them secure and intact until the environment becomes hospitable again (Lawler et al., 2020). A sub-lethal thermal shock of approximately 75–80°C for 10 minutes, or alternative stimuli such as high pressure or acidic environments, are requisite for the swift germination of these spores (Gibbs, 2009). Additionally, bile salts and certain amino acids induce spore germination. All these conditions lead to the transformation of dormant *Clostridioides difficile* endospores into exospores. These exospores then undergo germination into active vegetative cells (Lawler et al., 2020; Rui et al., 2024). Their vegetative forms do not survive prolonged exposure to oxygen outside the body. In the context of individuals who are in good health, the presence of stomach acid and commensal intestinal flora serves as a protective mechanism against the invasion of harmful microorganisms. The ingestion of vegetative *C. difficile* cells is effectively neutralized by the low pH environment, often ranging from pH 1 to 2. However, it is important to note that this acidic condition does not exhibit the same lethal effect on *C. difficile* endospores. Furthermore, *C. difficile*, a bacterium that typically resides in the gastrointestinal tracts of both humans and animals, generates toxins as a consequence of the disruption of the typical microbial community caused by prolonged and consistent antibiotic administration (Akkaya and Hampikyan, 2019). Therefore, *C. difficile* is capable of spreading throughout the gastrointestinal tract, causing a variety of gastrointestinal symptoms including diarrhea that can range in severity from moderate to severe. In specific instances, individuals

who are afflicted with severe illness may even die (De Boer et al., 2011). The CDC's Antibiotic Resistance Threats in the United States 2019 report states that in 2017, hospitals in the United States admitted approximately 223,900 adult people, and regrettably, this infection resulted in a minimum of 12,800 deaths (CDC, 2024). In contrast, the infant gut shows a natural resistance to *C. difficile* toxins. The low incidence of clinical infection in this demographic group is proof of this resistance. Colonization rates in healthy infants decline from birth and stabilize at a level corresponding to typical adult levels by the age of three years. There may be possible reasons for this. Initially, the lack of specific receptors in intestinal cells prevents toxins from binding to them. In addition, because the signaling pathways in the gastrointestinal tract of infants are incomplete, harmful agents cannot act sufficiently. Breast milk is a rich source of antibodies and other protective proteins, which neutralize toxic substances. Finally, the unique composition of the intestinal microflora in infants creates a line of defense that prevents the proliferation and activation of pathogens (Kociolek et al., 2019; Li et al., 2023). Therefore, the incidence of clinical infections is low and, in particular, infants younger than one year of age are often asymptomatic carriers of the bacteria, with more than 40% of individuals in this age group being such carriers (Stoesser et al., 2017). Despite all these, *Clostridioides difficile* is a commonly encountered bacterium in pediatric medicine and its negative effects on child health should not be ignored. According to population-based surveillance done by the CDC Emerging Infections Program in 2019, the rate of community-associated *Clostridioides difficile* infection in children was 25.8 per 100,000 and accounting for 75% of all CDI cases in children. This suggests that emerging infections are more likely to be community-associated than healthcare-associated (Shirley et al., 2023; CDC 2024).

The virulent strains of *C. difficile* are known to generate two substantial clostridial toxins, namely toxins A (*tcdA*) and B (*tcdB*) and these toxins are encoded by the genes *tcdA* and tcdB, respectively (Hensgens et al., 2012). The *tcdA* gene encodes the production of toxins A (enterotoxin), which leads to an increase in colonic fluid and cellular damage. Similarly, the *tcdB* gene encodes the production of toxins B (enterotoxin) which also contributes to cellular damage. These toxins are crucial in the pathogenesis of CDI. Certain strains also have cdtA/B genes, which encode the creation of binary toxins (actin-specific ADP-ribosyl transferase) (Barbut et al., 2005). Even though binary toxins by themselves have not been shown to cause disease (Eckert et al., 2015), their presence has been linked to more severe illness (Barbut et al., 2005). The presence of these toxins is largely responsible for the pathogenicity of this bacteria (Usui et al., 2020). Certain strains of *C. difficile* lack the ability to produce toxins, resulting in the absence of CDI symptoms (Jöbstl et al., 2010; Mooyottu et al., 2015). Nevertheless, non-toxigenic strains of *C.* difficile may have the ability to acquire toxins through horizontal gene transfer (Brouwer et al., 2013). Certain strains of *C. difficile* possess a DNA segment known as the pathogenicity locus (PaLoc), which harbors genes responsible for producing toxins A and B. Some strains of *C. difficile* with PaLoc can cause illness. *C. difficile* strains that possess this DNA sequence are toxigenic, indicating that they are capable of causing sickness. In spite of the fact that certain strains are only capable of including a single gene for the toxins (A−B+ or A+B−), it has been shown that they nonetheless cause serious sickness in people (Bolton and Marcos, 2023). However, not every strain of *C. difficile* possesses this specific DNA region. Therefore, PaLoc-free *C. difficile* strains are non-toxigenic and typically do not result in illness. Nevertheless, certain non-toxigenic strains of *C. difficile* have the ability to obtain this genetic material from another *C. difficile* strain that possesses PaLoc. Therefore, *C. difficile* that was previously non-toxigenic can undergo a transformation and begin generating toxins, thereby becoming toxigenic. There is concern that non-toxigenic strains of *C. difficile*, especially those that are resistant to many treatments, may obtain PaLoc and become toxic. This raises concerns that certain strains may be more resistant to treatment and potentially more harmful (Mooyottu et al., 2015).

Some *C. difficile* ribotypes have higher toxin production and effective sporulation, which renders them hypervirulent. In this subgroup, human pathogenic ribotypes such as RT027 and RT078 are prominent and are recognized as the cause of human CDI (Barbut et al., 2005; Rahimi et al., 2015; Hampikyan et al., 2018). The RT027 strain exhibits elevated rates of sporulation, resistance to fluoroquinolone antibiotics, heightened secretion of toxins A and B, and the ability to produce binary toxin, also known as *C. difficile* transferase (Lyon et al., 2016). The community-associated ribotypes RT027, RT078, and RT017 of *C. difficile* have additionally been identified in food products and farm animals (Goorhuis et al., 2008; Janezic et al., 2012; Rodriguez et al., 2014).

Ribotype 078 has been identified as the predominant etiological agent responsible for

Clostridium difficile-associated community-acquired diarrhea and infection (CACDI) in the Northern Hemisphere (Knight et al., 2015). The strain in question has been detected in ground turkeys located in Pennsylvania, USA (Varshney et al., 2014), as well as chicken carcasses situated in Ontario, Canada (Weese et al., 2010).

Presence of *C. difficile* in foods

The detection of genetically similar *C. difficile* strains in food and humans has led to an increased awareness of the potential for *C. difficile* as an unspecific foodborne agent (Songer and Anderson, 2006; Goorhuis et al., 2008; Rupnik et al., 2009; Knight et al., 2015). Numerous hypotheses have been proposed thus far regarding the transmission of *C. difficile*. Research conducted on various continents have exhibited escalating apprehension regarding the potential role of food as a reservoir for CDI. Food products were deemed to possess the capacity to function as a medium for the proliferation of *C. difficile* endospores. However, it appears that the ingestion of metabolically dormant endospores is primarily responsible for the transmission of CDI, likely due to the obligate anaerobic nature of the bacterium (Akkaya and Hampikyan, 2019). In addition, the rising prevalence of community-acquired *C. difficile* infection (CA-CDI) among younger individuals who have not been hospitalized suggests that retail foods may serve as significant reservoirs for this pathogen (Usui et al., 2020).

C. difficile has been detected in the environment (soil and water), a diverse array of sources/reservoirs including food animals (cattle, poultry, pigs, and sheep), meat and meat products (beef, chicken, lamb, pork, turkey, and veal), seafood (clams, mussels, salmon, and shrimp), vegetables, fruits, and packaged foods, according to studies conducted to date (Gould and Limbago, 2010; Knight et al., 2015; Hampikyan et al., 2018; Bingol et al., 2020). Recent meta-analytical studies have shed light on the relationship between various food types and the presence of *C. difficile*. A synthesis of 79 studies conducted from 1981 to 2019 by Rodriguez-Palacios et al. (2020) revealed a 4.1% prevalence in commonly consumed foods, including seafood, green leafy vegetables, and meats such as beef, pork, and poultry. These findings suggest not only an increasing incidence of *C. difficile*, but also a worrying trend of rising infection rates linked to these food products. Similarly, Borji et al. (2023) conducted a meta-analysis of 60 studies over a ten-year period from 2009 to 2019, focusing on the prevalence of *C. difficile* in commonly consumed foods, including seafood, poultry, red meat, dairy products, vegetables, salads, and other foods. Their research reported a *C. difficile* prevalence of 6.3 percent across all food types. According to the results of the analysis, seafood had the highest *C. difficile* prevalence with 10.3%, followed by poultry (6.2%), salads (6.1%), and red meat (5.5%). The study also calculated risk ratios, and seafood emerged as the highest risk carrier with a ratio of 12.88, indicating a risk approximately thirteen times greater than that of side dishes, which pose the least hazard for *C. difficile* contamination. Other significant risk carriers included ready-to-eat red meats and cooked poultry, which had risk ratios of 9.75 and 7.75, respectively, followed by salads and raw poultry meat.

C. difficile is acknowledged to be harbored by food animals. Studies conducted in Türkiye (Hampikyan et al., 2018), Belgium (Rodriguez et al., 2014), Canada (Rodriguez et al., 2019; Weese et al., 2009), Australia (Jöbstl et al., 2010), Costa Rica (Quesada-Gómez et al., 2013), and United States (Mooyottu et al., 2015) have detected *C. difficile* in various meat products, including beef, pork, veal, and sheep carcasses. In their study, Weese et al. (2009) successfully detected the presence of specific toxin genes (tcdA, tcdB, and cdtA/B) in the samples they analyzed. The presence of tcdA, tcdB, and cdtA/B was later confirmed in the findings of Rodriguez et al. (2014), although in a smaller percentage of pork and beef samples. It also identified a group of samples that tested positive for tcdA and tcdB but did not have the binary toxin genes, with one sample completely devoid of the genes in question. According to a study by Hampikyan et al. (2018), tcdB was discovered in more than half of the cattle isolates, and tcdA in nearly half of them. Furthermore, the majority of the isolates contained cdtA/B. Their study emphasized that a significant number of cattle and sheep samples contained all three virulence genes, while a small portion of sheep carcass isolates did not have any. Quesada-Gómez et al. (2013) discovered isolates positive for cdtA and tcdB but did not have the binary toxin genes. On the other hand, studies conducted by Jöbstl et al. (2010) and Mooyottu et al. (2015) have not found any evidence of these toxin genes. Contamination rates have ranged from 1.5% to 33.6%, indicating a widespread presence of *C. difficile* in the global meat supply chain. The persistent nature of *C. difficile* spores, which can endure harsh environmental conditions for extended periods, facilitates their transmission from personnel, equipment, and contaminated surfaces to food due to improper hygiene practices and posing a significant public health risk (EFSA, 2013; Hampikyan et al., 2018). The body of research investigating *C. difficile* contamination in vegetables and ready-to-eat salads remains limited. It has been observed that compost

fertilizers derived from farm animals have the potential to harbor *C. difficile* spores (Jöbstl et al., 2010; Quesada-Gómez et al., 2013). Hence, there is a potential risk of *C. difficile* entering the food chain due to the use of fertilizers on the land. The potential for contamination of vegetables and fruits arises from the utilization of contaminated water during irrigation or washing processes (Rupnik and Songer, 2010). Furthermore, even if good agricultural procedures are implemented, there is still a plausible possibility of spore transfer to fresh produce via fertilizer (Quesada-Gómez et al., 2013). The ingestion of minimally processed or uncooked vegetables or fruits has the potential to function as a means of transmission for CDI. Research conducted in Canada (Metcalf et al., 2010), South Wales (al Saif and Brazier, 1996), and Scotland (Bakri et al., 2009) has demonstrated raw vegetables contamination rates ranging from 2.3% to 7.5%, highlighting the potential for non-animal food sources to contribute to the spread of *C. difficile*, including antibiotic-resistant strains.

Although the studies have focused more on animal -derived foods and their different foodstuff, there is a scarcity of data on the prevalence and characteristics of *C. difficile* contamination in seafood. Several studies have reported the presence of *C. difficile* in various marine creatures, such as edible bivalve molluscs (e.g., scallops), shellfish (e.g., shrimp, clam, cockle, mussel, oyster), finfish, and fishes (e.g., perch, salmon), from different regions of the world. The prevalence of *C. difficile* contamination in seafood varies widely, ranging from 3.17% to 66.6%, depending on the location, species, and source of the samples. Italy (Pasquale et al., 2012; Troiano et al., 2015), Canada (Metcalf et al., 2011), United States (Montazeri et al., 2015; Norman et al., 2014), and Iran (Nayebpour and Rahimi, 2019) are some of the areas where *C. difficile* has been detected in seafood. These studies reveal that seafood, like other food products, can be contaminated by *C. difficile* and that there may be geographical differences in the contamination levels of this bacterium.

Presence of *C. difficile* in poultry meat and products

The detection of *C. difficile* in poultry raises concerns that poultry may be a possible reservoir for CDI. Studies demonstrate an association between *C. difficile* and its human pathogenic ribotypes in chicken carcasses, implicating chickens as a likely source of bacterial contamination and potentially contributing to the transmission of *C. difficile* to humans (Pasquale et al., 2012; Rahimi et al., 2015; Hampikyan et al., 2018).
The presence and characterization of *C. difficile* in poultry meat are summarized in Table 1. It has been observed that the presence of *C. difficile* in poultry products was detected within a range of 1% to 44.4%. Upon examination of these studies, it becomes apparent that the region of North America has exhibited the highest recorded prevalence rate, standing at an impressive 44.4% (Songer et al., 2009). Subsequently, Türkiye has been documented to possess a prevalence rate of 37.3% (Bingol et al., 2020), while Iran follows suit with a prevalence rate of 24.4% (Barezi et al., 2023). It is evident that the prevalence of *C. difficile* in meat collected in Asia is lower than that in meat collected in Europe, and the prevalence of *C. difficile* in Europe's meat is lower than that in United States-origined meat. In addition, the binary toxin (cdtA/B) was found in isolates originating more often from Canada and the United States (Songer et al., 2009; Weese et al., 2010; Varshney et al., 2014). Similarly, several investigations conducted in Europe have reported the presence of dual toxin genes (Bingol et al., 2020; Tkalec et al., 2020). In contrast, certain studies have documented the absence of binary toxin genes in isolates obtained from chicken meat (De Boer et al., 2011; Guran and Ilhak, 2015; Heise et al., 2021).

Recently, there has been increasing concern about the uncontrolled use of antibiotics in poultry. Different countries ban the use of antibiotics, yet the poultry industry uses various types of antibiotics to promote growth, treat diseases, and prevent disease. Because of this use, there is ongoing concern about the development of antibiotic resistance in *C. difficile* in poultry (Bingol et al., 2020). The European Society of Clinical Microbiology and Infectious Diseases (ESCMID) recommends vancomycin and metronidazole as the top choices for treating *C. difficile* infections in humans (Cho et al., 2020). Studies have shown that vancomycin and metronidazole are the antibiotics to which *C. difficile* strains are most frequently susceptible on all continents (Harvey et al., 2011; Quesada-Gómez et al., 2013; Varsheney et al., 2014; Ersöz and Coşansu, 2018; Lee et al., 2018; Usui et al., 2020; Attia et al., 2021; Barezi et al., 2023). This demonstrates their continued efficacy in treating *C. difficile* infections. On the other hand, resistance to clindamycin ranged from 2.2% to 50.0% (Lee et al., 2018; Usui et al., 2020), and some strains showed intermediate resistance (Lee et al., 2018; Attia et al., 2021; Filabadi et al., 2022). In addition to that, Harvey et al. (2011) and Bingol et al. (2020) both found that cefotaxime was not effective against many strains of C. *difficile*, with 100% (7/7) and 97.1% (67/69) of these strains being resistant. These results raise concerns that clindamycin and cefotaxime may show a higher frequency of resistance, limiting treatment options.

Table 1. Review of the presence and characterization of *Clostridioides difficile* in poultry meats from investigations conducted in countries on the European, Asian and American continents.

Reference	Study period	Country	Raw / Cooked	Sample material	Samples (n)	Positive samples (n^p)	Percentage of positive samples (%)	Toxin type (n^t)	PCR ribotype (n^r)
Von Abercron et al. (2009)	April - September 2008	Sweden	R	poultry meat	4	0	0.0	-	-
	April - September 2008	Sweden	C	poultry sausages					
Indra et al. (2009)	February 2008 - April 2008	Austria	R	chicken meat	6	0	0.0	-	-
De Boer et al. (2011)	October 2008 - March 2009	Netherlands	R	chicken meat	257	7	2.7	tcdA+ tcdB+ cdtAB- (4) tcdA- tcdB- cdtAB- (3)	001, 003 (2), 071, 087, NT** (2)
Guran and Ilhak (2015)	October 2012 - April 2013	Türkiye	R	chicken carcass (leg quarters, breast, wings, drumsticks, livers)	310	25	8.1	tcdA+ tcdB- (8) tcdA- tcdB+ (5)	-
Ersöz and Coşansu (2018)	April 2013 - February 2014	Türkiye	R	chicken (breast)	27	0	0.0	-	-
Bingol et al. (2020)	US*	Türkiye	R	chicken carcass	185	69	37.3	tcdA+ tcdB+ cdtAB+ (17) tcdA+ tcdB+ cdtAB- (14) tcdA+ tcdB- cdtAB+ (1) tcdA+ tcdB- cdtAB- (3) tcdA- tcdB+ cdtAB+ (13) tcdA- tcdB+ cdtAB- (11) tcdA- tcdB- cdtAB+ (0) tcdA- tcdB- cdtAB- (10)	003 (1), 010 (1), 020 (2), 027 (6), ML#027 (6), 085 (4), 087 (4), 470 (4), 456 (2), NT** (39)
Tkalec et al. (2020)	April 2015 - December 2015 March 2016 - December 2016 March 2016 - December 2017	Slovenia	R	chicken meat	60	3	5.0	-	001, 014/020, 015
				chicken meat preparations	120	5	4.2	-	001, SLO 052, 078
Heise et al. (2021)	July 2017 January 2018 - June 2018 June 2019 - July 2019	Germany	R	poultry meat (skin-out) (chicken and turkey)	42	0	0.0	-	-
				poultry meat (skin-on) (chicken and turkey)	322	51	15.8	tcdA+ tcdB+ (43)	002/2 (10); 001 (9); 005 (5); 014 (5); NT** (4); 087 (2); 049 (1); 020 (1); 464 (1); 503 (1); 212 (1); 220/1 (1); 625 (1); AI-29 (1); 205 (3); 701 (2); 010 (1); 578 (1); 629 (1)
Songer et al. (2009)	January 2007 - April 2007	USA	R	ground turkey	9	4	44.4	toxinotype V NAP7 tcdA+ tcdB+ cdtAB+ (4)	078
Weese et al. (2010)	November 2008 - June 2009	Canada	R	chicken carcasses (thigh, wing, and leg)	203	26	12.8	-	078 (26)
Harvey et al. (2011)	July 2010	USA	R	chicken meat	96	7	7.3	toxinotype V NAP7 (3) or NAP7-variant (4)	-
Varshney et al. (2014)	October 2011 - September 2012	USA	R	chicken meat (thighs)	77	6	7.8	tcdA+ tcdB+ cdtAB+ (4) tcdA- tcdB- cdtAB+ (1) tcdA- tcdB- cdtAB- (1)	-
				ground turkey	76	11	14.5	tcdA+ tcdB+ cdtAB+ (3) tcdA+ tcdB+ cdtAB- (1) tcdA+ tcdB- cdtAB+ (1) tcdA- tcdB- cdtAB+ (4) tcdA- tcdB- cdtAB- (2)	027 (1), 078 (2)
Mooyottu et al. (2015)	US*	USA	R	chicken meat (wing)	100	0	0.0	-	-
Quesada-Gómez et al. (2013)	November 2009 - April 2010	Costa Rica	R	chicken meat	67	1	1.5	tcdA+ tcdB+ cdtAB-	029

Continuation of Table 1

Hasanzade and Rahimi (2013)	US*	Iran	R	turkey meat	120	14	11.7	-	-
Hasanzadeh and Rahimi (2013)	US*	Iran	R	chicken meat	120	19	15.8	-	-
Rahimi and Khaksar (2015)	April - October 2012	Iran	C	chicken nugget	150	0	0.0	-	-
Razmyar et al. (2017)	2014	Iran	R	packed chicken parts (necks, thighs, wings)	65	10	15.4	tcdA+ tcdB+ (5) tcdA+ tcdB- (1) tcdA- tcdB- (2) tcdA+ tcdB+ cdtAB+ (2)	-
Lee et al. (2018)	April 2013 - March 2014	South Korea	R	chicken meat	149	25	16.8	tcdA+ tcdB+ (2)	-
Usui et al. (2020)	March 2015 - March 2016	Japan	R	chicken (liver)	28	1	3.6	-	-
				chicken meat	89	6	6.7	-	-
Attia (2021)	October 2019 - November 2019	Saudi Arabia	R	chicken carcass (legs, thighs, wings, breasts)	250	11	4.4	-	-
Ghorbani Filabadi et al. (2022)	July 2018 - July 2019	Iran	R	chicken meat	100	1	1.0	tcdA+ tcdB+	-
Ansarian Barezi et al. (2023)	July 2018 - July 2019	Iran	R	quail meat	60	1	1.7	-	-
				duck meat	60	12	20.0	-	-
				chicken meat	90	22	24,4	-	-
Hazarika et al. (2023)	July 2019 - De cember 2020	India	R	chicken meat	28	4	14.81	tcdA+ tcdB+ (2)	-
			C	chicken sausage	10	0	0.0	-	-
			C	chicken salami	10	0	0.0	-	-

ML#: most likely; np: number of positive samples; nr: number of ribotypes; nt: number of toxin types; NT**: Non-typable by the National Reference Laboratory for *Clostridium difficile*; US*: unspecified

***Prevalence of C. difficile in poultry meat in Europe*:** Research conducted in Europe has revealed that the presence of *C. difficile* is frequently observed in chicken meat with a range of 2.7 % to 37.3 % (Table 1). The ribotypes RT 001, 014, 027, and 078 identified in research conducted in Germany (Heise et al., 2021), the Netherlands (De Boer et al., 2011), Slovenia (Tkalec et al., 2020), and Türkiye (Bingol et al., 2020), are commonly detected in human that have been linked to human CDI.

Prevalence of C. difficile in poultry meat in America: *C. difficile* has been isolated from a variety of poultry and its products in America and Canada, including uncooked ground turkey, chicken meat, and chicken thigh, wing, and leg. The prevalence of *C. difficile* in these products ranges from 7.3 % to 44.4 % (Table 1). Several studies have also found that some of the *C. difficile* strains isolated from poultry and its products are toxigenic. These strains are responsible for the production of toxins that cause CDI. Additionally, some of these strains have been identified as ribotypes 027 and 078, which are two of the most virulent strains of *C. difficile* and are associated with significant CDI in humans (Songer et al., 2009; Weese et al., 2010; Varshney et al., 2014).

Prevalence of C. difficile in poultry meat in Asia: Numerous investigations have made contributions towards conducting a thorough examination of the occurrence of *C. difficile* in diverse reservoirs of poultry meat inside the Asian region. The observed prevalence of *C. difficile* in poultry meat exhibits considerable heterogeneity, ranging from a minimum of 1% to a maximum of 24.4% (Table 1). A greater number of research have been undertaken in Iran compared to other nations within the Asian continent (Hasanzadeh and Rahimi, 2013; Razmyar et al., 2017; Ghorbani Filabadi et al., 2022; Barezi et al., 2023). These studies have revealed a range of *C. difficile* prevalence rates, spanning from 1 % to 15.8 %, focusing on diverse poultry meat varieties such as chicken, turkey, quail, duck, and partridge (Barezi et al., 2023). Moreover, *C. difficile* has also been identified in South Korea (Lee et al., 2018), Japan (Usui et al., 2020), Saudi Arabia (Attia, 2021), and India (Hazarika et al., 2023).

In contrast to the prevailing trend, there exist studies that have documented an inability to identify the *C. difficile* strain within endeavors carried out in both the United States (Mooyottu et al., 2015) and the European (Indra et al., 2009; Von Abercron et al., 2009; Ersöz and Coşansu, 2018) continents. In the Asian continent, Rahimi and Khaksar (2015) investigated the heat-treated food (chicken nuggets), in contrast to earlier research, and indicated that no evidence of the presence of this pathogen was found. Additionally, upon closer examination of the African continent, it is worth noting that thus far only a solitary study has been documented (Abdel-Glil et al., 2018), wherein the existence of *C. difficile* was regrettably not ascertained.

Factors contributing to discrepancies in isolation rates across continents: The observed variabilities in *C. difficile* isolation rates across continents can perhaps be attributed to variations in the procedures employed for the isolation and identification of *C. difficile* within each continent. The absence of universally accepted ISO protocol for the identification of *C. difficile* in food products makes it difficult to compare the results of different studies, resulting in data inconsistencies. (Blanco et al., 2013) reported that the method used to isolate *C. difficile* may have a substantial effect on the prevalence statistics for this pathogen. The observed discrepancies in prevalence can also be ascribed to disparities in the biological material, collection techniques, hygiene practices, sampling methodologies, process size, and cultural practices utilized at each individual site.

The prevalence rates of *C. difficile* may be subject to variation due to factors such as geographical location and seasonal fluctuations. Research conducted at hospitals in Taiwan and Australia revealed that the prevalence of CDI exhibited a peak in the month of March, while the lowest incidence was observed during the last quarter of the year (Lee et al., 2016; Worth et al., 2016). Furuya-Kanamori et al. (2015) emphasized that the infection peaks in spring and is seen at a lower frequency in summer and autumn.

Rodriguez-Palacios et al. (2009) identified the prevalence of *Clostridioides difficile* in retail meat products, while Rodriguez et al. (2019) investigated the distribution of this pathogen in environmental soil samples. The results of both investigations indicate that there is a notable prevalence of *C. difficile* during the winter season. The observation that the detection of *C. difficile* in poultry was predominantly reported throughout the winter and spring seasons, particularly in the months of November to March, indicates a noteworthy correlation with the mentioned investigations.

Additionally, it is imperative to consider the age, breed, and other relevant characteristics of the animals that were included in the sample (Varshney et al., 2014; Ersöz and Coşansu, 2018; Hampikyan et al., 2018). Knight et al. (2013) observed that the prevalence of *C. difficile* decreases with the age of production animals, so meat from older animals poses much less risk. Colonization of *C. difficile* in chickens occurs predominantly during the initial two weeks following hatching, followed by a gradual decline as the poultry age.

Presence of *C. difficile* in heat-treated products

C. difficile is currently not recognized as a foodborne pathogen. Therefore, the available data regarding the viability of this strain in food is comparatively limited when compared to other pathogenic species within the Clostridium genus. In comparison to other pathogens, the most notable characteristic of *C. difficile* is the great resilience of its spores to a range of physical conditions, including heat and chemicals. Given the capacity of spores to endure the acidic environment of the stomach and the elevated temperatures encountered during cooking procedures, it is conceivable that these microorganisms may endure in food items even after being cooked (Rodriguez-Palacios et al., 2010; Rodriguez et al., 2013). Moreover, this persistence of spores presents a formidable obstacle in the thorough eradication of spores during the culinary preparation of food and the sanitation of food processing equipment and surfaces (Esfandiari et al., 2014).

The process of heating can lead to a decrease in the oxygen content within cooked food, which can result the creation of anaerobic conditions that can trigger the germination and growth of spores (Kouassi et al., 2014). In addition, heat treatments have the potential to enhance the resilience of some pathogens that can produce heat-shock proteins, hence leading to the possibility of pathogen selection during the process of heat treatments (Cowen and Lindquist, 2005). This selection increases the pathogenic properties of microorganisms exposed to heated foods, leading to their widespread presence in the food supply and increasing the risk of foodborne infections resulting from the consumption of these foods.

Flock et al. (2022) revealed the survival of *C. difficile* in fermented pork summer sausage even after exposure to a pH below 5 and cooking at 66.5°C for 45 minutes. In a study conducted by Rodriguez-Palacios et al. (2010), it was shown that vegetative cells of the bacterium C. difficile were able to withstand the recommended cooking temperatures for beef set by USDA, which is 71°C, for a duration of 2 hours. However, it was found that subjecting the food to a

reheating process at a temperature of 85°C resulted in the elimination of 90% of *C. difficile* spores within a span of 10 minutes. Similarly, it has been observed that *C. difficile* spores can withstand temperatures ranging from 60°C to 75°C for extended periods, with significant reduction in spore count only occurring at temperatures exceeding 85°C (Lawley et al., 2009). It was also demonstrated that the inhibition of *C. difficile* spore increased by subjecting them to a heat shock at a temperature of 96°C for a duration of 15 minutes. However, heat treatment is not always effective in killing spores, as some spores may exhibit persistence or regenerative ability even after exposure to high temperatures (Rodriguez-Palacios and LeJeune, 2011). Research on thermal inactivation kinetics further supports this notion, indicating that heating *C. difficile* spores to 100°C for 30 seconds resulted in a 3.75 log reduction, while a temperature of 105°C for the same duration achieved a 4.29 log reduction (Saad et al., 2023).

The heat resistance of *C. difficile* spores has been extensively documented in various studies. Songer et al. (2009) detected *C. difficile* in 14.3% ready-to-eat summer sausage and 62.5% ready-to-eat pork braunschweiger, highlighting its persistence after cooking processes. Ribotype 027 was identified in sausage, while both ribotype 078 and ribotype 027 were detected in braunschweiger. The prevalence of ribotype 078 in food sources indicates a potential for greater heat resistance compared to several other ribotypes, such as RT027 (Rodriguez-Palacios et al., 2016). This strain, possessing the ability to endure temperatures as high as 96°C, has been linked to instances of *C. difficile* infections (Brown and Wilson, 2018).

The fact that spores usually survive at temperatures recommended for cooking indicates the potential role of food in the transmission of the disease (Rodriguez-Palacios and LeJeune, 2011; Deng et al., 2015). Therefore, the current cooking recommendations ought to be revised to incorporate *C. difficile* and it is crucial to adopt more efficacious intervention measures aimed at mitigating spore contamination in food products. Moreover, the presence of *C. difficile* in ready-to-eat meat products underscores the potential role of food in the transmission of this pathogen, necessitating stricter control measures throughout the food chain. These combined efforts are crucial to mitigate the risk of foodborne *C. difficile* infections and safeguard public health.

Protection

Safeguarding consumer health throughout the food production continuum is paramount, necessitating strict adherence to prescribed food safety standards at every stage, from production and processing to storage, shipment, and consumption. In the realm of poultry meat and its products, sanitation protocols must be meticulously executed throughout the entirety of the poultry rearing process, following the farm-to-table methodology. During production, the utmost care must be exercised when extracting internal organs and removing feathers, ensuring no contamination of the carcass. This precautionary measure aims to avert potential contamination. Additionally, cleanliness of materials, machinery, and personnel hygiene must be maintained, and animal waste disposal must be handled with caution and attentiveness (Akkaya and Hampikyan, 2019).

The spores of *C. difficile* demonstrate an exceptional degree of resistance when confronted with adverse physical circumstances. The spores of *C. difficile* may survive in the environment for a period longer than five months (Kramer et al., 2006). A multitude of methodologies like subzero temperatures as low as -80°C, elevating temperatures to a maximum of 85°C, desiccation, exposure to UV radiation, utilization of alcohol gel, and application of various disinfectants, have been ascertained to be ineffective in eradicating said pathogenic bacterium (Deng et al., 2015; Connor et al., 2017).

Due to the ability of spores to survive cooking temperatures, *C. difficile* spores must be heated to a temperature above 85°C to ensure food safety (Rodriguez-Palacios and LeJeune, 2011; Deng et al., 2015). Nevertheless, the current cooking recommendations ought to be revised to incorporate *C. difficile*, and it is crucial to adopt more efficacious intervention measures aimed at mitigating spore contamination in food products. Furthermore, it is important to raise awareness among food handlers and consumers about proper food handling and cooking practices to prevent *C. difficile* contamination. These comprehensive measures will help ensure a safer food supply and protect individuals from potential infections.

Conclusions

The present review has provided an overview of the current understanding of *Clostridioides difficile* contamination in poultry meat and its products. While the role of contaminated poultry in human illness remains a subject of debate, the growing body of evidence suggests that poultry may serve as a potential reservoir for *C. difficile* infections within communities. The detection of identical *C. difficile* ribotypes, including RT001, RT014, RT027 and RT078,

in both human illnesses and poultry further supports this notion.

Nonetheless, rigorous hygiene management practices throughout the poultry production and handling process are crucial in minimizing the risk of *C. difficile* contamination and potential human exposure. Although there is limited research conducted to date on heat-treated poultry meat products, *C. difficile* exhibit resistance to heat treatment and have been identified in meat products derived from various animal sources, including pigs and cattle, suggests a potential concern regarding the potential presence of this bacterium in poultry meat products.

In conclusion, even though poultry is one of the agents that may play a role in the transmission of *C. difficile* infections, its potential role in foodborne transmission warrants further investigation and proactive intervention. The development and implementation of effective control strategies for the prevention of *C. difficile* cases related to poultry meat may be essential to protect public health and reduce the prevalence of *C. difficile* infections.

References

Abdel-Glil, M. Y., Thomas, P., Schmoock, G., Abou-El-Azm, K., Wieler, L. H., Neubauer, H., & Seyboldt, C. (2018). Presence of *Clostridium difficile* in poultry and poultry meat in Egypt. *Anaerobe*, 51, 21-25.

Akkaya, E., & Hampikyan, H. (2019). Clostridioides (Clostridium) difficile and its presence in food. *Turkish Microbiological Society Journal, 49*(4), 175-185.

al Saif, N., & Brazier, J. S. (1996). The distribution of Clostridium difficile in the environment of South Wales. *Journal of Medical Microbiology, 45*(2), 133-137.

Attia, A. E. T. (2021). Retail chicken meats as potential sources of Clostridioides difficile in Al-Jouf, Saudi Arabia. *Journal of Infection in Developing Countries,* 15, 972-978.

Bakri, M. M., Brown, D. J., Butcher, J. P., & Sutherland, A. D. (2009). Clostridium difficile in ready-to-eat salads, Scotland. *Emerging Infectious Diseases, 15*, 817-818.

Barbut, F., Decré, D., Lalande, V., Burghoffer, B., Noussair, L., Gigandon, A., Espinasse F., Raskine L., Robert J., Mangeol A., & Petit, J. C. (2005). Clinical features of *Clostridium difficile*-associated diarrhea due to binary toxin (actin-specific ADP-ribosyltransferase)-producing strains. Journal *of Medical Microbiology, 54*, 181-185.

Barezi, A. A., Shakerian, A., Rahimi, E., & Esfandiari, Z. (2023). Examining the extent of contamination, antibiotic resistance, and genetic diversity of Clostridioides (*Clostridium) difficile* strains in meat and feces of some native birds of Iran. *Biomed Research International*, 2023 (1), 3524091.

Bingol, E. B., Hampikyan, H., Muratoglu, K., Akkaya, E., Cetin, O., & Colak, H. (2020). Characterisation and antibiotic susceptibility profile of Clostridioides (*Clostridium*) *difficile* isolated from chicken carcasses. *Journal of Veterinary Research (Poland),* 64, 407-412.

Blanco, J. L., Álvarez-Pérez, S., & García, M. E. (2013). Is the prevalence of Clostridium difficile in animals underestimated? *Veterinary Journal, 197*, 694-698.

Bolton, D., & Marcos, P. (2023). The Environment, Farm Animals and Foods as Sources of Clostridioides difficile Infection in Humans. *Foods, 12*(5), 1094.

Borji, S., Kadivarian, S., Dashtbin, S., Kooti, S., Abiri, R., Motamedi, H., Moradi, J., Rostamian, M., & Alvandi, A. (2023). Global prevalence of Clostridioides difficile in 17,148 food samples from 2009 to 2019: a systematic review and meta-analysis. Journal of Health, Population and Nutrition, 42(1), 36.

Brouwer, M. S., Roberts, A. P., Hussain, H., Williams, R. J., Allan, E., & Mullany, P. (2013). Horizontal gene transfer converts non-toxigenic Clostridium difficile strains into toxin producers. *Nature Communications, 4*(1), 2601.

Brown, A. W. W., & Wilson, R. B. (2018). Clostridium difficile colitis and zoonotic origins a narrative review. *Gastroenterology Report (Oxford), 6*, 157-166.

Centers for Disease Control and Prevention. (2023, June 28). 2021 Annual Report for the Emerging Infections Program for *Clostridioides difficile* Infection. Retrieved from http://www.cdc.gov/hai/eip/Annual-CDI-Report-2021.html

Centers for Disease Control and Prevention (2024, March 20). Antibiotic resistance threats in the United States, 2019. Retrieved from www.cdc.gov/antimicrobial-resistance/data-research/threats/index.html

Cohen, S. H., Gerding, D. N., Johnson, S., Kelly, C. P., Loo, V. G., McDonald, L. C., Pepin, J., & Wilcox, M. H. (2010). Clinical practice guidelines for Clostridium difficile infection in adults: 2010 update by the Society for Healthcare Epidemiology of America (SHEA) and the Infectious Diseases Society of America (IDSA). *Infection Control and Hospital Epidemiology, 31*(4), 431-455.

Cho, J. M., Pardi, D. S., & Khanna, S. (2020). Update on Treatment of Clostridioides difficile Infection. *Mayo Clinic Proceedings, 95*(4), 758–769.

Connor, M., Flynn, P.B., Fairley, D.J., Marks, N., Manesiotis, P., Graham, W.G., Gilmore, B.F., & McGrath, J.W. (2017). Evolutionary clade affects resistance of *Clostridium difficile* spores to cold atmospheric plasma. *Scientific Reports, 7*(1), *41814.*

Cowen, L. E., & Lindquist, S. (2005). Hsp90 Potentiates the rapid evolution of new traits: Drug resistance in diverse fungi. *Science, 309*, 2185-2189.

De Boer, E., Zwartkruis-Nahuis, A., Heuvelink, A. E., Harmanus, C., & Kuijper, E. J. (2011). Prevalence of *Clostridium difficile* in retailed meat in The Netherlands. *International Journal of Food Microbiology, 144*, 561-564.

Deng, K., Plaza-Garrido, A., Torres, J. A., & Paredes-Sabja, D. (2015). Survival of Clostridium difficile spores at low temperatures. *Food Microbiology, 46*, 218-221.

Eckert, C., Emirian, A., Le Monnier, A., Cathala, L., De Montclos, H., Goret, J., Berger, P., Petit, A., De Chevigny,

R., Lalande, V., Marchandin, H., & Barbut, F. (2015). Prevalence and pathogenicity of binary toxin-positive Clostridium difficile strains that do not produce toxins A and B. *New Microbes and New Infections, 3*, 12-17.

Ersöz, Ş. Ş., & Coşansu, S. (2018). Prevalence of clostridium difficile isolated from beef and chicken meat products in Turkey. *Korean Journal of Food Science Animal Resources, 38*, 759-767.

Esfandiari, Z., Weese, S., Ezzatpanah, H., Jalali, M., & Chamani, M. (2014). Occurrence of Clostridium difficile in seasoned hamburgers and seven processing plants in Iran. *BMC Microbiology*, 14, 1-7.

European Food Safety Authority - EFSA. (2013). Scientific Opinion on the public health hazards to be covered by inspection of meat (bovine animals). *EFSA Journal, 11*(6).

Flock, G., Yin, H. B., Chen, C. H., Pellissery, A.J., & Venkitanarayanan, K. (2022). Survivability of Clostridioides difficile spores in fermented pork summer sausage during refrigerated storage. *Veterinary World, 15*, 162-167.

Furuya-Kanamori, L., McKenzie, S.J., Yakob, L., Clark, J., Paterson, D. L., Riley, T.V., & Clements, A. C. (2015). *Clostridium difficile* infection seasonality: Patterns across hemispheres and continents - A systematic review. *PLoS One, 10*, e0120730.

George, W. L., Sutter, V. L., Goldstein, E. J., Ludwig, S. L., & Finegold, S. M. (1978). Aetiology of antimicrobial-agent-associated colitis. *Lancet, 1*, 802-803.

Ghorbani Filabadi, P., Rahimi, E., Shakerian, A., & Esfandiari, Z. (2022). Prevalence, antibiotic resistance, and genetic diversities of Clostridium difficile in meat nuggets from various sources in Isfahan, Iran. *Journal of Food Quality, 2022*(1), 9919464.

Gibbs, P. (2009). Pathogenic Clostridium species, DOI: 10.1533/9781845696337.2.820

Goorhuis, A., Bakker, D., Corver, J., Debast, S. B., Harmanus, C., Notermans, D. W., Bergwerff, A. A., Dekker, F. W., Kuijper, E. J. (2008). Emergence of *Clostridium difficile* infection due to a new hypervirulent strain, polymerase chain reaction ribotype 078. *Clinical Infectious Diseases, 47*, 1162-1170.

Gould, L. H., & Limbago, B. (2010). Clostridium difficile in food and domestic animals: A new foodborne pathogen. *Clinical Infectious Diseases, 51*, 577-582.

Guran, H. S., & Ilhak, O. I. (2015). Clostridium difficile in retail chicken meat parts and liver in the Eastern Region of Turkey. *Journal Fur Verbraucherschutz Und Lebensmittelsicherheit, 10*, 359-364.

Hampikyan, H., Bingol, E. B., Muratoglu, K., Akkaya, E., Cetin, O., & Colak, H. (2018). The prevalence of *Clostridium difficile* in cattle and sheep carcasses and the antibiotic susceptibility of isolates. *Meat Science, 139*, 120-124.

Harvey, R. B., Norman, K. N., Andrews, K., Norby, B., Hume, M. E., Scanlan, C.M., Hardin, M. D., & Scott, H. M. (2011). *Clostridium difficile* in retail meat and processing plants in Texas. *Journal of Veterinary Diagnostic Investigation, 23*, 807-811.

Hasanzade, A., & Rahimi, E. (2013). Isolation of *Clostridium difficile* from turkey and ostrich meat sold in meat stores of Isfahan city. *International Journal of Advanced Biological and Biomedical Research, 1*, 963-967.

Hasanzadeh, A., & Rahimi, E. (2013). Isolation of *Clostridium difficile* from chicken meat sold in meat stores of Isfahan City. *Advances in Environmental Biology, 7,* 2372-2374.

Hazarika, R., Sarmah, H., Doley, M. K., Saikia, D. P., Hazarika, G., Barkalita, L. M., Deka, P., Manoharan, S., & Sharma, R. K. (2023). *Clostridioides difficile* in food and food products of animal origin in Assam, India. *Anaerobe, 81*, 102723.

He, M., Miyajima, F., Roberts, P., Ellison, L., Pickard, D. J., Martin, M. J., … Lawley, T. D. (2013). Emergence and global spread of epidemic healthcare-associated *Clostridium difficile*. *Nature Genetics, 45*, 109-113.

Heise, J., Witt, P., Maneck, C., Wichmann-Schauer, H., & Maurischat, S. (2021). Prevalence and phylogenetic relationship of Clostridioides difficile strains in fresh poultry meat samples processed in different cutting plants. *International Journal of Food Microbiology, 339*, 109032.

Hensgens, M. P. M., Keessen, E. C., Squire, M. M., Riley, T. V., Koene, M. G. J., De Boer, E., Lipman, L., & Kuijper, E. J. (2012). *Clostridium difficile* infection in the community: A zoonotic disease? *Clinical Microbiology and Infection, 18*, 635-645.

Indra, A., Lassnig, H., Baliko, N., Much, P., Fiedler, A., Huhulescu, S., & Allerberger, F. (2009). Clostridium difficile: A new zoonotic agent? *Wien Klin Wochenschr, 121*, 91-95.

Janezic, S., Ocepek, M., Zidaric, V., & Rupnik, M. (2012). *Clostridium difficile* genotypes other than ribotype 078 that are prevalent among human, animal, and environmental isolates. *BMC Microbiology, 12*, 1-8.

Jones, A. M., Kuijper, E. J., & Wilcox, M. H. (2013). Clostridium difficile: A European perspective. *Journal of Infection, 66*, 115-128.

Jöbstl, M., Heuberger, S., Indra, A., Nepf, R., Köfer, J., & Wagner, M. (2010). Clostridium difficile in raw products of animal origin. *International Journal of Food Microbiology, 138*, 172-175.

Knight, D. R., Elliott, B., Chang, B. J., Perkins, T. T., & Riley, T. V. (2015). Diversity and evolution in the genome of Clostridium difficile. *Clinical Microbiology Reviews, 28*, 721-741.

Knight, D. R., Thean, S., Putsathit, P., Fenwick, S., & Riley, T. V. (2013). Cross-sectional study reveals high prevalence of Clostridium difficile Non-PCR ribotype 078 strains in Australian veal calves at slaughter. *Applied and Environmental Microbiology, 79*, 2630-2635.

Kociolek, L. K., Espinosa, R. O., Gerding, D. N., Hauser, A. R., Ozer, E. A., Budz, M., Balaji, A., Chen, X., Tanz, R. R., Yalcinkaya, N., Conner, M. E., Savidge, T., & Kelly, C. P. (2019). Natural Clostridioides difficile Toxin Immunization in Colonized Infants. Clinical Infectious *Diseases, 70*(10), 2095-2102.

Kouassi, K. A., Dadie, A. T., N'Guessan, K. F., Dje, K. M., & Loukou, Y. G. (2014). Clostridium perfringens and

Clostridium difficile in cooked beef sold in CÔte d'Ivoire and their antimicrobial susceptibility. *Anaerobe, 28*, 90-94.

Kramer, A., Schwebke, I., & Kampf, G. (2006). How long do nosocomial pathogens persist on inanimate surfaces? A systematic review. *BMC Infectious Diseases, 6*.

Lawler, A. J., Lambert, P. A., & Worthington, T. (2020). A Revised Understanding of Clostridioides difficile Spore Germination. *Trends in Microbiology, 28*(9), 744-752.

Lawley, T. D., Croucher, N. J., Yu, L., Clare, S., Sebaihia, M., Goulding, D., Pickard, D. J., Parkhill, J., Choudhary, J., & Dougan, G. (2009). Proteomic and genomic characterization of highly infectious Clostridium difficile 630 spores. *Journal of Bacteriology, 191*, 5377-5386.

Lee, J. C., Hung, Y. P., Lin, H. J., Tsai, P. J., & Ko, W. C. (2016). *Clostridium difficile* infections in medical intensive care units of a medical center in Southern Taiwan: Variable seasonality and disease severity. *PLoS One, 11*, e0160760.

Lee, J. Y., Lee, D. Y., & Cho, Y. S. (2018). Prevalence of Clostridium difficile isolated from various raw meats in Korea. *Food Science and Biotechnology, 27*, 883-889.

Li, Z., Dong, N., Hao, J., Ouyang, Z., Qiang, C., Yang, Y., Mi, C., Niu, Y., Yang, J., Wen, B., Wang, L., Zhang, S., & Zhao, J. (2023). Clostridioides difficile infection in infants: a case report and literature review. *Gut Pathogens, 15*(1), 31.

Lyon, S. A., Hutton, M. L., Rood, J. I., Cheung, J. K., & Lyras, D. (2016). CdtR Regulates TcdA and TcdB Production in Clostridium difficile. *PLoS Pathogens, 12*.

McBee, R. H. (1960). Intestinal flora of some Antarctic birds and mammals. *Journal of Bacteriology, 79*, 311-312.

McDonald, L. C., Gerding, D. N., Johnson, S., Bakken, J. S., Carroll, K. C., Coffin, S. E., Dubberke, E. R., Garey, K. W., Gould, C. V., Kelly, C., Loo, V., Sammons, J. S., Sandora, T. J., & Wilcox, M. H. (2018). Clinical practice guidelines for *clostridium difficile* infection in adults and children: 2017 Update by the Infectious Diseases Society of America (IDSA) and society for healthcare epidemiology of America (SHEA). *Clinical Infectious Diseases/Clinical Infectious Diseases, 66*(7), e1-e48.

McFarland, L. V. (2007). Diarrhoea associated with antibiotic use. *BMJ, 335*, 54-55.

Metcalf, D., Avery, B.P., Janecko, N., Matic, N., Reid-Smith, R., & Weese, J.S. (2011). *Clostridium difficile* in seafood and fish. *Anaerobe, 17*, 85-86.

Metcalf, D.S., Costa, M.C., Dew, W.M.V., & Weese, J.S. (2010). Clostridium difficile in vegetables, Canada. *Letters in Applied Microbiology, 51*(4), 600-602.

Montazeri, N., Liu, D., & Janes, M. E. (2015). Occurrence of toxigenic Clostridium difficile in Louisiana Oysters (*Crassostrea virginica*) and environmental waters. *Food and Nutrition Sciences, 6*, 1065-1070.

Mooyottu, S., Flock, G., Kollanoor-Johny, A., Upadhyaya, I., Jayarao, B., & Venkitanarayanan, K. (2015). Characterization of a multidrug resistant *C. difficile* meat isolate. *International Journal of Food Microbiology, 192*, 111-116.

Nayebpour, F., & Rahimi, E. (2019). Prevalence, antibiotic resistance, and toxigenic gene profile of the Clostridium difficile isolated from molluscan shellfish. *Journal of Food Safety, 39*.

Norman, K. N., Harvey, R. B., Andrews, K., Hume, M. E., Callaway, T. R., Anderson, R. C., & Nisbet, D. J. (2014). Survey of Clostridium difficile in retail seafood in College Station, Texas. *Food Additives and Contaminants - Part A, 31*, 1127-1129.

Pasquale, V., Romano, V., Rupnik, M., Capuano, F., Bove, D., Aliberti, F., Krovacek, K., & Dumontet, S. (2012). Occurrence of toxigenic *Clostridium difficile* in edible bivalve molluscs. *Food Microbiology, 31*, 309-312.

Plants-Paris, K., Bishoff, D., Oyaro, M. O., Mwinyi, B., Chappell, C., Kituyi, A., Nyangao, J., Mbatha, D., & Darkoh, C. (2019). Prevalence of *Clostridium difficile* infections among Kenyan children with diarrhea. *International Journal of Infectious Diseases, 81*, 66-72.

Quesada-Gómez, C., Mulvey, M. R., Vargas, P., del M. Gamboa-Coronado, M., Rodríguez, C., & Rodríguez-Cavillini, E. (2013). Isolation of a toxigenic and clinical genotype of clostridium difficile in retail meats in Costa Rica. *Journal of Food Protection, 76*, 348-351.

Rahimi, E., & Khaksar, F. (2015). Detection of toxigenic Clostridium difficile strains isolated from meat and meat products in Iran. *Bulgarian Journal of Veterinary Medicine, 18*, 277-281.

Rahimi, E., Afzali, Z. S., & Baghbadorani, Z. T. (2015). Clostridium difficile in ready-to-eat foods in Isfahan and Shahrekord, Iran. *Asian Pacific Journal of Tropical Biomedicine, 5*, 128-131.

Razmyar, J., Jamshidi, A., Khanzadi, S., & Kalidari, G. (2017). Toxigenic Clostridium difficile in retail packed chicken meat and broiler flocks in northeastern Iran. *Iranian Journal of Veterinary Research, 18*, 271-274.

Rodriguez Diaz, C., Seyboldt, C., & Rupnik, M. (2018). *Non-human C. difficile reservoirs and sources: Animals, food, environment*. *In S. Hudson (Ed.),* Advances in Experimental Medicine and Biology, (pp. 227-243). New York, NY: Springer New York LLC.

Rodriguez, C., Avesani, V., Van Broeck, J., Taminiau, B., Delmée, M., & Daube, G. (2013). Presence of Clostridium difficile in pigs and cattle intestinal contents and carcass contamination at the slaughterhouse in Belgium. *International Journal of Food Microbiology, 166*, 256-262.

Rodriguez, C., Bouchafa, L., Soumillion, K., Ngyuvula, E., Taminiau, B., Van Broeck, J., Delmée, M., & Daube, G. (2019). Seasonality of Clostridium difficile in the natural environment. *Transboundary and Emerging Diseases, 66*, 2440-2449.

Rodriguez, C., Taminiau, B., Avesani, V., Van Broeck, J., Delmée, M., & Daube, G. (2014). Multilocus sequence typing analysis and antibiotic resistance of Clostridium difficile strains isolated from retail meat and humans in Belgium. Food Microbiology, 42, 166-171.

Rodriguez-Palacios, A., & LeJeune, J. T. (2011). Moist-heat resistance, spore aging, and superdormancy in

Clostridium difficile. *Applied and Environmental Microbiology, 77*, 3085-3091.

Rodriguez-Palacios, A., Ilic, S., & LeJeune, J. T. (2016). Subboiling moist heat favors the selection of enteric pathogen Clostridium difficile PCR Ribotype 078 spores in food. *Canadian Journal of Infectious Diseases and Medical Microbiology, 2016*, 1462405.

Rodriguez-Palacios, A., Mo, K. Q., Shah, B. U., Msuya, J., Bijedic, N., Deshpande, A., & Ilic, S. (2020). Global and historical distribution of clostridioides difficile in the human diet (1981–2019): Systematic review and meta-analysis of 21886 samples reveal sources of heterogeneity, high-risk foods, and unexpected higher prevalence toward the tropic. *Frontiers in Medicine (Lausanne), 7*, 9.

Rodriguez-Palacios, A., Reid-Smith, R. J., Staempfli, H. R., & Weese, J. S. (2010). *Clostridium difficile* survives minimal temperature recommended for cooking ground meats. *Anaerobe, 16*, 540-542.

Rodriguez-Palacios, A., Reid-Smith, R. J., Staempfli, H. R., Daignault, D., Janecko, N., Avery, B. P., Martin, H., Thomspon, A. D., McDonald, L. C., Limbago, B., & Weese, J. S. (2009). Possible seasonality of *Clostridium difficile* in retail meat, Canada. *Emerging Infectious Diseases, 15*, 802 -805.

Rui, W., Li, X., Li, Y., Meng, L., & Yang, J. (2024). Clostridioides difficile contamination in the food chain: Detection, prevention and control strategies. *Food Bioscience, 58*, 103680.

Rupnik, M., & Songer, J. G. (2010). Clostridium difficile: Its potential as a source of foodborne disease. *Advances in Food and Nutrition Research, 60*, 53-66.

Rupnik, M., Wilcox, M. H., & Gerding, D. N. (2009). Clostridium difficile infection: New developments in epidemiology and pathogenesis. *Nature Reviews Microbiology, 7*, 526-536.

Saad, J., Lendormi, T., Le Marechal, C., Pourcher, A. M., Druilhe, C., & Lanoiselle, J. L. (2023). Kinetic study of thermal inactivation of enterococci and clostridial spores. *MATEC Web of Conferences, 379*, 05004.

Shirley, D. A., Tornel, W., Warren, C. A., & Moonah, S. (2023). Clostridioides difficile infection in children: recent updates on epidemiology, diagnosis, therapy. *Pediatrics, 152*(3). 1-13.

Song, J. H., & Kim, Y. S. (2019). Recurrent *clostridium difficile* infection: Risk factors, treatment, and prevention. *Gut and Liver, 13*(1), 16-24.

Songer, J. G., & Anderson, M. A. (2006). Clostridium difficile: An important pathogen of food animals. *Anaerobe, 12*, 1-4.

Songer, J.G., Trinh, H.T., Killgore, G.E., Thompson, A.D., McDonald, L.C., & Limbago, B.M. (2009). Clostridium difficile in retail meat products, USA, 2007. *Emerging Infectious Diseases, 15*, 819-821.

Stoesser, N., Eyre, D. W., Quan, T. P., Godwin, H., Pill, G., Mbuvi, E., Vaughan, A., Griffiths, D., Martin, J., Fawley, W., Dingle, K. E., Oakley, S., Wanelik, K., Finney, J. M., Kachrimanidou, M., Moore, C. E., Gorbach, S., Riley, T. V., Crook, D. W., Peto, T. E. A., Wilcox, M. H., & Walker, A. S. (2017). Epidemiology of Clostridium difficile in infants in Oxfordshire, UK: Risk factors for colonization and carriage, and genetic overlap with regional *C. difficile* infection strains. *PloS One, 12*(8), e0182307

Tkalec, V., Jamnikar-Ciglenecki, U., Rupnik, M., Vadnjal, S., Zelenik, K., & Biasizzo, M. (2020). *Clostridioides difficile* in national food surveillance, Slovenia, 2015 to 2017. *Eurosurveillance, 25*.

Troiano, T., Harmanus, C., Sanders, I.M.J.G., Pasquale, V., Dumontet, S., Capuano, F., Romano, V., & Kuijper, E.J. (2015). Toxigenic Clostridium difficile PCR ribotypes in edible marine bivalve molluscs in Italy. International *Journal of Food Microbiology, 208*, 30-34.

Usui, M., Maruko, A., Harada, M., Kawabata, F., Sudo, T., Noto, S., Sato, T., Shinagawa, M., Takahashi, S., & Tamura, Y. (2020). Prevalence and characterization of Clostridioides difficile isolates from retail food products (vegetables and meats) in Japan. *Anaerobe, 61*, 102132.

Varshney, J. B., Very, K. J., Williams, J. L., Hegarty, J. P., Stewart, D. B., Lumadue, J., Venkitanarayanan, K., Jayarao, B. M. (2014). Characterization of Clostridium difficile isolates from human fecal samples and retail meat from Pennsylvania. *Foodborne Pathogens and Disease, 11*, 822-829.

Von Abercron, S. M. M., Karlsson, F., Wigh, G. T., Wierup, M., & Krovacek, K. (2009). Low occurrence of Clostridium difficile in retail ground meat in Sweden. *Journal of Food Protection, 72*, 1732-1734.

Weese, J. S., Avery, B. P., Rousseau, J., & Reid-Smith, R. J. (2009). Detection and enumeration of Clostridium difficile spores in retail beef and pork. *Applied and Environmental Microbiology, 75*, 5009-5011.

Weese, J. S., Reid-Smith, R. J., Avery, B. P., & Rousseau, J. (2010). Detection and characterization of Clostridium difficile in retail chicken. *Letters in Applied Microbiology, 50*, 362-365.

Worth, L.J., Spelman, T., Bull, A.L., Brett, J.A., & Richards, M.J. (2016). Epidemiology of Clostridium difficile infections in Australia: Enhanced surveillance to evaluate time trends and severity of illness in Victoria, 2010-2014. *Journal of Hospital Infection, 93*, 280-285.

Zilberberg, M. D., Shorr, A. F., & Kollef, M. H. (2008). Increase in adult Clostridium difficile-related hospitalizations and case-fatality rate, United States, 2000-2005. *Emerging Infectious Diseases, 14*, 929-931.

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CRISPR-Cas technology and use in antiviral development

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ABSTRACT

Throughout history, viral diseases have periodically reached pandemic proportions and have had devastating effects on human history. With the advancement of science and technology, antivirals have been developed and continue to be developed in the fight against viral diseases. The difficulty in the development of antirival has tried to use new technologies in the development of antiviral. One of these new technologies is the CRISPR/Cas system. CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) defines a series of DNA sequences called clusters of regularly interspaced palindromic repeats, and CAS defines endonucleases that use CRISPR sequences as a guide to recognize and cut specific DNA chains related to the CRISPR region. While protein engineering systems defined before CRISPR/Cas systems can be off-target and cause undesirable results, the CRISPR/Cas system reduces this risk by Watson-Crick base pairing. In the fight against viral infections of humans and animals, vaccine protection methods are widely used due to the problems in developing antivirals. On the other hand, the difficulty of vaccination, inadequacies in long-term immunity and the emergence of new infections or epidemics due to mutational changes in viruses pave the way for developing new antivirals. This article emphasizes the history and working areas of CRISPR-Cas technology and the potential applications of this method in antiviral development for human and animal viruses.

Keywords: CRISPR-Cas technology, antivirals, antiviral development

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Introduction

CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) refers to a series of DNA sequences called clusters of regularly interspaced palindromic repeats, CAS refers to endonucleases that use CRISPR sequences as a guide to recognise and cut specific DNA strands related to the CRISPR site. Before the discovery of CRISPR, RNA-targeted nucleases, zinc finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs) or self-directed meganucleases were widely used in protein engineering (Bogdanove and Voytas, 2011; Jinek et al., 2012 as cited in Gök and Tunalı, 2016). Although these methods are successfully applied, the proteins produced can sometimes cause off-target effects and toxic effects. On the other hand, CRISPR technology is based on simple Watson-Crick base pairing, which reduces the risks in different techniques. In the fight against viral infections of humans and animals, vaccine protection methods are widely used due to the problems in developing antivirals. On the other hand, the difficulty of vaccination, inadequacies in long-term immunity, and the emergence of new infections or epidemics due to mutational changes in viruses pave the way for developing new antivirals. In this article,

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developing new antivirals. In this article, the history and working areas of CRISPR-Cas technology and potential applications of this method in antiviral development are emphasized.

History of CRISPR-CAS technology

Firstly Ishino et al.(1987), while doing a gene sequencing study on *Escherichia coli*, noticed these gene regions repeating at certain intervals, but he could not make sense of these regions. In the following years, Mojica also noticed these repetitive gene regions in the 1990s and 2000 Mojica et al. named these regions SRSRs (Short Regularly Spaces Repeats) and focused his studies on SRSRs (F. J. Mojica et al., 1993, 1995, 2000).

Jansen et al. (2002) with Mojica et al. named these repetitive regions CRISPR. In this in-slico study, the genomes of more than 40 microorganisms were sequenced and as a result of these sequences, they found that CRISPR regions can be more than one and that there is a protein region following these CRISPR regions (CRISPR-Cas). Cas regions were found to bind to helicases, ligases and DNA.

In 2005, Mojica et al. proposed the idea that these repetitive regions are an immune site against phages in microorganisms (Mojica et al., 2005).

Various studies have been carried out around the world to find out the importance of CRISPR. As a result of these studies, it was discovered that these repetitive regions originated from bacteriophage. With this discovery, it started to be investigated whether CRISPR regions are related to the immunity of bacteria.

Barrangou et al., (2007) in their study on *Streptococus thermophilus*, as a result of their sequences, it was observed that as the number of CRISPR regions increased in the genome of the bacterium, the bacterium showed resistance to phages and did not die.

Since 2010, the basic mechanism of the CRISPR system has been investigated and revealed by various scientists. Doudna and Carpentier received the Nobel Prize in 2020 for their work on the mechanism of CRISPR, which largely revealed the functioning of the system. In almost the same period as Doudna and Carpentier, Siksyns also found the working principle of CRISPR and these three scientists received the Nanosience Prize (Cross Ryan, 2018; Nobel Prize, 2020).

During this period, studies have been carried out to reveal the CRISPR mechanism, defining the functional mechanisms of the CRISPR Type II system, the simplicity of this system for genome editing and the basic components. In a study on Streptococcus *thermophilus*, Cas9 was found to be the only enzyme in the cas gene clusters that enables the cutting of target DNA (Garneau et al., 2010).

Deltcheva et al. (2011) revealed trans-activating crRNAs (tracrRNAs). TracrRNAs are RNA hybrids formed from Cas9 and endogenous RNA III and are required for transcription of the CRISPR sequence into mature crRNAs in the CRISPR Type II system (a CRISPR system using Cas9).

Sapranauskas et al. (2011) transplanted the CRISPR Type II region from *Streptococus thermophilus* into *Esherichia coli*, demonstrating that type II CRISPR is transferable and can be rearranged in different bacterial strains.

In 2013, the CRISPR-Cas9 system started to be used in genome editing with the successful use of Streptococus thermophilis and *Streptecocus pyogenes* for editing mammalian cells with CRISPR in two separate studies (Cong et al., 2013).

In their paper, Ran et al. (2013), showed that CRISPR could be used to identify the mammalian genome.

CRISPR-CAS system

In fact, CRISPR-CAS is a defence mechanism that bacteria and some archaea involved in fermentation etc. develop against phages coming from outside. Studies have shown that this defence mechanism is provided by CAS proteins in the bacterial genome. (van der Oost et al., 2009) (Bikard and Marraffini, 2012).

In short, Cas (CRISPR-associated protein) in the CRISPR-Cas system can also be called the adapted antiviral immune system of prokaryotes. Cas is widespread in archaea (~90%) but is also present in some bacteria (~50%) (Bayat et al., 2018).

CRISPR sequences are formed by the insertion of foreign genomes, foreign genome products, transplanted products of non-repeating sequences into repetitive sequences; certain parts of the genetic material of the phage infecting bacteria or archaea are integrated into CRISPR sites together with repetitive regions (Gök and Tunalı, 2016).

A bacterial or archaeal genome may contain different CRISPR regions. While the repeat sequences are around 21-48 base pairs (bp), the gaps, which vary from a few to several hundred, are 26-72 bp in length. At the 5' end of the first repeat sequence in the CRISPR region, there is a leader sequence rich in Adenine and Thymine. This leader sequence is approximately 550 bp in bacterial genomes. A genome may contain single or multiple CRISPR sites (Jansen et al., 2002; Pourcel et al., 2005; Rath et al., 2015; as cited in Kılıç Tosun and Kesmen, 2022).

The genomic components of the CRISPR system

are formed by trans-activated crRNA (tracrRNA), which is formed by short direct repeat sequences led by the cas protein. In between these repeat regions, gaps are formed from non-repeating regions. These spacer regions are mainly composed of invasive elements of the virus or plasmid. The CRISPR-Cas system provides the organism with resistance to foreign genetic material that has already been transferred to the CRISPR site in the organism's genome. (Bayat et al., 2018). This immune system consists of three stages:

1) Gap fragments obtained from exogenous nucleic acid are inserted into the CRISPR site and adaptation is achieved. The gaps to be inserted are determined by protospacer adjacent motifs (PAM) in the genome of the invading phage or plasmid. PAM is specifically recognised. These conserved sequences of 2-5 nucleotides of the genome of the invading microorganism are adapted to the CRISPR site by repeating genes in the spacer portion of the invading genome. The lack of PAM in the CRISPR region prevents the region from being cut, mutations in the PAM region can cause the invading microorganism to escape from CRISPR and thus from the host organism's immunity. (Jiang and Doudna, 2015)

2) The target region in the DNA of the invading microorganism is inserted into the CRISPR site and transcribed into pre-CRISPR RNAs, the transcribed pre-crRNA Cas endoribonucleases are converted into cr-RNAs corresponding to the invader genome, showing base pairing with the target sequences.

3) In the last step, nucleic acids belonging to the invader genome are targeted with crRNA, homologous sequences are cut with Cas nucleases, thus preventing the replication of viruses and plasmids. Nucleic acids of the invader genome are targeted according to the Watson-Crick base pairing principle (Gök and Tunalı, 2016)

Types of CRISPR system

The components of CRISPR are basically divided into two parts. The first is the Cas enzyme, which cuts the DNA strand at specific locations in the genome. The second is Guide RNA (gRNA), which drives the Cas protein to the target region of the genome.

The widely used classification was developed by Haft et al. in 2005 using the topology of the Cas1 phylogenetic tree on around 40 archaea and bacteria and CRISPR-Cas system typing on eight genomes. (Haft et al., 2005)

The names of the four core Cas genomes were proposed by Jansen et al. in 2002. The other two core Cas gene names Cas 5 and Cas 6 were added later. The names were proposed for genes encoding proteins unique to each of the eight genes. For example, a unique system found in *E. coli* was found and named cse1 (CRISPR system of *E. coli* gene number 1), cse2, cse3, cse4 and cse5 (elsewhere these *E. coli* genes have also been named casA, casB, casE, casC, casD, but this difference has caused confusion).

Although the diversity of Cas proteins, the presence of different CRISPR regions in a genome and the ability to switch between living organisms make classification difficult, according to the organisation of the CRISPR region and the content of Cas genes, the CRISPR-Cas system is basically divided into three main systems and 11 subsystems. Apart from these, there are also Unclassified CRISPR-Cas Systems.

Type I CRISPR-Cas System: Type I: CRISPR-Cas systems contain 6 subsystems from 1A to I-F. Type I-CRISPR-Cas system gene region, typically the cas3 gene. This gene synthesises a wide range of proteins with helicase DNAase activities. In addition, these genes probably also synthesise Cascade-like complexes of different composition. These complexes include proteins in the RAMP (receptor-activated regulatory protein) superfamily, including many Cas5 and Cas6 family proteins. Cas7 protein was also detected by HHPred method. The CRISPR-Cas function contained in these complexes includes large proteins such as Cse1 as well as small alpha-helicase proteins or subunits such as Cse 2. In the cascade complex there is a RAMP protein with RNA endonuclease activity, catalase activity, and a defined main enzyme activity that transcribes long gap- repeat segments into mature cr-RNA. Mostly catalytic RAMP proteins are peripherally encoded by the respective operon; Cas5 and Cas7 do not belong to RMAP families. However, subtype I-C (also known as Dvulg or CASS1) may be an exception for the RNAase activity of Cas5 and Cas7 (Makarova et al. 2011). Type-I CRISPR-Cas systems appear to target DNA. Target cleavage is mediated by Cas3-based HD nuclease activity. In several type-I CRISPR-Cas systems, the Cas4 domain RecB nuclease fuses with Cas1. Cas4 is potentially involved in cavity acquisition. (Makarova et al. 2011) Basically, in the type-I CRISPR-Cas system, cascade and Cas3 cut foreign DNA. mature crRNAs are produced from pre-crRNAs via Cas6. Cas6 performs the cutting of repeat portions of pre-cRNAs. (Gök and Tunalı , 2016)

Type II CRISPR-Cas System: Type II: CRISPR-Cas systems include 3 subsystems, II-A to II-C. Type II system is the system whose mechanism is the most researched and known in detail among CRISPR-Cas systems (Gök and Tunalı, 2016). Type II CRISPR-Cas systems include the "HNH"-type system (Streptococcus-like; also known as Nmeni subtype, Neisseria meningitidis serogroup A str. Also known as. Z2491, or also called CASS4,). It contains a very large

protein containing Cas9, which produces crRNA and cleaves target DNA. Cas1, Cas2 and Cas9 contain at least two nuclease domains. The RuvC-like nuclease domain is located near the amino terminus and the HNH (McrA-like) nuclease domain is located in the centre of the protein. The HNH nuclease domain contains abundant restriction enzymes and has endonuclease activity and is responsible for cutting target DNA (Makarova et al., 2011). The type 2 system cleaves the double formation between pre-crRNA and tracrRNA. The first cleavage occurs at repeat sites in the processing pathways of pre-crRNA. This cleavage is catalysed by hausekeeping. It is mediated by the double-stranded RNA-specific RNAase 3 in Cas9. (Deveau et al., 2008).

In the type II system, the endonuclease cas9 together with a non-coding RNA combines with CRISPR RNA (crRNA) to form a ribonucleoprotein complex that recognises and cuts the foreign genome. (Rath et al., 2015).

Type III CRISPR-Cas system: Type III: CRISPR-Cas systems contain 2 subsystems, III-A to III-B. The type III system contains polymerase and RAMP modules that perform cascade complex-like transcription by processing the gap- repeat complex. Ribonucleases identified in the type III system (except cas2 protein) are RAMP proteins. Type III systems contain at least two RAMPs in addition to the Cas6 protein. These additional RAMP proteins are involved in the trancription process. In many organisms, type III CRISPR-Cas operons lack the Cas1 - Cas2 gene pair. However, in most cases an additional CRISPR locus is present and this additional locus comes from Cas1 or Cas2. It is thought that these Cas1 or Cas2 genes existing in the relevant genome were added in trans. In type III CRISPR-Cas systems of *Staphylococcus epidermidis*, *Mycobacterium tuberculosis*, *Alorhodospira halophila*, it is suggested that there is a single CRISPR-Cas locus. It is suggested that this locus (polymerase-RAMP module) combines with Cas1 and Cas2 and takes part in the formation of new cavities with full function. The production of small mature crRNAs in the type 3 CRISPR-Cas system is based on cutting the repeated sequences of pre-crRNAs into crRNAs with the Cas6 nuclease family. The resulting crRNA forms a complex with Cmr/Cas10 or Csm/Cas10 proteins and the Cas protein in the complex cuts the invading genome.

Unclassified CRISPR-Cas systems: Although most of the CRISPR-Cas systems found are classified up to subclasses, there are also systems that do not fit the existing classification. For example, the CRISPR-Cas system of *Acidithiobacillus ferrooxidans* needs a new class. And the name Type U was proposed by Makarova et al. This CRISPIR system was later referred to as the putative type IV CRISPR/Cas system in the 2015 paper by Makorova et al. In many bacteria and in the identified cpf1, the archival genome adjacent to Cas1, Cas2 and the CRISPR locus (e.g. cf. noncida Fx1 at the FNFX1 1431-FNFX1 1428 locus of Francisella) was named as the putative type V CRISPR/Cas system by Makrova et al. In the same paper, it was proposed to name CRISPR/Cas systems as Class 1 and Class 2. Again in 2015, in the classification of Class-2 CRISPR/Cas system by Shmakov et al. (2015) type II, type V were included in this class and the C2c2 gene locus synthesised by *Listeria seeligeri serovar* 1/2b str and expressed by *E. coli* was named as type 6 CRISPR/cas system and included in Class 2 CRISPR/Cas system. Class 1 CRISPR/Cas system type I, type III and type IV CRISPR/Cas system were also identified (Mohanraju et al., 2016).

CRISPR scans

In the development of antiviral agents, knowing the interactions, biology and reactions of the virus and host is important for the design of the studies to be carried out. At this point, CRISPR screening provides a great advantage for investigating the host and viral agent for these purposes. Genetic screening is one of the gold standards for finding host factors that restrict or promote viral infection. There are two types of advanced genetic screening. These are loss-of-function screening and gain-of-function screening. Loss-of-function screening is the most commonly used of these two screens (Puschnik et al., 2017; Chulanov et al.,2021). CRISPR screens are divided into 3 categories according to their mechanisms of action: CRISPRi, CRISPRa and CRISPR knockout (CRISPR-ko) screens (Chulanov et al. 2021). CRISPRi and CRISPR-ko screens are loss of function approaches. CRISPR-ko is classically based on the CRISPR-Cas9 system. This classical system, results in DBSs and indel mutations, or codons are converted into stop codons by cytidine-based regulators, enabling the production of truncated non-functional proteins (CRISPR-STOP or iSTOP approaches (Billon et al., 2017; Kuscu et al., 2017;as cited in Chulanov et al., 2021)

CRISPR screening is performed not only for the host but also for viral protein synthesis in viral replication. For example, Hoffmann et al (2021). utilised CRISPR analysis to examine the SARS-CoV-2 interactome in cells infected against COVID-19. For this, they specified 332 highly comprehensive, identified SARS-CoV-2 interactomes in the CRISPR-Cas9 library and designed targets. They stated that

thanks to the structure of the library, they screened on four related viruses. As a result of this study, they screened in HCoVNL63, HCoV-229E and HCoV-OC43 infection models to search for pan-coronavirus factors necessary for replication, and sterol regulatory element binding protein division activating protein (SCAP) was identified as the host factor important for the replication of all four coronaviruses. In healthy cells, SCAP regulates lipid and cholesterol haemostasis through secretion of binding proteins by sieving sterol regulation in the endoplasmic reticulum. It has been reported that SCAP may promote coronavirus infection by increasing SREBPs-dependent transport or cholesterol content in the cell membrane and increasing viral interaction.

Use of CRISPR-CAS Systems in antiviral field

The gene editing application of the CRISPR-Cas system was used in the development of antiviral therapies. It then revolutionized diagnostics as a gene detection system. At this point, CRISPR diagnostics enables accurate and rapid identification of any pathogen in clinical settings. CRISPR-Cas system is being studied for the development of new treatments against many viruses.

HIV and the CRISPR-Cas system

To date, the CRISPR-Cas system has been studied mostly for the treatment of HIV infections and today serves as an advanced treatment option. (Huang et al., 2017, 2022; Xu et al., 2019; Herrera-Carrillo et al., 2020 as cited in Kılıç Tosun and Kesmen, 2022)

In a study by Park et al. (2017) on HIV-1 (Human immunodeficiency virus-1), genome-wide CRISPR screening was performed to identify host factors and five factors, including HIV co-receptors CD4 and CCR5, were identified. Candidate pathways were validated by Cas9-mediated knockdown and antibody blockade in primary human CD4+ T cells.

Ophinni et al. (2018) reported that they inhibited HIV-1 replication by targeting Tat and Rev genes in HeLa cells with CRISPR/Cas9 vector.

In their 2016 study on HIV-1, Ueda et al. (2016) reported that the vector targeting the gag, pol and long terminal replication of HIV-1 with CRISPIR/Cas 9 was effective in the early stages of HIV-1 infection in the human T-cell line, but the vector was insufficient in the inhibition of wild-type (WT) HIV-1. They evaluated this inadequacy as a point to be considered when developing CRISPR-Cas system and treatment against HIV-1.

Xu et al. (2019) transplanted haematopoietic tissue and progenitor cells (HSPCs) with CCR5 protein with CRISPR/Cas9 system to an individual with acute lymphocytic leukaemia and HIV and treated lymphocytic leukaemia and HIV in the patient. In the study conducted by Jin et al. on HIV-1 in 2018, it was stated that they found two important genes (TSC1 and DEPDC5) that play a role in HIV-1 latency in their CRISPR screening study on HIV-1 latency. They stated that inactivation of TSC-1 or DEPDC5 genes increases reactivation in both T-cell line and monocyte cell line, and in general, both TSC1 and DEPDC5 agonists can be used in the development of new therapeutic approaches to activate HIV-1 latency.

In another study on HIV-1 latency, Z. Li et al. (2020) used genome-wide CRISPRi screening to show that inhibition of FTSJ3, TMEM178A and NICN1 is effective. They stated that these genes stimulate RNA polymerase II-mediated transcription of HIV and increase its latency. In other immunoprecipitation experiments performed in the study, it was reported that depletion of TMEM178A and NICN1 increased poimerase II signalling in the HIV-1 envelope region, but not in the long term rpeat (LTR) region. Mandan et al. developed CRISPR/Cas9 vectors targeting two clinically important genes (B2M and CCR5) against HIV-1 in primary human CD4+ T cells and CD34+ hematopoietic system and progenitor cells (HSPCs) to develop CRISPR-Cas9 system against HIV-1, and it was observed that the vector targeting CCR5 was 30% effective in HSPC cells (between 22-44%); It was reported that the success of the vector targeting B2M varied between 7-48%. When HSPC cells with inactivated CCR5 protein were transplanted into mice, they reported that the transplanted HSPC clones were resistant to HIV1.

In their study on HIV-1, McLaurin et al. (2024) showed that the CRISPR/Cas9 system they developed against HIV-1 mRNAs reduced the neurocognitive effect of HIV-1 in invitro and in vivo conditions.

In their study on HIV-1 by Liao et al. (2015) targeted the repeat regions of the viral genome in HIV-infected CD41T cell culture with CRISPR-Cas9 and as a result, viral replication and latency decreased. At the same time, HIV reservoir was obtained in pluripotent stem cells and CRISPR/Cas9 vector targeting the repeat regions of the HIV genome was transferred and it was reported that the new reservoir cells formed as a result were immune to HIV.

Coronaviruses and the CRISPR-Cas systems

The study conducted by J. Wei et al. (2021) is to find therapeutic pathways for SARS-CoV-2, SARS-CoV-2, Middle East respiratory syndrome CoV (MERS-CoV), They performed genome-wide CRISPR screens in Vero-E6 cells containing bat CoV HKU5 and vesicular stomatitis virus (VSV)-containing Vero-E6 cells

expressing SARS-CoV-1 enhancement, and identified known SARS-CoV-1 receptors, including the ACE2 receptor and the protease Katepsin L.2 host factors, in addition to discovering pro-viral genes and pathways specific to the SARS lineage and pan-coronavirus, including HMGB1 and the SWI/SNF chromatin remodeling complex, and that HGM1 regulates ACE2 expression, and found that it is critical for SARS-CoV-1 and SARS-CoV-2 entry. It was reported by Daniloski et al. that depletion of RAB7A decreased the cell surface expression of ACE2.

Abbott et al. (2020) stated that they developed PAC-MAN (prophylactic antiviral CRISPR in human cells) strategy and investigated its antiviral activity on SARS-CoV-2 and live influenza A virus (H1N1) and that this strategy developed with Cas13d protein reduced the load of H1N1 virus in respiratory epithelial cells and that six of the crRNAs they developed were effective against SARS-CoV-2, and that this system is promising for the inhibition of pan-coronaviruses.

Cas13 is a Cas protein widely used in genome editing. It is used in the type VI CRISPR/Cas system. Since it targets RNA, it is especially used in viruses with RNA (Xie et al., 2021; Zhang et al., 2021 as cited in Kılıç Tosun and kesmen, 2022).

Arboviruses and the CRISPR-Cas systems

Ganaie et al.(2021) used a CRISPR/Cas9 library for the mouse microgial cell line BV-2 cell line for Rift Valley Fever Virus (RVFV) and identified lipoprotein receptor-related protein 1 (Lpr1) protein, heat shock protein (Grp94) and receptor associated protein (RAP) as possible antiviral targets; They stated that the RVFV genome binds specifically to the Lpr1 protein and when they transduced the line with lentivirus with a single-guided gRNA targeting the Lpr1 gene, they found that the remaining cells were resistant to RVFV. Thus, it was stated that Lpr1 protein is an important host factor against RVFV.

In the study on human orovirusvirus (HCMV), Wu et al., (2018). Showed that PDGFRα as a host-dependent factor is important for trimer-mediated entry of HCMV into the cell and trimer-mediated passage of HCMV from cell to cell and that pentamer-coated viruses have low efficiency in PDGFRRα-deficient cells by CRISPR tracking study. In another study on HCMV, they showed that OR1411 is an important co-receptor for the HCV pentameric complex, which is related to the sensitivity orovirlial cells to pentamer HCMV in OR1411 protein-mediated infection (Chulanov et al., 2021).

In their study on Zika virus (ZV), dengue virus (DENV) and West Nile virus (WNV), Richardson et al. found that the functional gene pair between IFI 6 (encodes IFN-α inducing protein) is important for the inhibition of replication of Flaviviruses by CRISPR screening and showed that IFI 6 inhibits the replication of viruses invitro with the CRISPR-Cas system (Richardson et al., 2018).

van Diemen et al. (2016) has been shown that when Epstein-Barr virus (EBV) remains latent in cells, the latent EBV genome can be edited with the CRISPR-Cas system.

Lin et al. (2017) reported that in their CRISPR screen for host factors for Dengue virus (DENV), they identified the oligosaccharyltransferase (OST) complex as an essential host factor for DENV infection. However, the STT3B-associated OST subunit MAGT1 is also required for DENV propagation. MAGT1 expression requires STT3B and a catalytically inactive STT3B also enables MAGT1 expression, supporting the hypothesis that STT3B serves to stabilise MAGT1 in the context of DENV infection. Since cells expressing an AXXA MAGT1 mutant were unable to support DENV infection, and found that the oxidoreductase CXXC active site motif of MAGT1 is required for DENV propagation; cells expressing single cysteine CXXA or AXXC mutants of MAGT1 were able to support DENV propagation. Using the engineered peroxidase APEX2, they demonstrated the proximity between MAGT1 and NS1 or NS4B during DENV infection. They stated that these results revealed that the oxidoreductase activity of STT3B-containing OST is required for DENV infection and that this could guide the development of antiviral agents targeting DENV.

Labeau et al. (2020) similarly used CRISPR scanning to identify the host factor for DENV and identified two endoplasmic reticulum-resistant dolichol-phosphate mannose synthase (DPMS) complex subunits, DPM-1 and 3. They also found that DPMS complexes are important in regulating viral RNA replication and supporting the stability of folding of viral structural proteins.

Enteric viruses and CRISPR-Cas systems

Orchard et al., (2019) stated that they found 49 genomes that would prevent murine norovirus proliferation in human cells in their CRISPR-Cas scan. Hosmillo et al. (2019) stated that they identified G3BP1 as an important host factor for human noravirus and murine noravirus as a result of their CRISPR screening study. They identified G3BP1 protein as an important host factor for VPg-dependent translation of noravirus.

Ding et al. (2018) reported that STAG2 is an important part of the cohesin complex, an important nuclear protein complex that coordinates the sister chromatid during cell division and is an important

element of the replication of human rotavirus (HRV) in the cell.

Other viruses and the CRISPR-Cas systems

In a study on human papillomavirus (HPV) conducted by Kennedy et al. in 2014, the effects of vectors prepared with Streptococus pyogenes Cas9 protein targeting E6 and E7 genes of HPV-16 and HPV-18 were investigated and HeLa and SiHa cell cultures were used for this purpose. As a result, it was reported that the designed vector did not affect the E6 gene of HPV-18, HPV-16 affected the E6 gene and both viruses affected the E7 gene at a significant level (Kennedy et al., 2014).

Zhen et al. (2014) conducted invitro (SiHa cell line was used) and in vivo (nude mice were used) studies on HPV and found that CRISPR-Cas9, which they developed to target the E6 and E7 trancrept of HPV-16, significantly reduced the proliferation of HPV-16 both invitro and in vivo.

In the article written by Y. Wei et al. (2022) various studies for the treatment of HPV with CRISPR/ Cas9 technology are seen.

In a study conducted by Chou et al. (2016) on John Cunningham Polyomavirus (JCPyV), it was reported that CRISPR/Cas9 targeted the non-coding control region and the late open reading frame in the genome of JCPyV and observed that the administration of JCPyV-specific single-guide RNA Cas9 protein before or after infection significantly reduced virus replication and protein formation. In 2015, in a study on John Cunningham Poliomavirus, the N-terminal region of the T-antigen gene was targeted for CRISPR-Cas9 and it was reported that plasmid-mediated mutation in this region inhibited viral replication invitro (Wollebo et al., 2015).

In the study conducted by Roehm et al.,(2016) on Herpes Simplex Virus-1 (HSV-1) in 2016, it was reported that the virus developed a guide-mediated CRISPR-Cas9 system targeting the genome associated with the ICP0 protein of the virus and provided InDel mutation to the exon 2 region of the ICP0 genome and reduced the infection in an invitro environment. When this system for the ICP0 gene was combined with the version for ICP4 or ICP27, it was observed that the infection was completely eliminated.

In the study conducted by Das et al. (2020) , it was found in CRISPR screening that gangliosides are an important endosomal receptor for semi-enveloped or naked (non-enveloped) Hepatitis A Virus (HAV). This has revealed a point that can be used for antiviral strategies against HCV.

Animal Viruses and CRISPR-Cas systems

Marek and CRISPR-Cas Systems: Zhang et al. (2019) showed that the viral gene phosphoprotein 38 (pp38) is important in the latent/litic phase transitions of Marek's disease virus (MDV). It was emphasised that proliferation increased when pp38 was transfected with CRISPR/Cas9. This finding suggests that pp38 is a potential target for antiviral drugs to be developed against Marek.

In the study conducted by Luo et al. (2020) on MDV-1 , it was stated that virus-encoded micro-RNAs (miRNAs) play an important role in the latency, replication, etc. phases of herpesviruses, and they stated that if the Meq-cluster miRNAs of MDV-1 were interfered with CRISPR-Cas9, the replication of the virus decreased. They stated that deletion of miRNAs in the middle-cluster increased viral replication.

In the study conducted by Senevirathne et al. (2021), it was reported that when the pp38 gene of MDV was targeted with CRISPR-Cas9 using Salmonella spp. as a plasmid, the effectiveness of the plasmid in the spleen was seen between 1.7-13%, 1.8-8% in the spleen, and the highest effect was seen in chickens treated with plasmid treatment before MDV infection. This suggests that CRISPR-Cas9 application may be an effective treatment option against MDV when given according to the course of the disease.

In the study conducted by Teng et al. (2023) , CIRISPR-Cas9 study was performed with hybridoma technology on the Meq gene, which is effective in the oncogenic feature of MDV, and as a result of the study, 5 Meq-deleted hybridoma MDV-1 were obtained.

In a study conducted by Li et al. (2020) , they used MDV as a vector to create a vaccine against Reticuloendotheliosis virus (REV) and showed that this recombinant strain developed using CRISR-Cas9 significantly reduced the REV load.

Similarly, Liu et al. (2020) programmed MDV with CRISPR-Cas9 as a target for avian leukosis virus subgroup J (ALV-J) in 2020 and showed that the MDV strain obtained was effective in the resistance of the host cell against ALV-J and emphasised that MDV could be an important vector in CRISPR-Cas technology.

Bovine herpes virus (BHV) and CRISPR-Cas systems

In the study conducted by Dai et al. (2022), they stated that when they interrupted the UPL-41 protein of the BHV with CRISPR-Cas9 technology, they found that the proliferation of the virus in the host cell decreased. It has also been tried to develop a vaccine for BHV using CRISPR-Cas technology. In the study conducted by Ma et al. (2023), BHV-1's glycoprotein I, glycoprotein E, TK gene and UL-23 genes were targeted with CRISPR-Cas to create a vaccine strain

and Pseudorabies virus (PRV) was also included in the study and it was stated that CRISPR-Cas9 technology could be effective in the development of multivalent vaccines.

In their study conducted by Zhao et al. (2022). showed that BHV can be used as a vector for developing a vaccine for rabies virus. For this, they added rabies virus glycoprotein-g (RABVG) to BHV-1 virus by interfering with CRISPR-Cas technology. They stated that they observed that the recombinant BHV-1 had a protective effect against severe fatal infection in mice after 20 passages.

Yu et al. (2024) also studied gene editing on BHV and pseudo rabies virus (PRV) with CRISPR-Cas9 system in their study in 2024. In the study, thymidine kinase (TK) gene of PRV or glycoprotein I (gI) and glycoprotein E (gE) of BHV were targeted. With this approach, recombinant TK-/eGFP+ PRV and gIgE-/ eGFP+ BHV-1 mutants were generated and then characterised and their invitro and invivo biological activities were examined. As a result, it was reported that alpha herpes virus, including PRV and BHV-1, can be rapidly edited using the CRISPR/Cas9 approach and may contribute to the development of animal herpes virus vaccines.

Canin distemper virus (CDV) and CRISPR-Cas systems

In the study conducted by Cai et al. (2019) it was suggested that cell lines with mavs (mitochondrial antiviral signalling) activity produced with CRISPR-Cas technology could be used in the development of CDV vaccine.

Gong et al. (2020) reported that they developed a highly efficient recombinant canary pox virus containing CDV virus-like particles (VLPs) called "ALVAC CDV-M-F-H/C5-" with CRISPR/Cas9 technology, which enabled simultaneous expression of matrix (M), H and F genes.

Gradauskaite et al. (2023) stated in their study in 2022 that LPR6 is an important receptor for CDV and will be important in the development of attuned vaccines. They stated that when they silenced the LPR6 receptor in cells with CRISPR-Cas9, they eliminated cell entry in multiple cell lines and lost infectivity in LRP6KO cells pseudotyped with CDV-OP envelope glycoproteins after transfer to recombinant viral particles and vesicular stomatitis virus (VSV) and that the study identified LRP6 as the long-sought cell entry receptor of CDV OP in multiple cell lines.

Equine arteritis virus (EAV) and CRISPR-Cas systems

de Wilde et al. (2018) investigated the proliferation of EAV, human coronavirus 229E (HCoV-229E), and betacoronavirus Middle East respiratory syndrome coronavirus (MERS-CoV) by deleting the Cyclophilin A (CypA) receptor in Huh7 cells with CRISPR-Cas9 . As a result of the research, they stated that the proliferation of EAV and MERS-CoV in CypA deleted cell lines decreased around 3log.

Equine Herpesvirus (EHV) and CRISPR-Cas Systems

In the study conducted by Hassanien et al. (2024), they developed single-guide RNAs targeting ORF30, ORF31, ORF74 and ORF7 regions of EHV with CRISPR-Cas9 and stated that sgRNAs targeting ORF30 and ORF7 showed synergistic effect in reducing viral replication of EHV.

Feline leukoma virus (FeLV) and CRISPR-Cas systems

Helfer-Hungerbuehler et al. (2021) investigated the infection-reversing effect of the cat's immune system using CRISPR/Cas9-assisted gene therapy and evaluated different adeno-associated vectors (AAVs) for their ability to provide gene regulation. The CRISPR -Cas system was transferred into cat cells and then the efficiency of the CRISPR/SaCas9 system to target different regions of the FeLV provirus was investigated, for which nine natural AAV serotypes, two AAV hybrid strains and Anc80L65, an AAV ancestor predicted by Silico, were tested for their potential to infect different viruses. They reported that the CRISPR/SaCas9 system was used to target selected FeLV provirus regions, followed by T7-validated endonuclease 1 (T7E1) and Truncation of Indels (TIDE) analysis, showing that the gag and pol regions had the highest percentage (up to 80%) of non-homologous end joining (NHEJ) in the conserved region. Subsequent transduction experiments using AAV-DJ confirmed indel formation and showed a significant reduction in FeLV p27 antigen for some targets. Targeting of FeLV provirus was effective when using the CRISPR/SaCas9 approach invitro, while the means to overcome infection in vivo should be further investigated.

Feline calicivirus (FCV) - feline herpesvirus (FHV) and CRISPR-Cas9 system

Studies on FCV and FHV are more oriented towards viral diagnosis and viral screening.

In the study conducted by Huang et al. (2022), they stated that they developed a CRISPR-Cas13a and RPA reaction-based analysis for FCV detection. The recombinant plasmid they designed targeted the ORF1 gene of FCV. They stated that the positive detection rate of the FCV-Cas13a test they developed was higher than RT-PCR and showed that CRISPR-Cas systems can also be used in viral diagnosis.

In a study conducted by Fang et al. (2023) , they reported that they developed a nucleic acid detection system called 4 thermostatic steps (4TS) for the diagnosis of respiratory disease agents, including FCV and FHV, using the CRISPR-Cas12 system.

Poxvirus and CRISPR-Cas systems

In their publication by Siegrist et al. (2020) is showed that vaccine strains developed by using vaccinia irus (VACV) as a vector and targeting E3L, I2L, A17L genomes against orthopoxviruses with CRISPIR-Cas9 reduced CPEs.

Ohlson et al. (2023) reported that AAA ATPase and SPATA5 are important host factors for these virus groups as a result of CRISPR-Cas screening of poxviruses and flaviviruses.

Singh et al. (2023) developed a Cas12a nuclease-based assay associated with clustered, regularly spaced short palindromic repeats to detect monkey poxvirus (Mpox), They stated that they identified Mpox-specific conserved sequences that differ from all viruses present in the genus Orthopoxvirus by a single nucleotide polymorphism (SNP) and used this SNP in our assay to specifically distinguish mpox virus from other related orthopox viruses with a detection limit of 1 copy/µl in 30 minutes. They stated that this region may provide practicality in cases where the detection of Mpox virus needs to be sensitive and specific. This suggests that SNP may be a key point for the antiviral agent that can be developed against Mpox virus in the future.

In the study conducted by F. Zhao et al. (2023) , they stated that they developed a recombinase polymerase amplification (RPA)-coupled CRISPR-Cas12a study for the detection of Mpox virus and used 6DR and E9L, which are important for orthopoxviruses, and N3R and N4R gene regions specific for Mpox in the experiment. This suggests that these regions may be key in the development of tests for antiviral agents that can be developed against Mpox virus and other orthopoxviruses in the future.

Conclusion

The ability to develop CRISPR/Cas vectors for various stages of viral replication, including the latent stage, or to develop CRISPR/Cas vectors against various stages of viral replication, both in terms of its usefulness in revealing the virus-host relationship and in terms of guiding the development of antivirals for various stages of viral replication, including the latent stage, paves the way for the development of antivirals and paves the way for the development of antivirals, which are more difficult to develop than other antimicrobial agents due to technical conditions.

CRISPR/Cas studies have started to be used in the production of viruses to be used in vaccine development as well as antiviral development studies. With the transition from the laboratory-scale production stage to the high-scale production stage, an important stage will be passed in the fight against human viral diseases and animal viral diseases.

References

Abbott, T. R., Dhamdhere, G., Liu, Y., Lin, X., Goudy, L., Zeng, L., Chemparathy, A., Chmura, S., Heaton, N. S., Debs, R., Pande, T., Endy, D., La Russa, M. F., Lewis, D. B., & Qi, L. S. (2020). Development of CRISPR as an antiviral strategy to combat SARS-CoV-2 and influenza. *Cell, 181*(4), 865-876.

Barrangou, R., Fremaux, C., Deveau, H., Richards, M., Boyaval, P., Moineau, S., Romero, D. A., & Horvath, P. (2007). CRISPR provides acquired resistance against viruses in prokaryotes. *Science, 315*(5819), 1709-1712.

Bayat, H., Naderi, F., Khan, A. H., Memarnejadian, A., & Rahimpour, A. (2018). The impact of CRISPR-Cas system on antiviral therapy. *Advanced Pharmaceutical Bulletin, 8* (4), 591-597.

Bikard, D., & Marraffini, L. A. (2012). Innate and adaptive immunity in bacteria: mechanisms of programmed genetic variation to fight bacteriophages. *Current Opinion in Immunology, 24*(1), 15-20.

Billon, P., Bryant, E. E., Joseph, S. A., Nambiar, T. S., Hayward, S. B., Rothstein, R., & Ciccia, A. (2017). CRISPR-mediated base editing enables efficient disruption of eukaryotic genes through induction of STOP codons. *Molecular Cell, 67*(6), 1068-1079.e4.

Bogdanove, A. J., & Voytas, D. F. (2011). TAL effectors: Customizable proteins for DNA targeting. *Science, 333* (6051), 1843-1846.

Cai, C., Wang, X., Zhao, Y., Yi, C., Jin, Z., Zhang, A., & Han, L. (2019). Construction of a mavs-inactivated MDCK cell line for facilitating the propagation of canine distemper virus (CDV). Molecular Immunology, 114, 133-138.

Chou, Y., Krupp, A., Kaynor, C., Gaudin, R., Ma, M., Cahir-McFarland, E., & Kirchhausen, T. (2016). Inhibition of JCPyV infection mediated by targeted viral genome editing using CRISPR/Cas9. *Scientific Reports, 6*(1), 36921.

Chulanov, V., Kostyusheva, A., Brezgin, S., Ponomareva, N., Gegechkori, V., Volchkova, E., Pimenov, N., & Kostyushev, D. (2021). CRISPR Screening: Molecular Tools for Studying Virus-Host Interactions. *Viruses, 13*(11), 2258.

Cong, L., Ran, F. A., Cox, D., Lin, S., Barretto, R., Habib, N., Hsu, P. D., Wu, X., Jiang, W., Marraffini, L. A., & Zhang, F. (2013). Multiplex genome engineering using CRISPR/Cas systems. *Science, 339*(6121), 819-823.

Cross Ryan. (2018, July 1). CRISPR researchers receive Kavli Prize in Nanoscience. cen.asc.org/biological-chemistry/ biotechnology/CRISPR-researchers-recive-Kavli-Prize/96/ web/2018/06

Dai, H., Wu, J., Yang, H., Guo, Y., Di, H., Gao, M., & Wang, J. (2022). Construction of BHV-1 UL41 defective virus using the CRISPR/Cas9 system and analysis of viral replication properties. *Frontiers in Cellular and Infection Microbiology, 12*, 942987.

Das, A., Barrientos, R., Shiota, T., Madigan, V., Misumi, I., McKnight, K. L., Sun, L., Li, Z., Meganck, R. M., Li, Y., Kaluzna, E., Asokan, A., Whitmire, J. K., Kapustina, M.,

Zhang, Q., & Lemon, S. M. (2020). Gangliosides are essential endosomal receptors for quasi-enveloped and naked hepatitis A virus. Nature Microbiology, 5(9), 1069–1078.

de Wilde, A. H., Zevenhoven-Dobbe, J. C., Beugeling, C., Chatterji, U., de Jong, D., Gallay, P., Szuhai, K., Posthuma, C. C., & Snijder, E. J. (2018). Coronaviruses and arteriviruses display striking differences in their cyclophilin A-dependence during replication in cell culture. Virology, 517, 148-156.

Deltcheva, E., Chylinski, K., Sharma, C. M., Gonzales, K., Chao, Y., Pirzada, Z. A., Eckert, M. R., Vogel, J., & Charpentier, E. (2011). CRISPR RNA maturation by trans-encoded small RNA and host factor RNase III. Nature, 471(7340), 602-607.

Deveau, H., Barrangou, R., Garneau, J. E., Labonté, J., Fremaux, C., Boyaval, P., Romero, D. A., Horvath, P., & Moineau, S. (2008). Phage Response to CRISPR-Encoded Resistance in Streptococcus thermophilus. Journal of Bacteriology, 190(4), 1390-1400.

Ding, S., Diep, J., Feng, N., Ren, L., Li, B., Ooi, Y. S., Wang, X., Brulois, K. F., Yasukawa, L. L., Li, X., Kuo, C. J., Solomon, D. A., Carette, J. E., & Greenberg, H. B. (2018). STAG2 deficiency induces interferon responses via cGAS-STING pathway and restricts virus infection. Nature Communications, 9(1), 1485.

Fang, J., Liu, J., Cheng, N., Kang, X., Huang, Z., Wang, G., Xiong, X., Lu, T., Gong, Z., Huang, Z., Che, J., & Xiang, T. (2023). Four thermostatic steps: A novel CRISPR-Cas12-based system for the rapid at-home detection of respiratory pathogens. Applied Microbiology and Biotechnology, 107(12), 3983-3996.

Ganaie, S. S., Schwarz, M. M., McMillen, C. M., Price, D. A., Feng, A. X., Albe, J. R., Wang, W., Miersch, S., Orvedahl, A., Cole, A. R., Sentmanat, M. F., Mishra, N., Boyles, D. A., Koenig, Z. T., Kujawa, M. R., Demers, M. A., Hoehl, R. M., Moyle, A. B., Wagner, N. D., … Hartman, A. L. (2021). Lrp1 is a host entry factor for Rift Valley fever virus. Cell, 184 (20), 5163-5178.e24.

Garneau, J. E., Dupuis, M.-È., Villion, M., Romero, D. A., Barrangou, R., Boyaval, P., Fremaux, C., Horvath, P., Magadán, A. H., & Moineau, S. (2010). The CRISPR/Cas bacterial immune system cleaves bacteriophage and plasmid DNA. Nature, 468(7320), 67-71.

Gök, G., & Tunalı, Ç. (2016). CRISPR-Cas İmmün siteminin biyolojisi, mekanizması ve kullanım alanları. *Uluslararası Mühendislik Araştırma ve Geliştirme Dergisi, 8(*2), 11-23.

Gong, Y., Chen, T., Feng, N., Meng, X., Sun, W., Wang, T., Zhao, Y., Yang, S., Song, X., Li, W., Dong, H., Wang, H., He, H., Wang, J., Zhang, L., Gao, Y., & Xia, X. (2020). A highly efficient recombinant canarypox virus-based vaccine against canine distemper virus constructed using the CRISPR/Cas9 gene editing method. *Veterinary Microbiology, 251*, 108920.

Gradauskaite, V., Inglebert, M., Doench, J., Scherer, M., Dettwiler, M., Wyss, M., Shrestha, N., Rottenberg, S., & Plattet, P. (2023). LRP6 Is a Functional receptor for Attenuated canine distemper virus. *MBio, 14*(1), e0311422.

Haft, D. H., Selengut, J., Mongodin, E. F., & Nelson, K. E. (2005). A guild of 45 CRISPR-associated (Cas) protein families and multiple CRISPR/Cas subtypes exist in prokaryotic genomes. *PLoS Computational Biology, 1*(6), e60.

Hassanien, R. T., Thieulent, C. J., Carossino, M., Li, G., & Balasuriya, U. B. R. (2024). Modulation of equid herpesvirus-1 replication dynamics in vitro using CRISPR/Cas9-Assisted genome editing. *Viruses, 16*(3), 409.

Helfer-Hungerbuehler, A. K., Shah, J., Meili, T., Boenzli, E., Li, P., & Hofmann-Lehmann, R. (2021). Adeno-associated vector-delivered CRISPR/SaCas9 system reduces feline leukemia virus production in vitro. *Viruses, 13*(8), 1636.

Herrera-Carrillo, E., Gao, Z., & Berkhout, B. (2020). CRISPR therapy towards an HIV cure. *Briefings in Functional Genomics, 19*(3), 201-208.

Huang, J., Liu, Y., He, Y., Yang, X., & Li, Y. (2022). CRISPR-Cas13a based visual detection assays for feline calicivirus circulating in southwest China. Frontiers *in Veterinary Science, 9*, 913780.

Huang, Z., Tomitaka, A., Raymond, A., & Nair, M. (2017). Current application of CRISPR/Cas9 gene-editing technique to eradication of HIV/AIDS. *Gene Therapy, 24* (7), 377-384.

Hoffmann, H. H., Schneider, W. M., Rozen-Gagnon, K., Miles L. A. , Schuster, F., Razooky, B., Jacobson, E., Wu, X., Yi, S., Rudin, C. M., MacDonald, M. R., McMullan, L. K., Poirier, J. T., Rice, C. M. (2021). TMEM41B is a pan-flavivirus host factor. *Cell, 184*(1), 133-148.

Hosmillo, M., Lu, J., McAllaster, M. R., Eaglesham, J. B., Wang, X., Emmott, E., Domingues, P., Chaudhry, Y., Fitzmaurice, T. J., Tung, M. K., Panas, M. D., McInerney, G., Locker, N., Wilen, C. B., Goodfellow, I. G. (2019). Noroviruses subvert the core stress granule component G3BP1 to promote viral VPg-dependent translation. *Elife*. 8, e46681.

Ishino, Y., Shinagawa, H., Makino, K., Amemura, M., & Nakata, A. (1987). Nucleotide sequence of the iap gene, responsible for alkaline phosphatase isozyme conversion in Escherichia coli, and identification of the gene product. *Journal of Bacteriology, 169*(12), 5429-5433.

Jansen, Ruud., Embden, Jan. D. A. van, Gaastra, Wim., & Schouls, Leo. M. (2002). Identification of genes that are associated with DNA repeats in prokaryotes. *Molecular Microbiology, 43*(6), 1565-1575.

Jiang, F., & Doudna, J. A. (2015). The structural biology of CRISPR-Cas systems. *Current Opinion in Structural Biology, 30*, 100–111.

Jinek, M., Chylinski, K., Fonfara, I., Hauer, M., Doudna, J. A., & Charpentier, E. (2012). A Programmable Dual-RNA-guided DNA endonuclease in adaptive bacterial immunity. *Science, 337*(6096), 816-821.

Kennedy, E. M., Kornepati, A. V. R., Goldstein, M., Bogerd, H. P., Poling, B. C., Whisnant, A. W., Kastan, M. B., & Cullen, B. R. (2014). Inactivation of the human papillomavirus E6

or E7 gene in cervical carcinoma cells by using a bacterial CRISPR/Cas RNA-guided endonuclease. *Journal of Virology, 88*(20), 11965–11972.

Kılıç Tosun, Ö., & Kesmen, Z. (2022). CRISPR-cas uygulamaları, potansiyel riskler ve yasal düzenlemeler. *Helal ve Etik Araştırmalar Dergisi, 4*(2), 11-42.

Kuscu, C., Parlak, M., Tufan, T., Yang, J., Szlachta, K., Wei, X., Mammadov, R., & Adli, M. (2017). CRISPR-STOP: gene silencing through base-editing-induced nonsense mutations. *Nature Methods, 14*(7), 710-712.

Labeau, A., Simon-Loriere, E., Hafirassou, M.-L., Bonnet-Madin, L., Tessier, S., Zamborlini, A., Dupré, T., Seta, N., Schwartz, O., Chaix, M.-L., Delaugerre, C., Amara, A., & Meertens, L. (2020). A Genome-wide CRISPR-Cas9 screen identifies the dolichol-phosphate mannose synthase complex as a host dependency factor for dengue virus infection. *Journal of Virology, 94*(7), 1128.

Li, K., Liu, Y., Xu, Z., Zhang, Y., Yao, Y., Nair, V., Liu, C., Zhang, Y., Gao, Y., Qi, X., Cui, H., Gao, L., & Wang, X. (2020). Prevention of avian retrovirus infection in chickens using CRISPR-Cas9 delivered by Marek's disease virus. molecular therapy. *Nucleic Acids, 21*, 343-353.

Li, Z., Hajian, C., & Greene, W. C. (2020). Identification of unrecognized host factors promoting HIV-1 latency. *PLOS Pathogens, 16*(12), e1009055.

Liao, H.-K., Gu, Y., Diaz, A., Marlett, J., Takahashi, Y., Li, M., Suzuki, K., Xu, R., Hishida, T., Chang, C.-J., Esteban, C. R., Young, J., & Belmonte, J. C. I. (2015). Use of the CRISPR/ Cas9 system as an intracellular defense against HIV-1 infection in human cells. *Nature Communications,* 6(1), 6413.

Lin, D. L., Cherepanova, N. A., Bozzacco, L., MacDonald, M. R., Gilmore, R., & Tai, A. W. (2017). Dengue virus hijacks a noncanonical oxidoreductase function of a cellular oligosaccharyltransferase complex. *MBio, 8*(4).10-1128.

Liu, Y., Xu, Z., Zhang, Y., Yu, M., Wang, S., Gao, Y., Liu, C., Zhang, Y., Gao, L., Qi, X., Cui, H., Pan, Q., Li, K., & Wang, X. (2020). Marek's disease virus as a CRISPR/Cas9 delivery system to defend against avian leukosis virus infection in chickens. *Veterinary Microbiology, 242*, 108589.

Luo, J., Teng, M., Zai, X., Tang, N., Zhang, Y., Mandviwala, A., Reddy, V. R. A. P., Baigent, S., Yao, Y., & Nair, V. (2020). Efficient mutagenesis of Marek's disease virus-encoded microRNAs Using a CRISPR/Cas9-based gene editing system. *Viruses, 12*(4), 466.

Ma, Z., Bai, J., Jiang, C., Zhu, H., Liu, D., Pan, M., Wang, X., Pi, J., Jiang, P., & Liu, X. (2023). Tegument protein UL21 of alpha-herpesvirus inhibits the innate immunity by triggering CGAS degradation through TOLLIP-mediated selective autophagy. *Autophagy, 1*9(5), 1512-1532.

McLaurin, K. A., Li, H., Khalili, K., Mactutus, C. F., & Booze, R. M. (2024). HIV-1 mRNA knockdown with CRISPR/CAS9 enhances neurocognitive function. *Journal of Neurovirology, 30*(1), 71-85.

Mohanraju, P., Makarova, K. S., Zetsche, B., Zhang, F., Koonin, E. V., & van der Oost, J. (2016). Diverse evolutionary roots and mechanistic variations of the CRISPR-Cas systems. *Science, 353*(6299), aad5147.

Mojica, F. J., Díez-Villaseñor, C., Soria, E., & Juez, G. (2000). Biological significance of a family of regularly spaced repeats in the genomes of Archaea, Bacteria and mitochondria. *Molecular Microbiology, 36*(1), 244-246.

Mojica, F. J., Ferrer, C., Juez, G., & Rodríguez-Valera, F. (1995). Long stretches of short tandem repeats are present in the largest replicons of the Archaea Haloferax mediterranei and Haloferax volcanii and could be involved in replicon partitioning. *Molecular Microbiology, 17*(1), 85 -93.

Mojica, F. J., Juez, G., & Rodríguez-Valera, F. (1993). Transcription at different salinities of Haloferax mediterranei sequences adjacent to partially modified PstI sites. *Molecular Microbiology, 9*(3), 613-621.

Mojica, F. J. M., Diez-Villasenor, C., Garcia-Martinez, J., & Soria, E. (2005). Intervening sequences of regularly spaced prokaryotic repeats derive from foreign genetic elements. *Journal of Molecular Evolution, 60*(2), 174–182.

Nobel Prize. (2020, October 7). The Royal Swedish Academy of Science has decided to award to Nobel Prize in Chemistry 2020 to Emmanuel Carpentier and Jennifer A. Doudna 'for development of a method for genome editing'. The Nobel Prize. nobelprize.org/prizes/ chemistry/2020/press-release

Ohlson, M. B., Eitson, J. L., Wells, A. I., Kumar, A., Jang, S., Ni, C., Xing, C., Buszczak, M., & Schoggins, J. W. (2023). Genome-Scale CRISPR Screening reveals host factors required for ribosome formation and viral replication. *MBio, 14*(2), e0012723.

Ophinni, Y., Inoue, M., Kotaki, T., & Kameoka, M. (2018). CRISPR/Cas9 system targeting regulatory genes of HIV-1 inhibits viral replication in infected T-cell cultures. *Scientific Reports,* 8(1), 7784.

Orchard, R. C., Sullender, M. E., Dunlap, B. F., Balce, D. R., Doench, J. G., & Virgin, H. W. (2019). Identification of antinorovirus genes in human cells using genome-wide CRISPR activation screening. *Journal of Virology, 93*(1), 10.1128.

Park, R. J., Wang, T., Koundakjian, D., Hultquist, J. F., Lamothe-Molina, P., Monel, B., Schumann, K., Yu, H., Krupzcak, K. M., Garcia-Beltran, W., Piechocka-Trocha, A., Krogan, N. J., Marson, A., Sabatini, D. M., Lander, E. S., Hacohen, N., & Walker, B. D. (2017). A genome-wide CRISPR screen identifies a restricted set of HIV host dependency factors. *Nature Genetics, 49*(2), 193-203.

Pourcel, C., Salvignol, G., & Vergnaud, G. (2005). CRISPR elements in Yersinia pestis acquire new repeats by preferential uptake of bacteriophage DNA, and provide additional tools for evolutionary studies. *Microbiology, 151*(3), 653-663.

Puschnik, A. S., Majzoub, K., Ooi, Y. S., & Carette, J. E. (2017). A CRISPR toolbox to study virus–host interactions. *Nature Reviews Microbiology, 15*(6), 351-364.

Ran, F. A., Hsu, P. D., Lin, C.-Y., Gootenberg, J. S., Konermann, S., Trevino, A. E., Scott, D. A., Inoue, A., Matoba, S., Zhang, Y., & Zhang, F. (2013). Double nicking by RNA-guided

CRISPR Cas9 for Enhanced Genome Editing Specificity.Shmakov S., Abudayyeh O. O., Makarova K. S., Wolf Y. I., Gootenberg J. S., Semenova E., Minakhin L., Joung J., Konermann S., Severinov K., Zhang F., and Koonin E. V. (2015). Discovery and functional characterization of diverse class 2 CRISPR-Cas systems. *Mollecular Cell, 60*, 385-397.

Rath, D., Amlinger, L., Rath, A., & Lundgren, M. (2015). The CRISPR-Cas immune system: Biology, mechanisms and applications. Biochimie, 117, 119-128.

Richardson, R. B., Ohlson, M. B., Eitson, J. L., Kumar, A., McDougal, M. B., Boys, I. N., Mar, K. B., De La Cruz-Rivera, P. C., Douglas, C., Konopka, G., Xing, C., & Schoggins, J. W. (2018). A CRISPR screen identifies IFI6 as an ER-resident interferon effector that blocks flavivirus replication. Nature Microbiology, 3(11), 1214-1223.

Roehm, P. C., Shekarabi, M., Wollebo, H. S., Bellizzi, A., He, L., Salkind, J., & Khalili, K. (2016). Inhibition of HSV-1 Replication by Gene Editing Strategy. Scientific Reports, 6 (1), 23146.

Sapranauskas, R., Gasiunas, G., Fremaux, C., Barrangou, R., Horvath, P., & Siksnys, V. (2011). The Streptococcus thermophilus CRISPR/Cas system provides immunity in Escherichia coli. Nucleic Acids Research, 39(21), 9275-9282.

Senevirathne, A., Hewawaduge, C., & Lee, J. H. (2021). Genetic interference exerted by Salmonella-delivered CRISPR/Cas9 significantly reduces the pathological burden caused by Marek's disease virus in chickens. Veterinary Research, 52(1), 125.

Shmakov, S., Abudayyeh, O. O., Makarova, K. S., Wolf, Y. I., Gootenberg, J. S., Semenova, E., Minakhin, L., Joung, J., Konermann, S., Severinov, K., Zhang, F., and Koonin, E. V., (2015). Discovery and functional characterization of diverse class 2 CRISPR-Cas systems. *Mollelucalar Cell 60*, 385-397.

Siegrist, C. M., Kinahan, S. M., Settecerri, T., Greene, A. C., & Santarpia, J. L. (2020). CRISPR/Cas9 as an antiviral against Orthopoxviruses using an AAV vector. *Scientific Reports, 10*(1), 19307.

Singh, M., Misra, C. S., Bindal, G., Rangu, S. S., & Rath, D. (2023). CRISPR-Cas12a assisted specific detection of mpox virus. *Journal of Medical Virology, 95*(8), e28974.

Teng, M., Zhu, Z.-J., Yao, Y., Nair, V., Zhang, G.-P., & Luo, J. (2023). Critical roles of non-coding RNAs in lifecycle and biology of Marek's disease herpesvirus. Science China. *Life Sciences, 66*(2), 251-268.

Ueda, S., Ebina, H., Kanemura, Y., Misawa, N., & Koyanagi, Y. (2016). Anti-HIV-1 potency of the CRISPR/Cas9 system insufficient to fully inhibit viral replication. *Microbiology and Immunology, 60*(7), 483-496.

van der Oost, J., Jore, M. M., Westra, E. R., Lundgren, M., & Brouns, S. J. J. (2009). CRISPR-based adaptive and heritable immunity in prokaryotes. Trends *in Biochemical Sciences, 34*(8), 401-407.

van Diemen, F. R., Kruse, E. M., Hooykaas, M. J. G., Bruggeling, C. E., Schürch, A. C., van Ham, P. M., Imhof, S. M., Nijhuis, M., Wiertz, E. J. H. J., & Lebbink, R. J. (2016). CRISPR/Cas9-mediated genome editing of herpesviruses limits productive and latent infections. *Plos Pathogens, 12* (6), e1005701.

Wei, J., Alfajaro, M. M., DeWeirdt, P. C., Hanna, R. E., Lu-Culligan, W. J., Cai, W. L., Strine, M. S., Zhang, S.-M., Graziano, V. R., Schmitz, C. O., Chen, J. S., Mankowski, M. C., Filler, R. B., Ravindra, N. G., Gasque, V., de Miguel, F. J., Patil, A., Chen, H., Oguntuyo, K. Y., … Wilen, C. B. (2021). Genome-wide CRISPR Screens Reveal Host Factors Critical for SARS-CoV-2 Infection. *Cell, 184*(1), 76-91.e13.

Wei, Y., Zhao, Z., & Ma, X. (2022). Description of CRISPR-Cas9 development and its prospects in human papillomavirus-driven cancer treatment. *Frontiers in Immunology, 13*, 1037124.

Wollebo, H. S., Bellizzi, A., Kaminski, R., Hu, W., White, M. K., & Khalili, K. (2015). CRISPR/Cas9 System as an Agent for Eliminating Polyomavirus JC Infection. *Plos one, 10*(9), e0136046.

Wu, K., Oberstein, A., Wang, W., & Shenk, T. (2018). Role of PDGF receptor-α during human cytomegalovirus entry into fibroblasts. Proceedings of the National Academy of Sciences of the United States of America, 115(42), E9889–E9898. Xie, S., Ji, Z., Suo, T., Li, B., & Zhang, X. (2021). Advancing sensing technology with CRISPR: From the detection of nucleic acids to a broad range of analytes - A review. *Analytica Chimica Acta, 1185*, 338848.

Xu, L., Wang, J., Liu, Y., Xie, L., Su, B., Mou, D., Wang, L., Liu, T., Wang, X., Zhang, B., Zhao, L., Hu, L., Ning, H., Zhang, Y., Deng, K., Liu, L., Lu, X., Zhang, T., Xu, J., … Chen, H. (2019). CRISPR-edited stem cells in a patient with HIV and acute lymphocytic leukemia. *New England Journal of Medicine, 381*(13), 1240-1247.

Yu, W., Liu, J., Liu, Y., Forlenza, M., & Chen, H. (2024). Application of CRISPR/Cas9 for rapid genome editing of pseudorabies virus and bovine herpesvirus-1. *Viruses, 16,* (2), 311.

Zhang, Y., Luo, J., Tang, N., Teng, M., Reddy, V. R. A. P., Moffat, K., Shen, Z., Nair, V., & Yao, Y. (2019). Targeted editing of the pp38 gene in Marek's disease virus-transformed cell lines using CRISPR/Cas9 system. *Viruses, 11*(5), 391.

Zhang, Y., Wu, Y., Wu, Y., Chang, Y., & Liu, M. (2021). CRISPR-Cas systems: From gene scissors to programmable biosensors. *TrAC Trends in Analytical Chemistry, 137*, 116210.

Zhao, C., Gao, J., Wang, Y., Ji, L., Qin, H., Hu, W., & Yang, Y. (2022). A Novel rabies vaccine based on a recombinant bovine herpes virus type 1 expressing rabies virus glycoprotein. *Frontiers in Microbiology, 13*, 931043.

Zhao, F., Hu, Y., Fan, Z., Huang, B., Wei, L., Xie, Y., Huang, Y., Mei, S., Wang, L., Wang, L., Ai, B., Fang, J., Liang, C., Xu, F., Tan, W., & Guo, F. (2023). Rapid and sensitive one-tube detection of mpox virus using RPA-coupled CRISPR-Cas12 assay. *Cell Reports Methods, 3*(10), 100620.

Zhen, S., Hua, L., Takahashi, Y., Narita, S., Liu, Y.-H., & Li, Y. (2014). In vitro and in vivo growth suppression of human papillomavirus 16-positive cervical cancer cells by CRISPR/ Cas9. *Biochemical and Biophysical Research Communications, 450*(4), 1422-1426.

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Antimicrobial susceptibilities of methicillin resistant *Staphylococcus Aureus* (MRSA) isolates recovered from perineum and nasal mucosa swab samples of dogs in Niger

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ABSTRACT

Methicillin Resistant Staphylococcus aureus (MRSA) is an important opportunistic pathogens of dogs, other domestic animals and humans. The proximity between humans, livestock and antimicrobial use in animals facilitates the emergence and spread of MRSA. In this study, 400 swab samples taken from the perineum and nasal mucosa of dogs. Swab samples were inoculated on 5% blood agar (Sigma® Switzerland) for 24 hours of aerobic incubation at 37 °C, growth with yellowish-white colonies with smooth, slightly raised surfaces were further gram stain and biochemical test of Coagulase and catalase tests was performed. Positive isolates were then inoculated mannitol salt agar (MSA, Oxoid) and incubated for 24 hours at 37 °C and further confirmed using Oxacillin Resistance Screening Basal (ORSAB) medium (Oxoid, Basingstoke, United Kingdom). Antibiotics susceptibility testing was determined using the Kirby Bauer's disc diffusion method according to Clinical and Laboratory Standards Institute (CLSI) guideline and positive S. aureus were inoculated on Mueller-Hilton Agar (Oxoid) plates and Vanier callipers were used to measure the zones of inhibition in millimetres (mm). Out of the 200 samples each from the nasal mucosa and perineum, 55%(72) and 39.5% (49) were positive for MRSA respectively. Out of the 206 male and 194 female dog sampled, 57.0% (69) and 43.0%(52) were positive for MRSA respectively. Nigerian indigenous breed (Mongrel) has the highest proportion of MRSA isolates with 48.8% (59) while Golden retriever has the least proportion of MRSA isolates with 0.8% (1). Most of the MRSA isolates were resistant to oxacillin, cefoxitin, tetracycline, erythromycin and cephazolin but susceptibile to gentamycin, ciprofloxacin and chloramphenicol.

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Introduction

Staphylococcus aureus (*S. aureus*) belongs to the members of the microbiota of human body, usually isolated from the upper respiratory tract and the skin. Although they can be opportunistic organism, resulting in skin and respiratory infections (Oh et al., 2020). Methicillin-resistant *S. aureus* (MRSA) has raised public health concerned all over the world (Deleo and Chambers, 2009). About 20% to 30% of humans are carriers of *S. aureus*, which are the normal microbiota of the skin and nasal mucosa (Tong et al., 2015). They are frequently distributed in nosocomial infections and wound infections after surgery.

The close interactions between humans, livestock and antimicrobial use in animals favors the emergence and spread of MRSA (Bouchami et al., 2020; Lawal et al., 2021). Since the year 2000, there have been the emergence of multi drug resistant as an opportunistic organism in companion dogs, dog's owners, veterinary hospitals and veterinary clinicians (Sweeney et al., 2018). Its common in dog population of about 20% (Gomez-Sanz et al.,2013; Afshar et al., 2023). MRSA infection has given rise to serious public health

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concerned because infection rate increases every year (Decline et al., 2020). The irrational use of antibiotics has given rise to the emergence of resistance to antibiotics due to *S. aureus* refer to as methicillin-resistance *S. aureus* (MRSA) (Decline et al., 2020).Transmission between animal to human usually occur because pet animals are regarded as family members, resulting in to close contacts between humans and pets which cause bacterial transmission dynamics (Decline et al., 2020). This is a serious public health problem because human MRSA can be transmitted to pets, and there after pets can also serve as source of infections to humans, as such pet animals can be regarded as reservoir for spreading infections to humans when they come in contact (Reddy et al., 2016; Findik et al., 2018; Decline et al., 2020). Human infections with MRSA methicillin resistant *staphylococcus aureus* have been documented in several parts of Nigeria (Aliyu et al., 2022). Pets especially dogs are kept at home for security reasons due to increase urbanization and raise in antisocial behaviors and crimes. These days relationships bond between humans and pet animals is on the increase and this could facilitate the spread of infectious or zoonotic organisms to humans (Audu et al., 2022). Due to continuous changes in the prevalence and epidemiology of MRSA, and the discovery of new strains of MRSA in Nigeria. The present study was designed to assess the prevalence of MRSA, antibiotic susceptibility profile and potential risk factors associated with dogs in Gombe State, Nigeria.

Materials and Methods

Study area: Gombe state is located in the North east geopolitical zone of Nigeria, the state covers an area of 20, 265km2 and located between latitudes 9°301N and 12°301E and longitude 8°451 and 11°451E.The state has a total of eleven local Government areas (LGAs) and 114 wards. The LGA are Akko, Balanga, Billiri, Dukku, Funakaye, Gombe, Kaltungo, Kwami, Nafada,, Shongom and Yamaltu/Deba (Wikipedia, 2006).

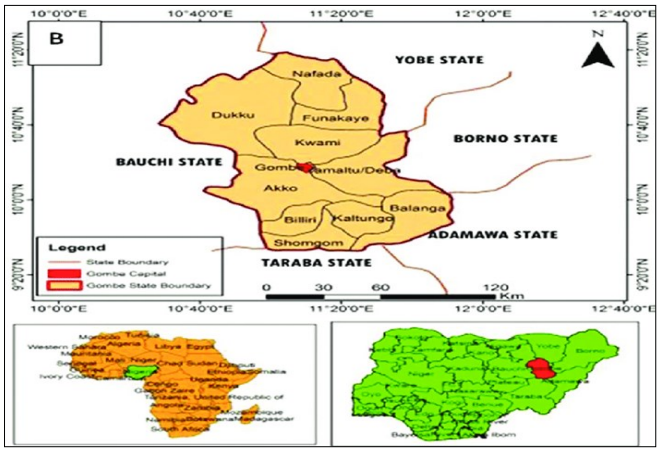

Figure 1. Map of Gombe State showing the study area

Sampling procedure and study population: A cross-sectional study was conducted. Six LGAs (Kwami, Gombe, Yamaltu Deba, Akko, Billiri and Kaltungo) out of the eleven LGAs were selected randomly (two from each senatorial zone). Facilities surveyed were the State and Private Veterinary clinics, households and dog markets. The quantity of samples gathered from every one of the six LGAs was determined by accessibility and availability. A systematic random sampling technique was used to choose individual canines, with one dog out of every two that were sighted being chosen (Pfeiffer, 2002). Using the 50% prevalence at 95% Confidence level and the Thrusfield (2005) formula, the sample size was calculated. For accuracy, the 384 sample size that was estimated was raised to 400.

Sample collection and processing: Dogs were sampled with the owners' permission. Every dog had one nostril opened, and a cotton-tipped sterile swab (Everson Industries Limited, Nigeria) was used to obtain a sample from the nasal mucosa, which was then quickly placed into an aseptic tube. The perianal area is rolled with a fresh, sterile cotton-tipped swab, which is then promptly placed back into its tube. The dates of collection and an identifying number were written on the labels of the tubes in accordance with laboratory protocol (Cheesbrough, 2010). The samples were transported to the Bacterial zoonosis lab of the Department of Veterinary Public Health and Preventive Medicine, Faculty of Veterinary Medicine, Ahmadu Bello University (ABU) Zaria on an ice pack for immediate processing. Using recognized microbiological techniques, such as colonial morphology, gram stained features, catalase and coagulase tests, the organisms were isolated aseptically (Cheesbrough, 2010). Oxacillin Resistance Screening Agar Base (ORSAB) was used to detect MRSA. The disk diffusion method was used to carry out the anti-microbial sensitivity test.

Isolation and identification: The manufacturer's instructions were followed to make an enriched solid medium containing 5% blood agar (Sigma® Switzerland) and inoculate the swabs into it (Cheesbrough, 2010). To obtain distinct colonies, the inoculums were streaked using a sterile wire loop. After 24 hours of aerobic incubation at 37°C, the infected plates were checked for yellowish-white colonies with smooth, slightly raised surfaces. While some positive colonies were non-hemolytic, others had entire zones of hemolysis.

Colony morphology: The samples that were taken underwent gram staining in order to determine the staphylococci based on their gram reaction. Coagulase and catalase tests were performed on samples that were organized into clusters resembling grapes.

Mannitol salt agar (MSA, Oxoid) is a selective medium for S. aureus. The plates were streaked with the positive isolates and then incubated for 24 hours at 37°C in an aerobic environment using mannitol salt agar (MSA, Oxoid). It was assumed that S. aureus was the source of the yellowish colonies that appeared on MSA.

Oxacillin resistance screening agar base (ORSAB): Methicillin resistance was presumably determined using ORSAB agar (OXOID), a commercial medium. ORSAB was made in compliance with the manufacturer's guidelines. MRSA can be screened using ORSAB, a medium that is nutrient-rich, selective, and contains growth-promoting agents for microbes. Mannitol and aniline blue are added to the medium together with a high concentration of salt and lithium chloride to inhibit non-staphylococcal growth and detect mannitol fermentation. The antibiotics included in the ORSAB selective supplement are polymyxin B to limit the growth of other bacteria that can tolerate such a high concentration of salt e.g. Proteus spp. and oxacillin at 2 mg/L to inhibit methicillin-sensitive *S. aureus*. The characteristic blue color of MRSA colonies against a colorless background made it easier to identify the bacteria.

Antibiotic susceptibility testing: Using the Bauer-Kirby approach, the antibiotic susceptibility of MRSA isolates was ascertained (Bauer et al., 1966). Utilizing the commercially produced disk (Oxoid, UK) that has an established antibiotic concentration. Three to four millilitres of sterile normal saline were used to emulsify freshly sub-cultured MRSA and well-isolated colonies from ORSAB plates. The suspension's turbidity was corrected to 0.5 McFarland, which is the standard equivalent (CLSI, 2019). A sterile cotton swab stick was dipped into the suspension of Mueller-Hinton agar medium. By applying pressure and spinning the swab against the tube's side above the suspension, extra fluid was eliminated. In order to guarantee even dispersion, the plate was rotated by about 60° after the swab was evenly streaked over the medium's surface in three directions on the dried Mueller-Hinton agar surface (Benkova et al., 2020). After being distributed into each inoculation plate, five antimicrobial discs were incubated for twenty-four hours at 35°C at a time. Vanier callipers were used to measure the zones of inhibition in millimetres (mm). The Clinical and Laboratory Standard Institutes (CLSI) criteria were utilized to interpret the sizes of the zones of inhibition. The following 10 antibiotics were tested; ciprofloxacin (CIP) 5 ug; erythromycin (ERY) 15 ug; gentamycin (GEN) 5 ug; tetracycline (TET) 30 ug; Clindamycin (DA) 2 ug; chloramphenicol (CHL) 30 ug; sulfamethoxazole/trimethoprim (STX) 25 ug; cefoxitin (CFX) 30 ug; cefazolin (CZO) 30 ug; oxacillin (OXA) 1 ug. Growth inside the zone of inhibition was thought to be suggestive of methicillin resistance for the interpretation of susceptibility toward the oxacillin disc. A diameter of inhibition zones of ≤10, 11–12, and ≥13 by 1 ug of oxacillin is classified as susceptible (S), intermediate (I), or resistant (R) to oxacillin correspondingly, based on the classification criteria provided by (CLSI, 2019). Regarding cefoxitin discs, staphylococci classified as either sensitive or resistant to oxacillin are indicated by inhibitory zone diameters of ≥24 and ≥25 mm, respectively. The cefoxitin disc diffusion test does not classify staphylococci into any intermediate category (CLSI, 2019).

Statistical analysis: Data generated were presented as frequency and percentages using descriptive statistics. Graph pad prism version 5 was used to analyzed the data generated. Chi-square/Fisher's exact test was employed to determine the association between MRSA colonization and site of isolation as well as sex of the dogs, values of $p<0.05$ were considered significant. The prevalence was calculated for all data as the number of MRSA colonized individuals divided by the number of sampled dogs in the category and was expressed in percentage by multiplying by 100.

Results

A total of 400 swab samples were collected from different breeds of dogs. Out of the 200 samples each from the nasal mucosa and perineum, 55%(72) and 39.5%(49) were positive for MRSA respectively (Table 1). There was no statistically significant association between MRSA colonization and site of isolation ($P>0.05$). Out of the 206 male and 194 female dogs sampled, 57.0% (69) and 43, 0%(52) were positive for MRSA respectively (Table 2). There was no statistically significant association between MRSA colonization and Sex of the dogs ($P>0.05$). Nigerian indigenous breed (Mongrel) has the highest proportion of MRSA positive isolates with 48.8%(59) while Golden retriever has the least proportion of MRSA positive isolates with 0.8%(1) (Table 3). Most of the MRSA positive isolates were resistant to oxacillin, oefoxitin, tetracycline, erythromycin and cephazolin. However, susceptibility to gentamycin, ciprofloxacin and chloramphenicol was remarkable (Table 4). Multidrug resistant pattern of the isolates was also evaluated and shows varying levels of resistance to multiple class of antimicrobials (Table 5)

Table 1. Prevalence of MRSA isolated from dogs according to site of isolation in Gombe State

Sites	Number of samples	*S. aureus* (%)	MRSA (%)
Nasal mucosa	200	129 (64)	72 (55.8)
Perineum	200	124 (62)	49 (39.5)
Total	400	253 (63.3)	121 (47.8)

P-value = 0.0667, 95% confidence interval (C. I.) = 0.4505-1.028, odds ratio (OR) = 0.6806

Table 2. Prevalence of MRSA isolated from dogs according to sex in Gombe State

Sex	Number of Samples	*S. aureus* (%)	MRSA (%)
Male	206	137 (45.8)	69 (57.0)
Female	194	116 (54.2	52 (43.0)
Total	400	253 (63.2)	121 (47.8)

P-value= 0.3002, 95% confidence interval (C. I.) = 0.5311-1.206, odds ratio (OR) = 0.8002

Table 3. Breed distribution of S. aureus and MRSA in dogs in Gombe State

Dog breeds	*S. aureus* isolates (%)	MRSA positives (%)
Mongrel	101 (39.9)	59 (48.8)
Alsatian	31 (12.3)	16 (13.2)
Rottweiler	18 (7.1)	5 (4.1)
Boerboel	13 (5.1)	2 (1.7)
Golden retriever	6 (2.4)	1 (0.8)
Caucasian	28 (11.1)	13 (10.7)
Lhasa apso	11 (4.3)	3 (2.5)
Alsatian/Mongrel cross	25 (9.9)	14 (11.6)
Caucasian/Mongrel cross	20 (7.9)	8 (6.6)
Total	256	121

Table 4. Antibiotic susceptibility pattern of MRSA isolated from dogs in Gombe State

Antibiotic	Resistant no. of isolates (%)	Intermediate resistant no. of isolates (%)	Susceptible resistant no. of isolates (%)
Chloramphenicol	19 (15.7)	27 (22.3)	75 (62.0)
Cephazolin	84 (69.4)	21 (17.4)	16 (13.2)
Oxacillin	121 (100)	0 (0)	0 (0)
Ciprofloxacin	1 (0.8)	13 (10.7)	107 (88.4)
Sulphamethaxazole/ Trimethoprim	6 (5.0)	21 (17.4)	94 (77.7)
Erythromycin	86 (71.1)	25 (20.7)	10 (8.3)
Gentamycin	0 (0)	1 (0.8)	120 (99.2)
Tetracycline	104 (86.0)	11 (9.1)	6 (5.0)
Cefoxitin	121 (100)	0 (0)	0 (0)
Clindamycin	34 (28.1)	39 (32.2)	48 (39.6)

Table 5. Multidrug resistant patterns observed in MRSA positive isolates from dogs in Gombe State

Number of antibiotics class	Resistance patterns	Number of bacterial isolates
3	CFX + CHL + CIP	1
3	OXA + CFX + DA	6
3	OXA + CFX + CZO	3
3	ERY + OXA + STX	5
3	OXA + TET + CFX	13
4	DA + OXA + CFX + TET	10
5	CZO + OXA + ERY + TET + CFX	30
5	CZO + OXA + ERY + TET + CFX	28
6	CZO + OXA + STX + ERY + TET + CFX	5
7	CZO + OXA + ERY + TET + CFX + DA + CHL	18

CIP= Ciprofloxacin; ERY= Erythromycin; GEN= Gentamycin; TET= Tetracycline; DA= Clindamycin; CHL= Chloramphenicol; STX= Sulfamethoxazole/trimethoprim; CFX= Cefoxitin; CZO= Cefazolin; OXA= Oxacillin

Discussions

Methicillin resistance has increasingly been reported in staphylococcal isolates from canines in several countries (Jang, et al., 2014; Ishihara et al., 2014; Decline et al., 2020; Afshar et al., 2023) including Nigeria (Yakubu et al., 2022). In this study, the overall prevalence of MRSA in dogs was 47.8%. This was greater than 36.9 % and 15 % (15/100) of MRSA that were reported in Maiduguri and Sokoto, Nigeria, respectively, by Mustapha et al. (2016) and Yakubu et al. (2022). It also surpassed the reports by Abbott et al. (2010), Kottler et al. (2010), Chah et al. (2014), and Penna et al. (2021), who recorded 1.1%, 3.3%, 12.8 %, and 3.4% of MRSA in dogs in Ireland, the United States, Nigeria, and Brazil, respectively.' This might probably be attributed to variation in the breeds of dogs used in this finding, indiscriminate antibiotic therapy, harsher environmental challenges and malnutrition, 'which weakens the immune system and induces stress in dogs' (Yakubu et al., 2022). Contrarily, higher MRSA carriage of 67.5 % and 51.1 %have been reported by Vincze et al. 2014 and Iverson et al. 2015, respectively.

The prevalence of MRSA in the nasal cavity with the rate of 55% was found to be higher than that of the perineum with the rate of 39.5%. This result in the current was in line with the Mustapha et al (2016)who reported the MRSA the rates of 50% and 30% determined in the nasal cavity and perineum, respectively. The prevalence of S. aureus and MRSA in the nasal cavity of dogs was determined to be extremely high. These findings showed that dogs are more likely to get contaminated through their nostrils and nasal sampling is a better way to detect MRSA Rich and Roberts, (2004). A single case of MRSA discovery from nasal swab samples of 255 dogs, rather than from the throat and skin of the same animals was confirmed by Rich and Roberts, (2004). The prevalence of MRSA in male dogs was 57% as against the 43% in the female. Breed distributions shows that Nigerian indigenous breed (Mongrel) has the highest proportion of MRSA positive isolates with 48.8% (59) whereas Golden retriever (exotic breed) has the least proportion of MRSA positive isolates with 0.8%(1). According to Kutdang et al. (2010) findings, native dogs raised in developing nations was more exposed to the outside environment and therefore, have a higher risk of contracting diseases than the exotic breeds of dogs.

The results of susceptibility patterns of MRSA to antibiotics showed a decreasing trend of resistance in the order; Oxacillin (100%), Cefoxitin (100%), Tetracycline (86%), Erythromycin (71.1%), Cephazolin (69.4%), Clindamycin (28.1), Chloramphenicol (15.7%), Sulphamethaxazole/Trimethoprim (5%), Ciprofloxacin (0.8%) and Gentamycin (0%). This may be as a result of the fact that, they are commonly used by veterinarians and dog owners. This study showed that commonly used antibiotics in the study area such as Tetracycline is no longer reliable in treating staphylococcal infections in this region as clearly seen in the 86% resistance of the isolates to this antibiotic. This result agrees with the results of Mustapha et al., (2016) in Maiduguri, Nigeria which shows high resistance to Tetracycline (73.4%). Further, the high resistance to the antibiotics can be explained in terms of their widespread and reckless use, as well as their affordability (Zedan et al., 2023). High percentage susceptibility of MRSA isolates Gentamycin (99.2 %), Ciprofloxacin (88.4%), Sulphamethaxazole/ Trimethoprim (77.7 %) and Chloramphenicol (62 %) was found to be in line with Yaser et al (2015)'s results. Gentamycin was the most sensitive among all the antibiotics used in this study. The increased sensitivity to gentamycin may be due to the fact that dog owners and veterinary professionals in the study area do not frequently use gentamycin for treatment. An acquired non-susceptibility to at least one agent in three or more antimicrobial categories is known as multidrug resistance, or MDR (Magiorakos et al., 2012). High rate of MDR was observed among the MRSA isolates as most of them were resistant to more than two classes of antimicrobial agents. Although the focus of the study has been on methicillin resistance, S. aureus may be resistant to any antimicrobial treatment. It might jeopardize the efficacious management of S. aureus infections in both humans and animals (Crespo□Piazuelo and Lawlor, 2021). Therefore, S. aureus that is resistant to antibiotics can live in animal reservoirs and spread to humans and other animals through contact. For improved management of Staphylococcal infection in humans and animals, it's critical to monitor for the emergence of resistant infections in animal reservoirs (Zedan et al., 2023).

Conclusion

This study established the prevalence of MRSA in dogs in Gombe State as 47.8 %. Gentamycin was the most sensitive among all the antimicrobials tested and therefore the drug of choice in the study area. Most of the isolates shows resistance to multiple class of antimicrobials, hence the need for proper policies and program on antimicrobial use by the regulatory authority.

Ethics Committee Approval: This research did not involve invasive procedure as such an ethics committee approval is not required.

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References

Abbott, Y., Leggett, B., Rossney, A. S., Leonard, F. C., & Markey, B. K. (2010). Isolation rates of meticillin-resistant Staphylococcus aureus in dogs, cats and horses in Ireland. *Veterinary Record, 166*(15), 451-455.

Afshar, M.F., Zakaria, Z., Cheng, H.C., and Ahmad, N.I. (2023) Prevalence and multidrug-resistant profile of methicillin-resistant Staphylococcus aureus and methicillin-resistant Staphylococcus pseudintermedius in dogs, cats, and pet owners in *Malaysia, Veterinary World, 16*(3), 536-545.

Aliyu, Y., Reuben, R. C., Abdullahi, I. O., Olayinka, B. O., & Abdullahi, M. S. (2022). A systematic review on the prevalence of multidrug-resistant Staphylococcus aureus from milk and milk products in Nigeria. *PAMJ-One Health, 7*(15), 1-10.

Audu, Y., KU, E., Dauda, J., UM, B., & EC, O. (2022). Seroprevalence of Brucella abortus in dogs and associated risk factors in Gombe State, Nigeria. *Journal of Sustainable Veterinary and Allied Sciences, 2*(2), 98-104.

Bauer, A. W., Kirby, W. M. M., Sherris, J. C., & Turck, M. (1966). Antibiotic susceptibility testing by a standardized single disk method. *American Journal of Clinical Pathology, 45*(4), 493-496.

Benkova, M., Soukup, O., & Marek, J. (2020). Antimicrobial susceptibility testing: currently used methods and devices and the near future in clinical practice. *Journal of Applied Microbiology, 129*(4), 806-822.

Bouchami, O., Fraqueza, M. J., Faria, N. A., Alves, V., Lawal, O. U., de Lencastre, H., & Miragaia, M. (2020). Evidence for the dissemination to humans of methicillin-resistant Staphylococcus aureus ST398 through the pork production chain: a study in a Portuguese slaughterhouse. *Microorganisms, 8*(12), 1892.

Cheesbrough, M. (2005). *District laboratory practice in tropical countries, part 2.* Cambridge university press. Cambridge, UK. p45

Crespo-Piazuelo, D., & Lawlor, P. G. (2021). Livestock-associated methicillin-resistant Staphylococcus aureus (LA-MRSA) prevalence in humans in close contact with animals and measures to reduce on-farm colonisation. *Irish Veterinary Journal, 74*, 1-12.

CLSI (2019) *CLSI document M100 – performance standards for antimicrobial susceptibility testing (29th edn).* Wayne, PA: CLSI

Decline, V., Effendi, M. H., Rahmaniar, R. P., Yanestria, S. M., & Harijani, N. (2020). Profile of antibiotic-resistant and presence of methicillin-resistant Staphylococcus aureus from nasal swab of dogs from several animal clinics in Surabaya, Indonesia. *International Journal of One Health, 6*(1), 90-94.

DeLeo, F. R., & Chambers, H. F. (2009). Reemergence of antibiotic-resistant Staphylococcus aureus in the genomics era. *Journal of Clinical İnvestigation, 119* (9), 2464-2474.

Findik, A., Ciftci, A., Önyay, T., Sezener, M. G., Koçak, Y., & Gülhan, T. (2018). Determination of methicillin resistance and some genotypic characteristics of staphylococci isolated from dogs and their owners. *Turkish Journal of Veterinary & Animal Sciences, 42*(6), 549-555.

Gomez-Sanz, E., Torres, C., Ceballos, S., Lozano, C., & Zarazaga, M. (2013). Clonal dynamics of nasal Staphylococcus aureus and Staphylococcus pseudintermedius in dog-owning household members. Detection of MSSA ST398. *PLoS One, 8*(7), e69337.

Ishihara, K., Saito, M., Shimokubo, N., Muramatsu, Y., Maetani, S., & Tamura, Y. (2014). Methicillin-resistant Staphylococcus aureus carriage among veterinary staff and dogs in private veterinary clinics in Hokkaido, *Japan. Microbiology and immunology, 58*(3), 149-154.

Iverson, S. A., Brazil, A. M., Ferguson, J. M., Nelson, K., Lautenbach, E., Rankin, S. C., ... & Davis, M. F. (2015). Anatomical patterns of colonization of pets with staphylococcal species in homes of people with methicillin-resistant *Staphylococcus aureus* (MRSA) skin or soft tissue infection (SSTI). Veterinary *microbiology, 176*(1-2), 202-208.

Jang, Y., Cho, J. K., Bahk, G. J., Lim, S. K., & Lee, Y. J. (2014). Characterization of methicillin-resistant Staphylococcus spp. isolated from dogs in Korea. Japanese Journal of Veterinary Research, 62 (4), 163-170.

Kottler, S., Middleton, J. R., Perry, J., Weese, J. S., & Cohn, L. A. (2010). Prevalence of Staphylococcus aureus and methicillin-resistant Staphylococcus aureus carriage in three populations. Journal *of Veterinary Internal Medicine, 24*(1), 132-139.

Kutdang, E. T., Bukbuk, D. N., & Ajayi, J. A. (2010). The prevalence of intestinal helminths of dogs (Canis familaris) in Jos, Plateau State, Nigeria. *Researcher, 2* (8), 51-56.

Lawal, O. U., Adekanmbi, A. O., & Adelowo, O. O. (2021). Occurrence of methicillin-resistant staphylococci in the pig-production chain in Ibadan, Nigeria. *The Onderstepoort Journal of Veterinary Research, 88*(1):a1959.

Magiorakos, A. P., Srinivasan, A., Carey, R. B., Carmeli, Y., Falagas, M. E., Giske, C. G., ... & Monnet, D. L. (2012). Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. *Clinical microbiology and infection, 18*(3), 268-281.

Mustapha, M., Bukar-Kolo, Y. M., Geidam, Y. A., & Gulani, I. A. (2016). Phenotypic and genotypic detection of methicillin-resistant Staphylococcus aureus in hunting dogs in Maiduguri metropolitan, Borno State, Nigeria. *Veterinary world, 9*(5), 501.

Oh, J. Y., Chae, J. C., Han, J. I., Song, W. K., Lee, C. M., & Park, H. M. (2020). Distribution and epidemiological relatedness of methicillin-resistant Staphylococcus aureus isolated from companion dogs, owners, and environments. *Journal of Veterinary Medical Science, 82*(9), 1379-1386.

Penna, B., Silva, M. B., Soares, A. E., Vasconcelos, A. T., Ramundo, M. S., Ferreira, F. A., ... & Figueiredo, A. M. (2021). Comparative genomics of MRSA strains from human and canine origins reveals similar virulence gene repertoire. *Scientific Reports, 11*(1), 4724.

Pfeiffer, D. U. (2002). *Veterinary epidemiology- An Introduction.* The Royal Veterinary College, University of London Pp54-64

Reddy, S. B., Kumari, K. N., & Sivajothi, S. (2016). Methicillin-resistant Staphylococcus aureus (MRSA) isolated from dogs with recurrent pyoderma. *Journal of Dairy Veterinary Animal Research, 3*(2), 62-65.

Rich, M., & Roberts, L. (2004). Methicillin-resistant Staphylococcus aureus isolates from companion Veterinary Record 154(10).310

Sweeney, M. T., Lubbers, B. V., Schwarz, S., & Watts, J. L. (2018). Applying definitions for multidrug resistance, extensive drug resistance and pandrug resistance to clinically significant livestock and companion animal bacterial pathogens. Journal of Antimicrobial Chemotherapy, 73(6), 1460-1463.

Thrusfield, M. (2005). Veterinarinary epidemiology, 3rd edition Blackwell science ltd London, UK Pp 228-246.

Tong, S. Y., Davis, J. S., Eichenberger, E., Holland, T. L., & Fowler Jr, V. G. (2015). Staphylococcus aureus infections: epidemiology, pathophysiology, clinical manifestations, and management. *Clinical Microbiology Reviews, 28*(3), 603-661.

Vincze, S., Stamm, I., Kopp, P. A., Hermes, J., Adlhoch, C., Semmler, T., ... & Walther, B. (2014). Alarming proportions of methicillin-resistant Staphylococcus aureus (MRSA) in wound samples from companion animals, Germany 2010–2012. *PloS one, 9*(1), e85656.

Wikipedia; 2006. Available from: http:// www.en.m.wikipe-dia/wiki/GombeState. [Last accessed on 12th May, 2024].

Yakubu, Y., Gaddafi, M. S., Musawa, A. I., Garba, B., Bitrus, A. A., Emeka, A. J., ... & Barka, S. A. (2022). Evidence of methicillin resistant (MRSA) in pet and stray dogs within sokoto metropolis, Nigeria. *Folia Veterinaria, 66*(2), 54-60.

Yaser, T. H., Ahmed, A. M., Humam, A. S., & Adnan, J. S. (2015). Molecular study on methicillin-resistant Staphylococcus aureus strains isolated from dogs and associated personnel in Jordan. Asian *Pacific Journal of Tropical Biomedicine, 12*, 855-860.

Zedan, A., Alatfeehy, N., & Marouf, S. (2023). Isolation and antibiogram profiles of staphylococcus aureus isolates from cow milk and dog samples. *Journal of Applied Veterinary Sciences, 8*(1), 38-44.

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A Comparative approach to phyllodes tumors in women and phyllodes-like tumors in female dogs

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ABSTRACT

Mammary gland tumors, a significant concern for both humans and dogs, often carry a malignant prognosis. The diagnosis of these tumors is primarily based on their histopathological appearance in both species. This study delves into the pathological features of Phyllodes tumors in female dogs, a type not typically included in the histopathological classification of canine mammary tumors but well-documented in women. By comparing the recently described Phyllodes tumor cases in dogs with those in women, we aim to shed light on the shared vulnerability of women and female dogs to these tumors. The increasing number of studies exploring the common features between breast cancer in women and mammary tumors in female dogs makes the comparative evaluation of this rare tumor type in both species not only interesting but also crucial.

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Introduction

Mammary glands play a vital role in providing essential nutrition and passive immunity to newborns. Structurally, they are ductal, lobular, and alveolar organs embedded in fibrovascular connective and fatty tissue (Sorenmo et al., 2011). Similar to the human mammary gland, in dogs, the ductal system begins with the papillary ducts of the nipple and culminates with the development of secretory alveoli. This process is vital for the health and well-being of the animal (Silver, 1966). What is more, studies conducted in recent years have revealed that there are shared clinicopathological, morphological, and biochemical features between human and canine mammary gland cancers (Kumaraguruparan et al., 2006; Liu et al., 2014; Reis et al., 2020; Seung et al., 2020). This shared knowledge and collaboration between human and veterinary medicine fields are crucial in advancing our understanding and treatment of mammary tumors in both species. In recent years, breast cancer has been the most frequently diagnosed type of cancer, with an estimated number of 2.3 million new cases worldwide, according to GLOBOCAN 2020 data, and ranks 5th (fifth) in the list of cancer-related deaths (Sung et al., 2021). Among domestic animals, dogs have the highest incidence of mammary tumors, with a rate of 25-42%. As in humans, mammary tumors are among the most common tumors in dogs (Klopfleisch et al., 2011). Mammary tumors are common in females but rare in male dogs (Goldschmidt et al., 2016). The frequency of mammary gland tumors in dogs varies by geographical region and is directly linked to the age at which ovariectomy is performed. Extensive studies have consistently shown that early ovariectomy significantly reduces the risk of later mammary gland neoplasia. Reports indicate a staggering 99.5% reduction in the risk of developing mammary tumors when

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ovariectomy is performed before the first estrus cycle. Furthermore, the risk is reduced by 92% and 74% when ovariectomy is performed before the second and third cycles, respectively. Even after the fourth cycle, ovariectomy still provides some level of protection against developing mammary neoplasms (Beaudu-Lange et al., 2021; Schneider et al., 1969). The average age for the occurrence of mammary tumors in dogs is typically between 10 and 11 years. Benign neoplasms tend to appear in dogs between 7 and 9 years of age, while malignant neoplasms are more common in older dogs, typically between 9 and 11 years of age. The development of tumors varies based on the specific breed and size of the dog. Mammary gland neoplasms tend to occur at a younger age in large breeds compared to small breeds (Goldschmidt et al., 2016; Murphy, 2008). The use of exogenous hormones, both progestins and estrogen, may cause mammary gland neoplasms to appear at an earlier age. Please remember the following important information: Low doses of progestin can encourage the growth of non-cancerous neoplasms, while combinations of progestin and estrogen may influence the development of cancerous neoplasms. When many cats and dogs are brought to the clinic due to masses, these masses are typically found in more than one gland. Tumors are usually detected in the caudal mammary glands. Smaller neoplasms (less than 1 cm in diameter) are more likely to be non-cancerous (benign), while larger neoplasms (greater than 3-5 cm in diameter) are more often cancerous (malign). It is essential to have all mammary nodules evaluated histologically to distinguish malignancy, regardless of their size (Murphy, 2008).

The features listed so far, such as its higher incidence compared to other cancers, its prevalence in middle and upper middle age, the risk of tumor development in the mammary gland after exogenous hormone use, and the relationship between mass size and malignancy, have many commonalities in both species.Phyllodes tumors of the breast are a captivating and rare category of fibroepithelial neoplasms. They bear a striking resemblance to intracanalicular fibroadenomas at the benign end of the spectrum, but they display enhanced stromal cellularity and a distinctive leaf-like architecture. Within this fascinating tumor, the stromal part is considered neoplastic and plays a pivotal role in determining whether the tumor is benign, borderline, or malignant based on the intricate pathophysiology of epithelial-stromal interactions (Tan et al., 2020). This rare tumor accounts for less than 1% of all breast tumors in women and 2-3% of all fibroepithelial tumors (Cook et al., 2024).

Although mammary tumors in women and dogs have many histologic and clinical features in common, the fact that this unusual tumor, which is also rare in women, has never been included in the classification of canine mammary tumors caught the authors' attention. This article aims to delve into the intriguing topic of phyllodes tumors of the breast and explore existing literature for evidence of its presence in female dogs.

Classification of mammary gland tumors

In women, breast cancer presents as a complex disease with numerous subtypes, each displaying distinct biological properties that significantly impact treatment responses and clinical results. The heterogeneity of breast cancer affects diagnosis, treatment, and, therefore, prognosis (Eliyatkın et al., 2015). Identifying whether a mammary gland tumor is benign or malignant in dogs can be complex and risky. Although rapid growth, tumor size, and skin ulceration may indicate malignancy, distinguishing between benign and malignant tumors using cytological diagnostic methods is challenging. Studies have shown a diagnostic accuracy rate as low as approximately 20% due to factors such as cellular differentiation and mixed tumor structure. (Goldschmidt et al., 2016). Histopathology stands as the gold standard for establishing an accurate diagnosis, including tumor type, malignancy, grade, and prognosis. Through histopathological evaluation, we gain insight into the tissue architecture of the tumor and its intricate connections with the surrounding tissue. Benign tumors are identifiable by their well-defined boundaries, soft margins, and dense fibrous connective tissue. In contrast, malignant tumors exhibit irregular margins, immature fibrous connective tissue, and multifocal necrosis fueled by rapid neoplastic cell proliferation. These characteristics underscore the critical importance of histopathology in guiding treatment decisions and predicting patient outcomes (Goldschmidt et al., 2016; Murphy, 2008).
Histopathological classification of mammary gland tumors

The traditional classification of breast cancer is performed using a microscopic method, known as histological or morphological classification. This method is based on the cancer cells' size, shape, and arrangement. It also takes into account the region in which breast cancer develops in women. Two critical questions are addressed in this classification: First, is the tumor confined to the area where it originated within the breast epithelial component, or if it has

spread to surrounding tissues? Second, it determines whether the tumor originated in the milk duct or the mammary gland (do Nascimento & Otoni, 2020). If the cancer develops in the milk ducts, it is classified as ductal; if it develops in the mammary gland alveoli, it is termed lobular. It is worth noting that, although rare, breast cancer can develop in the connective tissue outside the mammary glands and ducts (do Nascimento & Otoni, 2020).

The spread of breast cancer also influences its type. In this regard, breast cancer is classified into two types: in situ and invasive. In situ, breast cancer is limited to the area where it originated and did not spread to the rest of the mammary gland tissue. Invasive breast cancer, on the other hand, refers to the type that spreads into the surrounding breast tissue. Among the types of breast cancer, the most predominant type, accounting for 70% of cases, is invasive ductal carcinoma NOS (no specific type). Invasive lobular carcinoma, a specific histological subtype, ranks second, accounting for approximately 10% of cases. Subtypes such as tubular, mucinous, cribriform, and papillary carcinoma are generally associated with favorable prognoses. Medullary and mucinous carcinoma incidence is approximately 5%, while tubular carcinoma varies between 1-5%. Other microscopic types are rare (DeSantis et al., 2019; do Nascimento & Otoni, 2020).

Canine mammary tumors have been classified histologically for many years, starting with studies conducted by the World Health Organization (Misdorp et al., 1999). This initial classification focused on histological and descriptive morphological features of breast tumors and histological and prognostic features related to increased malignancy. Goldschmidt et al. (2011) developed a more detailed alternative classification that emphasizes the morphology of neoplastic cells and the involvement of myoepithelial cells in the neoplastic process. In the 2018 consensus on "Classification and grading of canine mammary tumors" organized by the Oncology-Pathology working group of the Veterinary Cancer Society (VCS) and the American Academy of Veterinary Pathology (ACVP), it was suggested to use Goldschmidt et al. (2011) classification. According to this classification, mammary tumors in dogs are classified under eight main headings, including Hyperplasia/Dysplasia, Benign tumors, Malignant epithelial tumors, Malignant epithelial tumors-special types, Malignant mesenchymal tumors-sarcoma, Malignant mixed mesenchymal tumors-Carcinosarcoma, Nipple tumors, and Hyperplasia/dysplasia of the nipple (Goldschmidt et al., 2011).

Beyond classifying by histomorphological features, the histological grading system is a crucial prognostic indicator for canine mammary tumors. This system uses a numerical method to evaluate three morphological features: tubule formation, nuclear pleomorphism, and mitotic number. It allows for evaluating the heterogeneity of mammary tumors in dogs, as well as how to assess complex and mixed tumors, and the variability in the size of nuclei and nucleoli. This simplifies histological interpretation and enables the prediction of the biological behavior of mammary gland carcinomas based on determining the tumor grade along with its histological subtype (Goldschmidt et al., 2016).

Phyllodes tumors in women

The Phyllodes tumor is a rare fibroepithelial neoplasm, accounting for less than 1% of all primary breast tumors (Tan et al., 2020). It was initially described by Chelios in 1828 as a hydatid cyst-like structure in the breast and later named Cystosarcoma Phyllodes by Johannes Müller in 1838 (Nabi et al., 2013). Histologically, it is a biphasic tumor consisting of benign epithelial elements and a cellular stroma formed by spindle cells (Tavassoli, 1999).

Although similar to fibroadenomas, Phyllodes tumors require careful examination due to their stromal cellularity, local recurrence, and malignant potential. These tumors occur in women aged 4-5 decades, but can also be seen in adolescents, though less frequently (WHO, 1983). Studies have shown different origins of stromal cells in Phyllodes tumors, with fibroblasts and myofibroblasts being the most common (Aranda et al., 1994).

Phyllodes tumors present as a palpable mass in the breast, and other symptoms include dilated skin veins, skin discoloration, nipple retraction, fixation to the skin or muscle, skin ulcers, skin necrosis, or palpable lymph nodes (Chen et al., 2005).

Prognosis is influenced by tumor size, development pattern, stromal atypia, and mitotic activity, although there is no clear correlation between histological parameters and clinical course. The local recurrence rate in benign and malignant cases is reported to be 15-25%, and metastasis in high-grade tumors has been reported at 20% (Norris & Taylor, 1967). Progesterone and estrogen-binding proteins have been detected in Phyllodes tumors (Lewko et al., 1990).

Phyllodes tumors may grow rapidly during adolescence but typically have a low malignancy potential. The effects of hormonal stimulation during adolescence and exposure during the intrauterine and

prepubertal periods on breast morphology and malignancy are controversial. A genetic predisposition may be present if there is a history of ovarian cancer in the patient's mother or grandmother (Tan & Köprülü, 2013). Long-term follow-up is important, as local recurrence and distant metastasis may occur, and in some cases, the disease may result in death (Barth, 1999).

The connection between clinical behavior and histological type in phyllodes tumors is debatable (Contarini et al., 1982). Even though numerous biological markers have been scrutinized in phyllodes tumors, along with their correlation to tumor grade, their application in defining grade and predicting clinical behavior in individual cases remains constrained (Tan et al., 2016). While metastases are more common in those with malignant histological structures, they have also been observed in benign and borderline types (Norris & Taylor, 1967). No distinct clinical features can differentiate phyllodes tumors from other breast tumors. Phyllodes tumors are unequivocally considered de-novo lesions originating from the periductal and specialized lobular stroma. The initiation of tumorigenesis is contingent upon epithelial–stromal interactions. However, the histological similarity between fibroadenoma and phyllodes tumors undeniably raises the question of pathogenetic closeness (Tan et al., 2016). However, histological examination sets it apart from fibroadenoma by the presence of a broad leaf-like stroma rich in stromal cells (Sarsu et al., 2015). On the other end of the spectrum, the malignant phyllodes tumor could easily be mistaken for primary breast sarcoma or spindle cell metaplastic carcinoma. These compelling similarities highlight the importance of accurate diagnosis and further research (Tan et al., 2016). In certain aggressive phyllodes tumors, the stromal overgrowth is particularly conspicuous, making it challenging to distinguish the epithelial component. The stroma of these tumors may exhibit diverse sarcomatous differentiation, including prevalent liposarcoma and less common myosarcoma, angiosarcoma, chondrosarcoma, and osteosarcoma. A spindle cell metaplastic breast carcinoma presents with varying proportions of a malignant epithelial component such as squamous, glandular, or adenosquamous cells. These unique carcinomas can also lack typical epithelial elements or exhibit diverse mesenchymal differentiation. The presence of ductal carcinoma in situ alongside a malignant mammary spindle cell tumor strongly signals a diagnosis of metaplastic carcinoma. Additionally, primary breast sarcomas are exceedingly rare, and sarcomas metastatic to the breast are exceptionally uncommon. They do not exhibit unique histological features that differentiate them from phyllodes tumors or metaplastic breast carcinomas. It's important to consider a patient's history of previous or metastatic sarcoma, and thorough imaging and clinical correlation can be extremely beneficial. Besides, demonstrating diffuse cytokeratin or p63 immunoreactivity in the malignant spindle cells supports a confident diagnosis of metaplastic carcinoma. Harnessing the power of immunohistochemical characterization of tumors is a crucial tool for accurately distinguishing phyllode tumors from other malignant breast tumors.

Mammography and ultrasound, which are employed in diagnosing breast masses, are not very reliable for grading phyllodes tumors and differentiating them from fibroadenomas. Although fine needle aspiration biopsy is not the preferred preoperative histopathological diagnostic method for phyllodes tumors due to its high false negativity rate, core biopsy results are more reliable for diagnosis. The most effective treatment is wide surgical excision. Lymph node involvement is rare, so lymph node dissection is not recommended. Even though age, surgical approach, tumor diameter, and mitotic activity are important for developing local recurrence, tumor cell positivity at the surgical margin is the most crucial factor. To prevent local recurrence, wide excision with negative surgical margins (at least 1-2 cm) is recommended, irrespective of the histopathological type in the surgical approach (Foxcroft et al., 2007).

The effectiveness of chemotherapy and radiotherapy is uncertain. Radiotherapy may enhance local control but may not improve overall survival in borderline and malignant phyllodes tumors. The 5-year overall survival rates for patients with phyllodes tumors were 91% and 82% for benign and malignant cases, respectively, in the MD Anderson series; in the analysis of SEER data, it was 91% for malignant cases. The primary factors affecting overall survival and systemic metastasis are findings of stromal overgrowth, positive surgical margins, and cytonuclear atypia. Malignant phyllodes tumors mainly spread to the lungs through hematogenous spread and may rarely spread by the lymphatic route (Macdonald et al., 2006).

Histopathology of phyllodes tumor

Phyllodes tumors can be macroscopically benign, round, encapsulated, and with limited masses. Malignant and borderline Phyllodes tumors have a cross-sectional surface that is curved, circumscribed, and protruding to the outer surface in an infiltrative,

leaf-like pattern. Skin ulcers, bleeding, and cystic changes may occur in large lesions (Yoneyama et al., 2020). Phyllodes tumors generally exhibit benign cytomorphology. They have a lower epithelial/stromal ratio than fibroadenomas and fibromyxoid stromal clusters. Higher-grade phyllodes tumors may show higher nuclear atypia and cellularity. Multinucleated tumor cells and significant stromal anaplasia have been reported in malignant phyllodes tumors (El Hag et al., 2010).

Phyllodes tumors are categorized as benign, borderline, or malignant based on specific criteria. Benign tumors have 0-4 mitosis/10x magnification, minimal or moderate stromal cellularity, minimal or moderate stromal overgrowth, and intact surgical margins. Borderline tumors exhibit 5-9 mitoses/10x magnification, moderate stromal cellularity, atypia, overdevelopment, and intact or invaded surgical margins. Malignant tumors feature over 10 mitoses/10x magnification, moderate or advanced stromal cellularity, atypia, overgrowth, and invaded surgical margins.

The following markers are used in immunohistochemistry to diagnose Phyllodes tumors. Epithelial cells: Cytokeratin, Estrogen receptor (ER), Progesterone receptor (PR), Gross cystic disease fluid protein 15 (GCDFP-15). Stromal cells: Vimentin, CD34, Bcl-2, Estrogen receptor beta, c-KIT, p53, Ki-67 (High c-KIT, p53, and Ki-67 expressions mean high-grade phyllodes tumor.) Negatively labeled in immunohistochemistry: Stromal cells: Cytokeratin, p40, p63 (except malignant phyllodes tumor) (Cimino-Mathews et al., 2014; Chia et al., 2012). Since malignant proliferation is generally in the stromal component (Cook et al., 2024), the immunohistochemical reaction of markers such as Bcl-2, c-KIT, p53, and Ki-67 are preferred to evaluate in the stromal cells. Detection of diffuse cytokeratin or p63 immunoreactivity in the malignant spindle cells strongly supports a diagnosis of metaplastic carcinoma (Tan et al., 2016). However, pathologists should be cautious when interpreting focal keratin or p63 expression, as these features have been observed in stromal cells of phyllodes tumors. The use of p40 in a similar diagnostic context is currently under investigation, showing higher specificity but lower sensitivity than p63. Nevertheless, it is important to note that p63 and p40 may occasionally exhibit staining in stromal cells of phyllodes tumors. CD34 reactivity, well established in the stromal cells of phyllodes tumors, has been found to have an inverse relationship with adverse histological features. This holds significance if CD34 is to be employed for diagnostic purposes in distinguishing high-grade spindle cell lesions of the breast, given the lower likelihood of CD34 expression in malignant phyllodes tumors. Further markers, such as Bcl-2 (frequently expressed in phyllodes tumors), CD117 (demonstrating increased expression in higher-grade phyllodes tumors), and sarcoma-specific molecular cytogenetic alterations, can serve as valuable diagnostic adjuncts (Cimino-Mathews et al., 2014; Chia et al., 2012; Noronha et al., 2011).

Phyllodes tumor cases identified in dogs

Mixed tumors containing epithelial and mesenchymal components are included in the histological classification of canine mammary tumors and are frequently encountered. However, neoplasms in which epithelial and mesenchymal (fibroepithelial) components are organized similarly to phyllodes tumors have rarely been described in dogs. According to our research, Shahzamani et al. (2013) were the first to describe a benign phyllodes tumor in a female dog. This study reported that this case was the first description of a phyllodes tumor in the canine species. In another study published by De Araújo et al. (2014), the anatomopathological features and immune phenotype of five phyllodes tumors diagnosed in female dogs were reported for the first time. All phyllodes tumors diagnosed in this study were detected as solitary. In canine mammary tumors, the expression of hormone receptors is associated with a good prognosis. According to the literature, both benign and malignant phyllodes tumors have a well-differentiated epithelial component with estrogen and progesterone receptors. In the study, immunohistochemistry results were consistent for ER and PR. The stroma of high-grade malignant phyllodes tumors in women may have different biological behavior than in dogs. Studies involving the expression of the c-KIT proto-oncogene and the development of phyllodes tumors in dogs may elucidate this conundrum. However, while c-KIT, which marks the stromal component of tumors, was positive in phyllodes cases in women, De Araújo et al. (2014) did not label any of the cases presented, and this result is consistent with Tse et al. (2004), who found an increase in c-KIT expression. Some morphological features of phyllodes tumors in dogs are defined as follows (De Araújo et al., 2014). High-grade malignant phyllodes tumors are characterized by marked stromal cellularity, high mitotic index, and marked stromal cellular pleomorphism. Pseudoangiomatous stromal hyperplasia may also be seen in high-grade malignant phyllodes tumors. Occasionally, the stroma contains

angiosarcoma, liposarcoma, chondrosarcoma, myosarcoma, or osteosarcoma. Mixed canine mammary tumors, characterized by a stromal component with myoepithelial and mesenchymal cell proliferation giving rise to a myxoid, chondroid, or bone matrix with epithelial cell proliferation, are among the most common tumor types (De Araújo et al., 2014).

Conclusion

The striking parallels in the epidemiological and clinicopathological features of spontaneous tumors in companion animals and their human counterparts, along with the shared exposure to similar risk factors, have elevated companion animals as crucial models in human cancer research. Cats and dogs, as companion animals, experience expedited cancer development due to their shorter lifespans. This unique attribute presents valuable opportunities for comparative oncology studies (Cannon, 2015).

Veterinary pathologists must be cautious in recognizing this tumor because phyllodes tumors have epithelial, mesenchymal, and even associated myoepithelial components like mixed tumors. Definitive diagnosis of phyllodes tumors can be achieved by identifying excessive intratumoral stroma and accompanying leaf-like structures in the epithelial component, rarely seen in mixed tumors. According to the data presented in this article, the observed morphological and immunophenotypic features indicate similarities between phyllodes tumors diagnosed in female dogs and women. The presence of this neoplasm in dogs may serve as a model for women, and revealing such similarities for future studies on common features in pathogenesis is of great importance for the discipline of pathology.

References

Aranda, F. I., Laforga, J. B., & López, J. I. (1994). Phyllodes tumor of the breast. *Pathology - Research and Practice, 190*(5), 474-481.

Barth, R. J. (1999). Histologic features predict local recurrence after breast conserving therapy of phyllodes tumors. *Breast Cancer Research and Treatment, 57*(3), 291-295.

Beaudu-Lange, C., Larrat, S., Lange, E., Lecoq, K., & Nguyen, F. (2021). Prevalence of reproductive disorders including mammary tumors and associated mortality in female dogs. *Veterinary Sciences, 8*(9), 184.

Cannon, C. (2015). Cats, cancer and comparative oncology. *Veterinary Sciences, 2*(3), 111-126.

Chen, W. H., Cheng, S. P., Tzen, C. Y., Yang, T. L., Jeng, K. S., Liu, C. L., & Liu, T. P. (2005). Surgical treatment of phyllodes tumors of the breast: retrospective review of 172 cases. *Journal of Surgical Oncology, 91*(3), 185–194.

Chia, Y., Thike, A. A., Cheok, P. Y., Yong-Zheng Chong, L., Man -Kit Tse, G., & Tan, P. H. (2012). Stromal keratin expression in phyllodes tumours of the breast: a comparison with other spindle cell breast lesions. *Journal of Clinical Pathology, 65*(4), 339-347.

Cimino-Mathews, A., Sharma, R., Illei, P. B., Vang, R., & Argani, P. (2014). A Subset of malignant phyllodes tumors express p63 and p40: A diagnostic pitfall in breast core needle biopsies. *American Journal of Surgical Pathology, 38*(12), 1689-1696.

Cook, W., Lee, C.S., Tan, P.H. (2024). Epithelial carcinomas arising within phyllodes tumours of the breast: A review of their pathological characteristics. *Pathobiology, 91*(2), 144-157.

Contarini, O., Urdaneta, L. F., Hagan, W., & Stephenson Jr., S. E. (1982). Cystosarcoma phylloides of the breast: A new therapeutic proposal. *American Surgeon, 48*(4), 157–166.

De Araújo, M. R., Damasceno, K. A., De Oliveira Gamba, C., Campos, C. B., Campos, L. C., Reis, D. C. D., De Souza, C. M., & Cassali, G. D. (2014). Morphological and immuno-histochemical characterization of five phyllodes mammary gland tumors in dogs. *Online Journal of Veterinary Research, 18*(8), 688–695.

DeSantis, C. E., Ma, J., Gaudet, M. M., Newman, L. A., Miller, K. D., Goding Sauer, A., Jemal, A., & Siegel, R. L. (2019). Breast cancer statistics, 2019. *CA: a cancer journal for clinicians, 69*(6), 438-451.

Do Nascimento, R. G. D., & Otoni, K. M. (2020). Histological and molecular classification of breast cancer: what do we know? Mastology, 30, 1-8.

El Hag, I. A., Aodah, A., Kollur, S. M., Attallah, A., Mohamed, A. A., & Al-Hussaini, H. (2010). Cytological clues in the distinction between phyllodes tumor and fibroadenoma. *Cancer Cytopathology, 118*(1), 33–40.

Eliyatkın, N., Yalçın, E., Zengel, B., Aktaş, S., & Vardar, E. (2015). Molecular Classification of Breast Carcinoma: From Traditional, Old-Fashioned Way to A New Age, and A New Way. *The Journal of Breast Health, 11*(2), 59–66.

Foxcroft, L. M., Evans, E. B., & Porter, A. J. (2007). Difficulties in the pre-operative diagnosis of phyllodes tumours of the breast: a study of 84 cases. *Breast (Edinburgh, Scotland), 16*(1), 27-37.

Goldschmidt, M. H., Peña, L., Rasotto, R., & Zappulli, V. (2011). Classification and grading of canine mammary tumors. *Veterinary pathology, 48*(1), 117-131.

Goldschmidt, M. H., Peña, L., & Zappulli, V. (2016). *Tumors of the mammary gland. In D. J. Meuten (Ed.),* Tumors in Domestic Animals (5th ed.). John Wiley & Sons, Inc.

Klopfleisch, R., von Euler, H., Sarli, G., Pinho, S. S., Gärtner, F., & Gruber, A. D. (2011). Molecular carcinogenesis of canine mammary tumors: news from an old disease. *Veterinary Pathology, 48*(1), 98–116.

Kumaraguruparan, R., Karunagaran, D., Balachandran, C., Manohar, B. M., & Nagini, S. (2006). Of humans and canines: a comparative evaluation of heat shock and

canine mammary tumors: news from an old disease. *Veterinary Pathology, 48*(1), 98–116.

Kumaraguruparan, R., Karunagaran, D., Balachandran, C., Manohar, B. M., & Nagini, S. (2006). Of humans and canines: a comparative evaluation of heat shock and apoptosis-associated proteins in mammary tumors. *Clinica Chimica Acta; International Journal of Clinical Chemistry, 365*(1-2), 168–176.

Lewko, W. M., Vaghmar, R., Maleckar, J. R., Husseini, S., Montgomery, C. A., Thurman, G. B., & Oldham, R. K. (1990). Cultured breast cystosarcoma phylloides cells and applications to patient therapy. *Breast Cancer Research and Treatment, 17*(2), 131–138.

Liu, D., Xiong, H., Ellis, A. E., Northrup, N. C., Rodriguez, C. O., Jr., O'Regan, R. M., Dalton, S., & Zhao, S. (2014). Molecular homology and difference between spontaneous canine mammary cancer and human breast cancer. *Cancer Research, 74*(18), 5045–5056.

Macdonald, O. K., Lee, C. M., Tward, J. D., Chappel, C. D., & Gaffney, D. K. (2006). Malignant phyllodes tumor of the female breast: association of primary therapy with cause-specific survival from the Surveillance, Epidemiology, and End Results (SEER) program. *Cancer, 107*(9), 2127–2133.

Misdorp, W., Else, R. W., Hellmén, E., & Lipscomb, T. P. (1999). *Histological classification of mammary tumors of the dog and the cat.* In Armed Forces Institute of Pathology eBooks (Vol. 7, pp. 1–59).

Murphy, S. (2008). Mammary tumours in dogs and cats. In *Practice, 30*(6), 334–339.

Nabi, J., Akhter, S. M., & Authoy, F. N. (2013). A case of large phyllodes tumor causing "rupture" of the breast: a unique presentation. *Case Reports in Oncological Medicine, 2013*, 871292.

Noronha, Y., Raza, A., Hutchins, B., Chase, D., Garberoglio, C., Chu, P., Weiss, L., Wang, J. (2011). CD34, CD117, and Ki-67 expression in phyllodes tumor of the breast: an immunohistochemical study of 33 cases. *International Journal of Surgical Pathology, 19*(2), 152-158.

Norris, H. J., & Taylor, H. B. (1967). Relationship of histologic features to behavior of cystosarcoma phyllodes.Analysis of ninety-four cases. *Cancer, 20*(12), 2090-2099.

Reis, J., Oliveira, T., Pereira, A., Infante, P., Leal, N., & Faísca, P. (2020). Microtomographic characterization of calcifications in canine mammary tumours. *Veterinary and Comparative Oncology, 18*(3), 281-291.

Sarsu, S. B., Parmaksız, M. E., & Ucak, R. (2015). A giant cystosarcoma phylloides of the mammary gland in an adolescent: A Case Report. *Çocuk Cerrahisi Dergisi*.29(2), 57-60.

Schneider, R., Dorn, C. R., & Taylor, D. O. (1969). Factors influencing canine mammary cancer development and postsurgical survival. Journal of the National Cancer Institute, 43(6), 1249–1261.

Seung, B. J., Cho, S. H., Kim, S. H., Lim, H. Y., & Sur, J. H. (2020). Quantitative analysis of HER2 mRNA expression by RNA in situ hybridization in canine mammary gland tumors: Comparison with immunohistochemistry analysis. PloS one, 15(2), e0229031.

Shahzamani, P., Takhtfooladi, M. A., & Daneshi, M. H. (2013). Phyllodes tumor of mammary gland in a dog: Case report. *Global Veterinaria, 10*(2), 239–242.

Silver, I. A. (1966). The anatomy of the mammary gland of the dog and cat. *The Journal of Small Animal Practice, 7* (11), 689–696.

Sorenmo, K. U., Rasotto, R., Zappulli, V., & Goldschmidt, M. H. (2011). Development, anatomy, histology, lymphatic drainage, clinical features, and cell differentiation markers of canine mammary gland neoplasms. *Veterinary pathology, 48*(1), 85–97.

Sung, H., Ferlay, J., Siegel, R. L., Laversanne, M., Soerjomataram, I., Jemal, A., & Bray, F. (2021). Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. *CA: a Cancer Journal for Clinicians, 71*(3), 209–249.

Tan, B.Y., Acs, G., Apple, S.K., Badve, S., Bleiweiss, I.J., Brogi, E., Calvo, J.P., Dabbs, D.J., Ellis, I.O., Eusebi, V., Farshid, G., Fox, S.B., Ichihara, S., Lakhani,. S.R., Rakha, E.A., Reis-Filho, J.S., Richardson, A.L., Sahin, A., Schmitt, F.C., Schnitt, S.J., Siziopikou, K.P., Soares, F.A., Tse, G.M., Vincent-Salomon, A., Tan, P.H. (2016). Phyllodes tumours of the breast: a consensus review. *Histopathology, 68*(1), 5–21.

Tan, M., & Köprülü, Ş. (2013). Phyllodes tumor: A case report. *Haydarpasa Numune Medical Journal, 53*(1), 58–63.

Tan, P. H., Ellis, I., Allison, K., Brogi, E., Fox, S. B., Lakhani, S., Lazar, A. J., Morris, E. A., Sahin, A., Salgado, R., Sapino, A., Sasano, H., Schnitt, S., Sotiriou, C., van Diest, P., White, V. A., Lokuhetty, D., & Cree, I. A., & WHO Classification of Tumours Editorial Board (2020). The 2019 World Health Organization classification of tumours of the breast. *Histopathology, 77*(2), 181–185.

Tavassoli, F. A. (1999). *Biphasic tumors. In Tavassoli F. A. (Ed.), Pathology of the breast (2nd ed.)*. Stamford, CT: Appleton & Lange; p. 571–623.

Tse, G. M. K., Putti, T. C., Lui, P. C. W., Lo, A. W. I., Scolyer, R. A., Law, B. K. B., Karim, R., & Lee, C. S. (2004). Increased c-kit (CD117) expression in malignant mammary phyllodes tumors. *Modern Pathology, 17*(7), 827–831.

The World Health Organization. Histological typing of breast tumors. (1983). *Neoplasma, 30*(1), 113–123.

Yoneyama, K., Nakagawa, M., & Hara, A. (2020). Benign phyllodes tumor with hemorrhagic cyst in a 14-year-old girl: A case report. *International Journal of Surgery Case Reports, 67*, 114–116.