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Navigating the resistance: Current perspectives on ectoparasite control in veterinary medicine

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ABSTRACT

Ectoparasites present a global challenge to animal health, affecting a wide range of species. These parasites can infect both animals and humans, leading to significant health issues. The impact of ectoparasites extends beyond health, as they can also reduce the productivity of livestock. Moreover, many ectoparasite species act as vectors for bacterial, viral, or parasitic pathogens, thereby posing significant risks to human and animal health. Historically, agents such as organochlorides, organophosphates, carbamates, insect growth regulators, neonicotinoids, spinosad, fipronil, avermectins, isoxazolines, and synthetic pyrethroids have been extensively used to treat and prevent ectoparasitic infestations. However, the extensive use of these chemicals has resulted in the development of resistance among many target species, potentially reducing the effectiveness of these treatments. This article aims to survey the current status of resistance in ectoparasites to active pharmaceutical ingredients used in veterinary medicine. It also seeks to update the understanding of resistance mechanisms and explore measures that can be implemented to prevent the development of resistance.

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Introduction

The issue of ectoparasitic drug resistance has been progressively increasing and has become a problem over the last three decades (McNair, 2015). Ectoparasitic drug resistance is characterized as “the capacity of a parasite strain to survive and/or proliferate despite the administration and absorption of a drug at doses equal to or higher than those typically recommended but within the subject's tolerance” (WHO, 2001). Ectoparasitic drug resistance refers to the selection of specific heritable traits within a population of ectoparasites in response to exposure to an active pharmaceutical ingredient. Ectoparasites develop resistance to drugs by different mechanisms, including receptor modifications that prevent the binding of drugs to their targets or metabolic changes that lead to enzymatic degradation and rapid elimination of the drug. As a result of resistance, the recommended standard dosage of the active pharmaceutical ingredient may lead to a significant increase in the proportion of the population that does not respond to the active pharmaceutical ingredient used (Coles and Dryden, 2014). Resistance to one ectoparasiticide may develop towards another ectoparasiticide, either via side or cross-resistance. Side resistance describes a reduced susceptibility to multiple ectoparasiticides within the same chemical class, whereas

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cross-resistance refers to a decreased susceptibility to multiple ectoparasites from distinct chemical groups with a similar mode of action (Abbas et al., 2014).

The development of resistance reduces drug efficacy and leads to increase in parasite populations, which increases treatment costs in companion and livestock animals and also economic losses due to decreased productivity in food animals. The aim of this article is to describe the current status of ectoparasite resistance and perspectives on ectoparasite control. The scope of this article is to provide information on the status of ectoparasiticide resistance in ticks, mites, lice, and fleas, possible resistance mechanisms, methods of detection, strategies to delay the resistance, and monitoring programs. Mites, arthropods, mosquitoes that are non-habitable on animals, and endoparasites are beyond the scope of this article.

Current status of ectoparasite resistance

Ticks

The majority of reports on tick resistance mainly focus on *Rhipicephalus* (formerly *Boophilus*) *microplus* which is a single-host tick that prefers cattle and buffalo. The fact that this tick completes its complete life cycle on a single species of animal, makes it more susceptible to post-treatment resistance selection compared to ticks that feed on multiple hosts. At present *Rhipicephalus microplus* is not endemic to Europe, but it is prevalent in subtropical and tropical regions worldwide. Notably, this species has been eradicated in the United States. Various studies have examined the global resistance status of this tick (FAO, 2004; Abbas et al., 2014). Research carried out in Brazil has revealed the resistance of *R. microplus* to all existing classes of systemic-acting and contact acaricides, organophosphates, and combinations of pyrethroids (Valsoni et al., 2020). In addition, *R. microplus*, collected from the Indian states of Madhya Pradesh, Punjab, and Uttar Pradesh, was found to be resistant to both deltamethrin and cypermethrin (Sagar et al., 2019). Reports of resistance to avermectins in this tick species have emerged from Brazil (Martins and Furlong, 2001; Klafke et al., 2006; Cruz et al., 2015) and Mexico (Perez-Cogollo et al., 2010). Ivermectin resistance was first reported in ten brown dog tick (*Rhipicephalus sanguineus*) populations in Mexico (Rodriguez-Vivas et al., 2017). An *in vitro* study of dogs, conducted in Spain, reported a high prevalence of resistance to deltamethrin and a variable susceptibility to propoxur in *R. sanguineus* ticks. Notwithstanding, all tested *R. sanguineus* have been shown to remain sensitive to amitraz (Estrada-Pena, 2005). Permethrin resistance was investigated in the United States. All thirty-one of *R. sanguineus* were identified as resistant to permethrin, and nine of these were highly resistant to permethrin (Eiden et al., 2015). Ticks were collected from Porto Alegre, Brazil, to research acaricide resistance in *R. sanguineus* s.s. populations, seven samples with resistance ratios (RR) ranging from 2.32 to 5.67 were considered to be resistant to deltamethrin. Three of the five analyzed tick populations were resistant to fipronil, with resistance rates ranging from 2.56 to 13.83, whereas ivermectin resistance rates ranged from 1.54 to 2.14. This is the first study to document the presence of acaricide-resistant *R. sanguineus* s.s. populations in Brazil (Becker et al., 2019).

Mites

Dermanyssus gallinae: In poultry farming worldwide, especially in layers, *Dermanyssus* (*D.*) *gallinae* reduces productivity by sucking the blood of animals and poses a threat to public health as vector. The poultry mite *Dermanyssus gallinae* was found to be resistant to synthetic carbamates and pyrethroids for the first report in Italy (Genchi et al., 1984). In a study conducted in former Czechoslovakia, the resistance of *D. gallinae* to trichlorfon, tetramethrin, and permethrin was observed on several farms. Meanwhile, resistance to DDT is common even though the use has been banned (Zeman, 1987). It has been demonstrated that *D. gallinae* is resistant to synthetic pyrethroids in France (Beugnet et al., 1997), Sweden (Nordenfors et al., 2001), Italy (Marangi et al., 2009; Schiavone et al., 2023), China (Wang et al., 2021), and Türkiye (Koç et al., 2022). The presence of permethrin, cypermethrin, bendiocarb, and malathion resistance in field-isolated *D. gallinae* was demonstrated through comparisons with lab-grown sensitive mites in the United Kingdom (Fiddes et al., 2005). *D. gallinae* mites demonstrated resistance to all acaricides and insecticides examined in Japan, it showed resistance against carbaryl, trichlorfon, and a combination of fenitrothion, permethrin, and phthalathrin, but weak growth was observed than other acaricides and insecticides. 19 (13.7 %) of 139 poultry farms from 2007 to 2010 and 22 (18.5%) of 119 poultry farms from 2011 to 2013, the emergence of resistance to all commercial ectoparasiticides targeting the control of *D. gallinae* mites has been confirmed in Japan (Murano et al., 2015). It was reported exceptionally elevated levels of pyrethroid resistance in Greece, in addition to the identification and geographic distribution of pyrethroid resistance mutations in poultry red mite populations in Europe (Kastavou et al., 2019). Similarly, the mechanism of acaricide resistance of *D. gallinae* populations in Türkiye was investigated. Results obtained from this study show that a high level of resistance has been demonstrated for alpha-cypermethrin and phoxim (Koç et al., 2022).

Varroa destructor: One of the most destructive honeybee disease is varroosis, caused by the ectoparasitic mite *Varroa destructor*. Four populations of *Varroa destructor* were examined for their susceptibility to the organophosphorus acaricide coumaphos in Italy. This is the first study to report *V. destructo*r's resistance to the coumaphos (Spreafico et al., 2001). In a six-hour exposure study conducted in the United States to determine coumaphos resistance in *V. destructor*, it was determined that resistance existed based on the results (Elzen and Westervelt, 2002). A study involving amitraz revealed significant LC_{50} differences between susceptible and resistant mites in Argentina. The LC_{50} was found to be 35-39 times greater than its initial value. These results indicate that the mites in Argentina have resistant to amitraz (Maggi et al., 2010). *V. destructor* mites from colonies in the Czech

Republic were recently tested *in vitro* for resistance to formamidine amitraz and, the synthetic pyrethroids acrinatrin and tau-fluvalinate (Kamler et al., 2016). In addition, an update was provided on the actual spread of target site resistance to tau-fluvalinate in *V. destructor* samples collected from the Lombardy region in Italy (Panini et al., 2019). Resistance to coumaphos, amitraz, and tau-fluvalinate was investigated in Spain. In the analyzed samples, coumaphos mortality ranged from 2% to 89%, while tau-fluvalinate mortality ranged from 5% to 96%. In contrast, amitraz caused 100 percent mortality in every case. These outcomes indicate the presence of fluvalinate- and coumaphos-resistant *Varroa* mites in the majority of sampled apiaries, regardless of the reality that these active constituents have not been used over the span of years (Higes et al., 2020).

Psoroptes ovis: Flumethrin-resistant *Psoroptes* spp. populations have already shown side resistance to high cis-cypermethrin (Bates, 1998). Furthermore, moxidectin, ivermectin, and doramectin resistances were observed in *Psoroptes* mites in the United Kingdom (Doherty et al., 2018; Sturgess-Osborne et al., 2019). The presence of macrocyclic lactone resistance was detected in Belgian Blue cattle on sixteen cattle farms in Belgium and the Netherlands (van Mol et al., 2020). It was determined *in vitro* whether ivermectin and two other macrocyclic lactones (doramectin and moxidectin) were effective against *P. ovis* in sheep in Argentina. In light of this study, the presence of resistance to these active substances was determined (Soler et al., 2022).

Sarcoptes scabiei: Two dogs treated with 300 µg⁄kg bw ivermectin with case reports indicated that *S. scabiei* was clinically resistant to treatment in these dogs (Terada et al., 2010). Moreover, increased transcription of mu-1, delta-1, and delta-3 Glutathione S-transferase molecules, indicating permethrin resistance, was observed (Mounsey et al., 2010).

Lice

A study conducted in South Australia investigated cypermethrin resistance in sheep lice from 71 flocks infested with lice between 1990 and 1991, 16 flocks with suspected ectoparasitic resistance, and 31 flocks from Kangaroo Island. The frequency of resistance was determined to be 34% in lice collected from flocks with lice infestations, 50% in lice collected from flocks with suspected resistance, and 68% in lice collected from flocks on Kangaroo Island (James et al., 1993). A retrospective study in Australia examined the treatment history and response to cypermethrin of a strain resistant to pyrethroids of sheep lice over an 18-year period. In the study, the resistance of lice strains resistant to cypermethrin decreased when cypermethrin was not used but highly increased when pyrethroid was used again (Levot 2012).

Due to reports of decreased efficacy against triflumuron and diflubenzuron, the Insect Growth Regulator group of *Bovicola (B) ovis* ectoparasites, a resistance study was conducted in Australia. In order to evaluate sensitivity to active pharmaceutical ingredients, a laboratory test based on the moulting inhibition test was devised and used in the study. The lice used in the experiment were collected from sheep treated ineffectively with triflumuron and diflubenzuron. This resulted in the confirmation of the emergence of resistance in lice (James et al., 2008).

Simultaneously, a study utilizing the louse hatch test was conducted based on the inability of treated with diflubenzuron *B. ovis* to produce nymphs. The test is able to detect populations resistant to benzoylphenyl urea. The results of the test demonstrated the development of resistance. (Levot and Sales, 2008). In Scotland, it was suspected that sheep colonies were infested with *B. ovis* resistant to synthetic pyrethroids. Laboratory data and dependable field data have been shown to be effective methods for testing for potential deltamethrin resistance (Bates, 2001). In 16 (94%) of the 17 colonies examined during a pilot study in Ireland, lice were found. Lice species *Bovicola ovis* and *Linognathus vituli* were identified. Four farms' *B. ovis* samples exhibited tolerance to deltamethrin, as determined by *in vitro* contact biological tests. In addition, repeated evaluations of lice infestations in farm-treated animals confirmed this (Mckiernan et al., 2021). In Ethiopia, it has also been shown that *B. ovis* is resistant to 1% ivermectin (Legesse et al., 2020).

Fleas

Ctenocephalides (C.) felis and *C. canis*

Ctenocephalides (C.) *felis*, known as the cat flea exhibits resistance to various insecticides, including organophosphates, carbamates, pyrethroids, organochlorine, and pyrethrins. In general, resistance ratio, which is the LC50 of field isolates divided by the LC50 of a susceptible strain and indicates the level of insecticide resistance, is less than 20, and cross-resistance between carbaryl and organophosphate insecticides has been observed (Coles and Dryden, 2014). Due to the distinct mechanisms of action of the substances, one strain resistant to fipronil, the phenylpyrazole insecticide, was susceptible to nitenpyram, a neonicotinoid (Schenker et al., 2001). Bioassays for fipronil and imidacloprid were conducted on both adult and larval *C. felis* in a study. Adult and larval bioassays for fipronil and imidacloprid yielded comparable results. Both laboratory strains and field isolates have been found susceptible to fipronil treatment. However, the majority of strains and isolates were substantially more sensitive to imidacloprid (Rust et al., 2014). In a separate study, resistance to certain ectoparasites was investigated via topical application of 12 cat flea isolates collected from the field and 4 laboratory strains. No resistance to fipronil or imidacloprid was detected in the course of the experiments conducted. All isolates and strains exhibited pyrethroid resistance to permethrin and deltamethrin. Although the Rdl mutation, which confers site-specific cyclodiene insecticide resistance, is present in the majority of isolates and strains, the response to fipronil, which affects the identical receptor protein as cyclodiene insecticides, has not been changed (Rust et al., 2015). In a study involving Germany, France, England, the United States, and Australia, a bioassay was conducted on 1687 of 2307 cat flea isolates collected between 2002 and 2013 to determine their

resistance to imidacloprid. The results showed that the cat flea is still susceptible to imidacloprid despite decades of widespread use (Kopp et al., 2013).

The presence of T929V and L1014F mutations in the *para* gene of cat fleas, which correspond to pyrethroid resistance, was examined in flea samples collected from goats on seven farms in Türkiye where cypermethrin was widely used. According to the results, all collected fleas were *C. felis* (Alak et al., 2020).

From five localities in the northwest and west of Iran, 67 *C. canis* fleas were collected. Adult fleas were exposed to cypermethrin at a concentration of 0.75 percent for 1 and 8 hours, and mutation sites in the VGSC gene were analyzed. The mortality of this dose for *C. canis* was 33.33%, 41.17%, and 66.66%, 80.33% after 1 and 8 hours of exposure, respectively. Sequence analysis of VGSC revealed one mutation site in susceptible fleas and two mutation sites in resistant fleas (Seidy et al., 2022).

Mechanisms of resistance

Research on the mechanisms of resistance in ectoparasites is of concern for the development of new drugs and control strategies. Although these mechanisms have not been fully explored, some resistance mechanisms are outlined below. Due to a similar mode of action, parasitic resistance can develop within the same chemical class (Stafford and Coles, 2009).

Two main mechanisms of resistance have been identified:

1. Metabolic resistance: Ectoparasiticides cannot reach their target sites due to detoxification enzyme-based resistance, such as esterases, oxidases, and glutathione S-transferases (GST). This may be the result of the amplification of a single amino acid, which modulates the catalytic center activity of the enzyme, or multiple gene copies in resistant ectoparasites.

2. *Point mutation:* Point mutations inhibit the migration of ectoparasiticide within the target area. These mutations may occur at a single point in the DNA sequence or in the protein structure targeted by the ectoparasite. Consequently, the drug may not bind effectively to its target and may lead to reduced lethality in the parasite. Over time, the frequency of these mutations in the population may increase, and resistant parasites may transfer the mutations as they continue their genetic lineage, leading to an increase in ectoparasitic resistance in the population. Therefore, understanding and monitoring point mutations is important for the development of new drugs. In a study, it was found that resistance development was faster in the case of resistance due to only one gene mutation, especially if this single gene mutation constitutes a dominant allele (Jonsson and Hope, 2007). When multiple genes are involved in resistance formation, the propagation of the resistance rate within the population will decrease.

Pyrethroids: Pyrethroid resistance mechanism of *Rhipicephalus* (formerly *Boophilus*) *microplus* was investigated. Tick genotypes known to be resistant to pyrethroids were utilized for this purpose. These DNAs were sequenced after obtaining partial *para*-homologous sodium channel cDNA from these ticks. In the extremely preserved domain IIIS6 of the homologous sodium channel of ticks that are extremely resistant to pyrethroids, a point mutation that results in an amino acid change from Phe to Ile was identified (He et al., 1999).

In a study involving cat fleas from the United States and the United Kingdom, the mechanism of pyrethroid resistance was investigated. This study involved the cloning of domain II sequences from the cat flea *para* gene and the identification of two mutations, L1014F and T929V, pyrethroid resistance in residues previously implicated (Bass et al., 2004).

A study was conducted to ascertain the pyrethroid resistance of *Varroa destructor*. In this study, valid new methodologies for determining DNA methylation in resistant and susceptible strains were also identified. In the case of five mitochondrial gene fragments, polymerase chain reaction single-stranded conformational polymorphism (PCR-SSCP) was used to identify nucleotide substitutions in the DNA of pyrethroid-resistant, pyrethroid-susceptible, and control mites. The drug-resistant strains contained more bands than the other two categories. In pyrethroid-resistant mites, global DNA methylation levels were observed to be lower (Stracheka et al., 2015). TaqMan analyses performed on tau-fluvalinate-resistant *V. destructor* mites in the Lombardy region of Italy demonstrated the presence of the L925V mutation. (Lupi, 2019).

Organophosphates: Organophosphates (OP) are derived from one of the phosphorus acids and contain phosphorus. Organophosphates are specific for the acetylcholinesterase (AChE) protein. Due to the complexity and multifactorial nature of organophosphate resistance, an extensive investigation has been conducted to identify the mechanisms underlying organophosphate resistance, however, there has been little progress at the molecular level (Guerrero et al., 2012). The existence of OP resistance is supported by biochemical and bioanalytical data (Jamroz et al., 2000).

Biochemical, bioassay, and molecular assays were used to determine the OP resistance status of *R. microplus* ticks collected in the Indian state of Punjab. As a measure, the Adult Immersion Test (AIT) was utilized. Malathion resistance was detected in 12 isolates following the test. The gene sequences of AChE3 and AChE activity were analyzed to determine the potential resistance mechanism. A significantly higher level of uninhibited AChE activity was observed in all collected ticks compared to the susceptible population. While V71A mutation was observed in seven isolates collected from the field, R86Q mutation was found in all isolates collected from the field (Singh et al., 2016).

Macrocyclic lactones: In a study involving two tick strains, one resistant and one susceptible, a lethal time bioassay was conducted. The significance of the ivermectin detoxification enzymes cytochrome P450, esterases, glutathione-S-transferase, and ATP Binding Cassette Transporters in resistance was investigated. As a consequence, it was determined that the most important

detoxification mechanisms in resistant strains are those mediated by ABC transporters. It was discovered that esterases, glutathione-S-transferases, and cytochrome-oxidases perform a lesser role in detoxification (Le Gall et al., 2018).

Carbamates: Carbamates are derived from carbamic acid. Inhibiting acetylcholinesterase (AChE) is their mode of action. Ticks that are resistant to organophosphates may also be resistant to carbamates due to their analogous mode of action (Li et al., 2005).

The emergence of resistance to propoxur, permethrin, DDT, and malathion in *R. microplus* was examined using the Larval Packet Test in Sri Lanka. The 30% insensitivity of tick populations' acetylcholinesterases, the target site of carbamates and organophosphates, to propoxur inhibition suggests that altered acetylcholinesterases are partially responsible for the carbamate and organophosphate resistance observed in tick populations (Bandara and Karunaratne, 2017).

Phenylpyrazoles: Fipronil is the only phenylpyrazole compound used to treat and/or control animal ectoparasite infestations. Very little is known about fipronil resistance in ectoparasites. In 100% of field populations of German cockroaches, A302S target site mutations of the GABA-gated chloride channel associated with fipronil resistance were identified (Gonzales-Morales et al., 2022).

Amitraz: It has been hypothesized that the target of amitraz activity is one of the biogenic amine receptors, obviously the adrenergic or octopaminergic receptors. Two nucleotide substitutions were discovered in the octopamine receptor sequence of resistant tick strains, causing amino acids that are distinct from those of all susceptible strains (Chen et al., 2007; Corley et al., 2013). These mutations provided the initial proof of a modified target site as a tick amitraz resistance mechanism. In addition, because the target site of amitraz has not been conclusively identified, the precise mechanism of resistance to amitraz remains unknown (Leeuwen et al., 2010; Guerrero et al., 2012; Pohl et al., 2012).

Methods of detecting resistance

In vivo studies are conducted by applying the product directly to animals in accordance with the recommended route of administration and dosage, and the number of parasites before and after treatment (Abolins et al., 2007).

In vitro studies vary depending on the specific chemical and ectoparasite being studied. The majority of the tests require laboratory conditions. A number of these tests can be conducted in the field, such as the CDC bottle test (CDC, 2012) and the Fly Box mobile testing device (Jandowsky et al., 2010). Thresholds (e.g. dose discrimination) differ between ectoparasite species and ectoparasiticides with different modes of action. When defined reference strains of susceptible or resistant ectoparasites are used to assess the validity of these methods, they are all valid.

Exposure of larvae or adults to treated surfaces

Adults: This strategy typically relies on direct contact of ectoparasites with a chemically treated surface under research. It involves exposing ectoparasites to surfaces treated with different subjected to various dilutions of the researched chemical for a specified time period. Mortality of ectoparasites is assessed at defined diagnostic time points. Various materials may be used for these surfaces, e.g. glass, fabric, or paper, although the principle has not changed (Thompson et al., 2002 Jandowsky et al., 2010; Sternberg et al., 2014; Rust et al., 2014).

Larvae: The larval pack test (LPT) is a test that tests that assesses the susceptibility of tick larvae to treated surfaces (FAO, 2004). It is recommended that this test can be used in conjunction with the concept of discriminating concentration as a low-cost and rapid resistance diagnostic technique (Eiden et al., 2015). The discriminating concentration consists of a single ectoparasitic concentration that will kill the most susceptible genotype while leaving the resistant genotypes remain viable.

This category of tests is not suitable to test the resistance of Insect Growth Regulators (IGR) acting by interfering with the moulting process and/or hatching of eggs. To test IGR resistance in transient pests such as flies, fly eggs are commonly incubated in raring media with increasing IGR concentrations (Jandowsky et al., 2010). Specific test conditions may be required for ectoparasites that remain consistently in the host, eg; the use of scrapings of the host's wool or skin is considered mandatory for the lice hatching (James et al., 2008; Levot and Sales, 2008).

Topical application to adults or larvae

Adults: Topical application to a specific location on the surface of the body of ectoparasites is a frequently used method. Using different dilutions, the chemical under investigation is administered in small droplets by micro-syringe to ectoparasites immobilized, e.g. by carbon dioxide or cooling. The mortality rate of ectoparasites is evaluated at the conclusion of the test (Pessoa et al., 2015).

Immersion testing is the second form of topical application. At the time of this test, ectoparasites are immersed in various dilutions of the researched ectoparasiticide (Castro-Janer et al., 2009).

Larvae: Larval Immersion Test (LIT) is a comparable evaluation for larvae (Shaw, 1966). This test is not extensively utilized and does not receive recommendations from FAO.

Feeding tests with treated rearing media

The tested chemical is added at varying concentrations to the culture medium for the larval stages of the ectoparasite, according to the fundamental principle. Such biological assays can be used to evaluate larvicide activity (Kelly et al., 1987; Rust et al., 2014).

Biochemical and molecular assays

These experiments have the potential to investigate the mechanisms of resistance in specific ectoparasite interference, thereby validating resistance. Nevertheless, it is currently used only for research purposes. WHO (1998) has described a number of biochemical and immunological assays to test for an increase or change in ectoparasite enzymes that are associated with higher tolerance to ectoparasites. Biochemical microtiter plate assays, for

experiments to test enzyme activity, e.g. to detect modified acetylcholinesterase, increased esterase, and glutathione-S-transferase. The enzyme activities are measured either visually or by spectrophotometer. Moreover, It should be emphasized that biochemical tests are not available for all identified resistance mechanisms and thus cannot replace conventional susceptibility testing.

Monitoring programs for resistance

Currently, there are no systematic surveillance programs for resistance to ectoparasites. Multiple initiatives monitor the environment and health status of honeybee colonies, including the national distribution of Varroa mite infestation in countries such as Spain, Germany, and Italy. On the other hand, they do not examine resistance levels specifically, and there is no global surveillance initiative that uniformly collects evidence on Varroa resistance.

System of pharmacovigilance

The World Health Organization (WHO) defines pharmacovigilance as " the science and activities concerned with the detection, evaluation, comprehension, and prevention of adverse effects and other drug-related issues".

The anticipated dearth of efficacy must be notified within the system of pharmacovigilance. These reports may provide evidence of the potential development of resistance to a particular active ingredient.

It is arduous to detect resistance in the field, and the expected lack of effectiveness is frequently underreported. Consequently, it is probable that the true incidence of inefficacy is underreported. Therefore, the current pharmacovigilance system's capacity for detection and monitoring of resistance is limited.

Management strategies to delay the development of resistance

Locating: Regular resistance monitoring is recommended before selecting a suitable ectoparasitic drug for administration (FAO, 2004; Abbas et al., 2014; Karakus et al., 2017). Monitoring requires a defined standard methodology, including a recognized laboratory in charge of resistance testing, a susceptible reference strain, and if required, a known resistant strain (FAO, 2004).

Usage of ectoparasites:

Reducing the number of applications: Reducing selection pressure for resistance in the field can delay the emergence of resistance, according to a consensus (FAO, 2004; Thullner et al., 2007). Additionally, reducing ectoparasitic use (e.g., treatment timing based on epidemiology) and avoiding treatment of non-infested animals have been suggested (FAO, 2004; Thullner et al., 2007; Heath and Levot, 2015). A case-control study supports this assertion conducted on farms in Australia where territorial variations in the prevalence of acaricide resistance in *R.* (*Boophilus*) *microplus* were observed. Certain sites and frequencies of ectoparasiticide treatment have been consistently linked to resistance; for instance, The risk of resistance to synthetic pyrethroids and amitraz has been observed to increase if more than five acaricides were administered the year before, the number of acaricide-resistant insects will increase (Jonsson et al., 2000).

Application technique of the veterinary medicinal product: FAO (2004) and Jonsson et al. (2000) cite the method of administration as an additional strategy for preventing the emergence of resistance. For tick eradication programs, topical application by dip or pulverizer is more effective in terms of efficacy than application by spray apparatus, because application by spray apparatus results in poor dispersion and/or poor wetting of animals, exposing ticks to non-lethal concentrations. According to Jonsson et al. (2000) and the World Health Organization (WHO) (2014), ineffective application methods may contribute to the emergence of resistance.

Rotation of distinct ectoparasite classes: Alternate or alternative use of various ectoparasiticide groups without cross-resistance has also been argued to prevent resistance (Kunz and Kemp, 1994; Abbas et al., 2014). This strategy demonstrates that, in a population of ectoparasites, the frequency of individuals resistant to each previously used chemical will decrease as alternative agents are administered (Kunz and Kemp, 1994). In this context, methods for susceptible external parasites to maintain their refugium in order to dilute resistant alleles have been considered (Kunz and Kemp, 1994; FAO, 2004; WHO report, 2014); however, a study indicates that this is challenging to implement in practice (Heath and Levot, 2015). Neither strategy has been sufficiently demonstrated to effectively reduce resistance (Cloyd, 2010).

Rotating pyrethroids (deltamethrin) and organophosphates (coumaphos) may postpone the emergence of pyrethroid resistance, according to a laboratory study involving identified *R. microplus* tick strains. To validate such a strategy, however, field trials are required (Thullner et al., 2007).

Products with more than one active pharmaceutical ingredient: The use of products comprising two or more ectoparasitic agents (multi-drug-containing products) with various mechanisms of action against the identical ectoparasite is another strategy under consideration for delaying resistance. According to the premise that it is unlikely for a single parasite to contain resistant alleles for two or more insecticides or acaricides with distinct modes of action (Kunz and Kemp, 1994; Abbas et al., 2014), this approach entails the use of a single acaricide or insecticide. This strategy necessitates that the active pharmaceutical ingredients in a multi-drug product be used in compatible, equivalent persistence, and at prescribed concentrations (to prevent non-lethal concentrations of an ingredient from selecting resistant heterozygotes). However, the potential risk of developing multiple resistance cannot be ruled out entirely, and further clarification of this approach appears necessary prior to drawing definitive conclusions about its utility.

Synergists

Piperonyl butoxide: Piperonyl butoxide (PBO) is frequently employed as a synergist with a number of ectoparasites

(e.g., carbamates, pyrethroids) for insect control. PBO has no inherent insecticidal properties and is virtually nontoxic to mammals and birds (NPIC, 2017). PBO inhibits many enzymes capable of degrading the active substance in insects prior to its activation. PBO impedes the detoxification of ectoparasites via binding to Mixed Function Oxidases (MFO), which contribute to the degradation of active pharmaceutical ingredients (Wexler et al., 2005). By incorporating PBO into a product, resistance according to the increased activity of insect MFOs can be partially overcome, protecting the product from the deleterious effects of synthetic pyrethroids and carbamates.

Environmental control measures

Increased measures that could reduce the infestation burden and, consequently, the incidence of ectoparasiticide administration in accordance to postpone the emergence of resistance have been discussed in the literature. Pasture management (e.g., adequate aeration, complete manure removal, optimum animal density, and minimal stress) (Jonsson et al., 2000; Abbas et al., 2014) has been shown to improve animal health (Jonsson et al., 2000; Abbas et al., 2014). As with fleas, it is common practice to treat the environment to reduce or eliminate re-infestations in order to reduce infestation pressure. Mosquito traps, horsefly traps, and fly traps (lights, adhesive strips) are examples of control methods (Heath and Levot, 2015). In addition, quarantine of newly-acquired livestock can be considered a preventative measure against possible infestation and the subsequent need to treat the entire herd (FAO, 2004). This is practiced in South America and Africa to prevent the transmission of *Ampblyomma*, *B. ovis*, or *Sarcoptes* (an obligate non-flying ectoparasite), and is recommended for ticks, lice, and mites with a single host.

Alternative management strategies

Alternative methods of ectoparasite control include the utilization of natural enemies and vaccinations:

Natural enemies: In poultry production, predatory mites such as *Androlaelaps casalis*, which consume *D. gallinae,* are utilized. Even though predatory mites are commercially available, additional research is necessary before they can be used in the field (Sparagano et al., 2014). In separate research, it was determined that *Cheyletus malaccensis* is the most potent natural enemy for *Dermanyssus gallinae* and *Megninia ginglymura*, which have been identified as a global economic threat to the poultry industry (Faleiro et al., 2015).

Vaccination: Vaccines against parasites have benefits such as preventing the development of resistance against ectoparasites and preventing environmental contamination (de La Fuente et al., 2017). Numerous years have been spent on the emergence of a vaccine against the single-host tick *R. microplus*, which has a significant negative effect on animal productivity (de La Fuente et al., 2007; Vargas et al., 2010; McNair, 2015; Schetters et al., 2016). Currently, only one vaccine containing the intestinal antigen Bm86 of *R. microplus* is commercially available (Guerrero et al., 2012b). Nonetheless, according to Guerrero et al. (2012 b), the effectiveness of this vaccine varies from strain to strain, and its adoption is limited. Advanced bovine tick vaccines are the subject of continuous research in order to develop them.

Similar strategies are believed to be effective for other infestations, such as sheep scabies and sea lice (McNair, 2015). Nonetheless, the determination of appropriate antigens as vaccine candidates is frequently a main limitation (Smith et al., 2001; Smith and Pettit, 2004), and no vaccines against ectoparasites are presently available.

Conclusion and Recommendations

Increasing global resistance to ectoparasitic agents found in veterinary medicinal products is a serious concern for animal welfare, animal productivity, and to some extent human health. There have been reports of site-specific ectoparasite resistance due to differences in reproduction and environmental conditions, as well as the life cycle and incidence of the ectoparasite. The global resistance status of ectoparasite species appears to have been investigated extra thoroughly.

Resistance mechanism

It is known that the host, the parasite, the frequency of use of antiparasitic products, and the environment/breeding system influence the development of antiparasitic resistance. In external parasites, i) point mutations and ii) enzyme-based detoxification mechanisms have been identified as the two most prevalent mechanisms of resistance. For a number of ectoparasite species, resistance mechanisms against a number of the relevant ectoparasiticide classes have been identified. Ticks have shown clinically significant resistance to amitraz and macrocyclic lactone compounds, for which the underlying mechanism of resistance is currently unknown. Consider the potential that resistance to an ectoparasiticide or class of ectoparasiticides may be caused by multiple mechanisms. In conclusion, this subject requires more information, including the inheritance of resistance genes, so that resistance management programs can be established. Therefore, continued inquiry into the extremely complex process of resistance development is required.

Determination of resistance

In general, resistance is suspected due to a lack of clinical efficacy. Ineffectiveness may also result from improper application of a product, such as insufficient dosing, inappropriate dosing frequency, inappropriate treatment timing, or poor administration procedures. However, these inappropriate practices can also result in the emergence of ectoparasite species with resistance. In a study conducted on fleas, it was determined that the lack of ectoparasitic activity was likely not due to the development of resistance, but rather to the absence of environmental flea control (Dryden and Rust, 1994).

According to the conducted studies, the prolonged usage of ectoparasites of the same class over an extended period of time is a risk factor for the emergence of ectoparasitic resistance in Australia (Levot et al., 1995;

Wilson et al. 1997; Jonsson et al., 2000). It can also be an issue when identical products are used to control various ectoparasites and the epidemiology of different types of infestations, such as resistance in external parasites when macrocyclic lactone anthelmintics are used (FAO, 2004). In order to determine the cause of an observed lack of efficacy in an ectoparasitic, it may be beneficial to be aware of previous treatment methods.

Complicating the situation is the fact that it is frequently challenging to corroborate that the observed ineffectiveness in the field is the result of resistance to the veterinary medicinal product. Presently, the majority of available methods to corroborate suspected resistance require lengthy laboratory conditions. Moreover, prior to resistance testing, laboratory propagation of ectoparasite populations requires specialized knowledge. Therefore, it is necessary to develop resistance detection methods that can provide timely results regarding the susceptibility/resistance conditions of an ectoparasite population and can be conducted routinely in the field.

Monitoring the resistance

The published information on resistance in external parasites is inconsistent and primarily focuses on mites, marine lice, lice, and flies, with a lesser emphasis on ticks, fleas, and mosquitoes.

Only a few countries offer structured resistance surveillance programs and for exclusive to particular ectoparasites. Aside from that, there is a severe lack of data regarding the resistance status and probable temporal trends of the majority of ectoparasite species in relation to currently employed ectoparasites. Therefore, systematic monitoring is requhas on a global scale. Countries such as Australia and New Zealand have demonstrated that such knowledge is essential for managing the development of resistance. (Jonsson et al., 2000; FAO, 2004; Abbas et al., 2014; Karakuş et al., 2017).

Strategies to delay resistance development

In addition to the correct administration of the veterinary medicinal product, a properly affirmed diagnosis is the most natural method for minimizing the risk of developing resistance and achieving the anticipated therapeutic effect.
To ensure that an appropriate ectoparasite is chosen, it is beneficial to regularly monitor the development of resistance to the various ectoparasiticide classes in the region.

On the assumption that increased exposure increases the risk of emerging resistance, it is also necessary to reduce the superfluous routine use of chemical controls as a preservative, according to the prevalent belief. the concept of targeted selective therapy, such as the concept of refugia, which has been demonstrated to delay the emergence of anthelmintic resistance. A portion of the ectoparasite population is left as a control group, in accordance with the refugia concept, in order to decrease the selective pressure on resistance-conferring alleles. The information currently available is insufficient to draw definitive conclusions regarding the utility of this concept for ectoparasites; nevertheless, refugia may be useful for delaying resistance to ectoparasites such as fleas, lice, and mites (Kunz and Kemp, 1995; Cloyd, 2010; Abbas et al., 2014; McNair, 2015). Still, additional clinical research is considered necessary (Cloyd, 2010).

Evaluation of ectoparasite product applications

The prospective emergence of clinically significant resistant arthropod species is a prerequisite for marketing authorization applications. Marketing authorization holders are also required to supply information on the known resistance mechanism. Also beneficial would be the presentation of scientifically supported risk reduction measures to reduce the risk of resistance development. However, due to a dearth of surveillance and efficient detection methods, it is acknowledged that the capacity to provide information on resistance is limited. Although the scientific literature contains some information on resistance mechanisms, the database is presently narrow, particularly in terms of information on inheritance patterns. However, marketing authorization holders should be encouraged to support all data regarding the development of resistance to the active pharmaceutical ingredient in the to-be-approved product. A sufficient quantity of various presentations should be made available in order to treat varying numbers of animals without leaving behind residues that could be misused.

References

Abbas, R. Z., Zaman, M. A., Colwell, D. D., Gilleard, J., & Iqbal, Z. (2014). Acaricide resistance in cattle ticks and approaches to its management: the state of play. *Veterinary parasitology, 203*(1-2), 6-20.

Abolins, S., Thind, B., Jackson, V., Luke, B., Moore, D., Wall, R., & Taylor, M. A. (2007). Control of the sheep scab mite *Psoroptes ovis* in vivo and *in vitro* using fungal pathogens. *Veterinary Parasitology, 148*(3-4), 310-317.

Alak, E. S., Köseoğlu, A. E., Kandemir, Ç., Taşkın, T., Demir, S., Döşkaya, M., ... & Can, H. (2020). High frequency of knockdown resistance mutations in the para gene of cat flea (*Ctenocephalides felis*) samples collected from goats. *Parasitology Research, 119*, 2067-2073.

Bandara, K. J., & Karunaratne, S. P. (2017). Mechanisms of acaricide resistance in the cattle tick *Rhipicephalus* (Boophilus) microplus in Sri Lanka. *Pesticide Biochemistry and Physiology, 139*, 68-72.

Bass, C., Schroeder, I., Turberg, A., Field, L. M., & Williamson, M. S. (2004). Identification of mutations associated with pyrethroid resistance in the para-type sodium channel of the cat flea, *Ctenocephalides felis*. *Insect Biochemistry and Molecular Biology, 34*(12), 1305-1313.

Bates, P. G. (1998). Acaricide resistance in sheep scab mites. Proc. Sheep Vet. Soc. 21, 117-122.

Bates, P. G. (2001). The epidemiology and control of sheep chewing lice in Great Britain: Recent research. Proc. Sheep Vet. Soc., 24: 163-168.

Becker, S., Webster, A., Doyle, R. L., Martins, J. R., Reck, J., & Klafke, G. M. (2019). Resistance to deltamethrin, fipronil and ivermectin in the brown dog tick, Rhipicephalus

sanguineus sensu stricto, Latreille (Acari: Ixodidae). *Ticks and Tick-Borne Diseases, 10*(5), 1046-1050.

Beugnet, F., Chauve, C., Gauthey, M., & Beert, L. (1997). Resistance of the red poultry mite to pyrethroids in France. *Veterinary Record, 140*(22), 577-579.

Castro-Janer, E., Rifran, L., Piaggio, J., Gil, A., Miller, R. J., & Schumaker, T. T. S. (2009). *In vitro* tests to establish LC50 and discriminating concentrations for fipronil against Rhipicephalus (Boophilus) microplus (Acari: Ixodidae) and their standardization. *Veterinary Parasitology, 162*(1-2), 120-128.

Centers for Disease Control and Prevention, CDC (2012). Guideline for evaluating insecticide resistance in vectors using the CDC bottle bioassay.

Chen, A. C., He, H., & Davey, R. B. (2007). Mutations in a putative octopamine receptor gene in amitraz-resistant cattle ticks. *Veterinary Parasitology, 148*(3-4), 379-383.

Cloyd, R. A. (2010). Pesticide mixtures and rotations: Are these viable resistance mitigating strategies. *Pest Technol, 4*(1), 14Г18.

Coles, T. B., & Dryden, M. W. (2014). Insecticide/acaricide resistance in fleas and ticks infesting dogs and cats. *Parasites & Vectors, 7*(1), 1-10.

Corley, S. W., Jonsson, N. N., Piper, E. K., Cutullé, C., Stear, M. J., & Seddon, J. M. (2013). Mutation in the RmβAOR gene is associated with amitraz resistance in the cattle tick Rhipicephalus microplus. *Proceedings of the National Academy of Sciences, 110*(42), 16772-16777.

Cruz, B. C., Lopes, W. D. Z., Maciel, W. G., Felippelli, G., Fávero, F. C., Teixeira, W. F. P., ... & De Oliveira, G. P. (2015). Susceptibility of Rhipicephalus (Boophilus) microplus to ivermectin (200, 500 and 630 µg/kg) in field studies in Brazil. *Veterinary Parasitology, 207*(3-4), 309-317.

de La Fuente, J., Almazán, C., Canales, M., de la Lastra, J. M. P., Kocan, K. M., & Willadsen, P. (2007). A ten-year review of commercial vaccine performance for control of tick infestations on cattle. *Animal Health Research Reviews, 8* (1), 23-28.

de La Fuente, J., Contreras, M., Estrada-Peña, A., & Cabezas-Cruz, A. (2017). Targeting a global health problem: Vaccine design and challenges for the control of tick-borne diseases. *Vaccine, 35*(38), 5089-5094.

Doherty, E., Burgess, S., Mitchell, S., & Wall, R. (2018). First evidence of resistance to macrocyclic lactones in *Psoroptes ovis* sheep scab mites in the UK. Veterinary *Record, 182*(4), 106-106.

Eiden, A. L., Kaufman, P. E., Oi, F. M., Allan, S. A., & Miller, R. J. (2015). Detection of permethrin resistance and fipronil tolerance in *Rhipicephalus sanguineus* (Acari: Ixodidae) in the United States. *Journal of Medical Entomology, 52*(3), 429-436.

Elzen, P. J., & Westervelt, D. (2002). Detection of coumaphos resistance in *Varroa destructor* in Florida. *American Bee Journal, 142*(4), 291-292.

Estrada-Pena, A. (2005). Etude de la résistance de la tique brune du chien, *Rhipicephalus sanguineus aux* acaricides. *Revue de Médecine Vétérinaire, 156*(2), 67-69.

Faleiro, D. C., Toldi, M., Da Silva, G. L., & Ferla, N. J. (2015). The ectoparasites *Dermanyssus gallinae* and *Megninia ginglymura*: bioecology and natural enemies in commercial egg-laying hens. Systematic *and Applied Acarology, 20*(8), 861-874.

FAO (2004): Guidelines resistance management and integrated parasite control in ruminants. FAO, Animal Health and Health Division, Rome, Italy.

Fiddes, M. D., Le Gresley, S., Parsons, D. G., Epe, C., Coles, G. C., & Stafford, K. A. (2005). Prevalence of the poultry red mite (Dermanyssus gallinae) in England. Veterinary *Record, 157*(8), 233.

Genchi, C., Traldi, G., & Huber, H. (1984). [The efficacy of Flumethrin (Bayticol, Bayer) for the control of chicken mite, Dermanyssus gallinae, De Geer, 1778 (Acarina, Dermanyssidae)].[Italian]. *Archivio Veterinario Italiano*.

González-Morales, M. A., DeVries, Z. C., Santangelo, R. G., Kakumanu, M. L., & Schal, C. (2022). Multiple Mechanisms Confer Fipronil Resistance in the German Cockroach: Enhanced Detoxification and Rdl Mutation. *Journal of Medical Entomology, 59*(5), 1721-1731.

Guerrero, F. D., Lovis, L., & Martins, J. R. (2012a). Acaricide resistance mechanisms in Rhipicephalus (Boophilus) microplus. *Revista Brasileira de Parasitologia Veterinária, 21*, 1-6.

Guerrero, F. D., Miller, R. J., & de León, A. A. P. (2012b). Cattle tick vaccines: many candidate antigens, but will a commercially viable product emerge?. *International Journal for Parasitology, 42*(5), 421-427.

He, H., Chen, A. C., Davey, R. B., Ivie, G. W., & George, J. E. (1999). Identification of a point mutation in the para-type sodium channel gene from a pyrethroid-resistant cattle tick. *Biochemical and Biophysical Research Communications, 261*(3), 558-561.

Heath, A. C. G., & Levot, G. W. (2015). Parasiticide resistance in flies, lice and ticks in New Zealand and Australia: mechanisms, prevalence and prevention. *New Zealand Veterinary Journal, 63*(4), 199-210.

Higes, M., Martín-Hernández, R., Hernández-Rodríguez, C. S., & González-Cabrera, J. (2020). Assessing the resistance to acaricides in *Varroa destructor* from several Spanish locations. *Parasitology Research, 119*(11), 3595-3601.

James, P. J., Saunders, P. E., Cockrum, K. S., & Munro, K. J. (1993). Resistance to synthetic pyrethroids in South Australian populations of sheep lice (Bovicola ovis). *Australian Veterinary Journal, 70*(3), 105-108.

James, P. J., Cramp, A. P., & Hook, S. E. (2008). Resistance to insect growth regulator insecticides in populations of sheep lice as assessed by a moulting disruption assay. *Medical and Veterinary Entomology, 22*(4), 326-330.

Jamroz, R. C., Guerrero, F. D., Pruett, J. H., Oehler, D. D., & Miller, R. J. (2000). Molecular and biochemical survey of acaricide resistance mechanisms in larvae from Mexican

trains of the southern cattle tick, *Boophilus microplus*. *Journal of Insect Physiology, 46*(5), 685-695.

Jandowsky, A., Clausen, P. H., Schein, E., & Bauer, B. (2010). Occurrence and distribution of insecticide resistance in house flies (Musca domestica) on dairy farms in Brandenburg, Germany. *Praktische Tierarzt, 91*(7), 590-598.

Jonsson, N. N., Mayer, D. G., & Green, P. E. (2000). Possible risk factors on Queensland dairy farms for acaricide resistance in cattle tick (Boophilus microplus). *Veterinary Parasitology, 88*(1-2), 79-92.

Jonsson, N. N., & Hope, M. (2007). Progress in the epidemiology and diagnosis of amitraz resistance in the cattle tick Boophilus microplus. *Veterinary Parasitology, 146*(3-4), 193-198.

Kamler, M., Nesvorna, M., Stara, J., Erban, T., & Hubert, J. (2016). Comparison of tau-fluvalinate, acrinathrin, and amitraz effects on susceptible and resistant populations of *Varroa destructor* in a vial test. *Experimental and Applied Acarology, 69*, 1-9.

Karakus, M., Gocmen, B., & Özbel, Y. (2017). Insecticide susceptibility status of wild-caught sand fly populations collected from two leishmaniasis endemic areas in Western Turkey. *Journal of Arthropod-Borne Diseases, 11* (1), 86.

Katsavou, E., Vlogiannitis, S., Karp-Tatham, E., Blake, D. P., Ilias, A., Strube, C., ... & Vontas, J. (2020). Identification and geographical distribution of pyrethroid resistance mutations in the poultry red mite *Dermanyssus gallinae*. *Pest Management Science, 76*(1), 125-133.

Kelly, J. A., Stubbs, M. R., & Maff, D. B. P. (1987). Laboratory evaluation of cyromazine against insecticide-resistant field strains of Musca domestica. *Medical and Veterinary Entomology, 1*(1), 65-69.

Klafke, G. M., Sabatini, G. A., de Albuquerque, T. A., Martins, J. R., Kemp, D. H., Miller, R. J., & Schumaker, T. T. (2006). Larval immersion tests with ivermectin in populations of the cattle tick Rhipicephalus (Boophilus) microplus (Acari: Ixodidae) from State of Sao Paulo, Brazil. *Veterinary Parasitology, 142*(3-4), 386-390.

Koç, N., İnak, E., Nalbantoğlu, S., Alpkent, Y. N., Dermauw, W., & Van Leeuwen, T. (2022). Biochemical and molecular mechanisms of acaricide resistance in *Dermanyssus gallinae* populations from Turkey. *Pesticide Biochemistry and Physiology, 18*0, 104985.

Kopp, S., Blagburn, B., Coleman, G., Davis, W., Denholm, I., Field, C., ... & Williamson, M. (2013). Monitoring field susceptibility to imidacloprid in the cat flea: a world-first initiative twelve years on. *Parasitology Research, 112*, 47-56.

Kunz, S. E., & Kemp, D. H. (1994). Insecticides and acaricides: resistance and environmental impact. *Revue Scientifique et Technique (International Office of Epizootics), 1*3(4), 1249-1286.

Le Gall, V. L., Klafke, G. M., & Torres, T. T. (2018). Detoxification mechanisms involved in ivermectin resistance in the cattle tick, *Rhipicephalus (Boophilus) microplus. Scientific Reports, 8*(1), 12401.

Legesse, S., Hailemelekot, M., Tamrat, H., & Ferede, Y. (2020). Epidemiology of Sheep Lice and Efficacy Evaluation of 60% Diazinon and 1% Ivermectin against Bovicola ovis in Sayint District, South Wollo, Ethiopia.

Levot, G. W., Johnson, P. W., Hughes, P. B., Powis, K. J., Boray, J. C., & Dawson, K. L. (1995). Pyrethroid resistance in Australian field populations of the sheep body louse, Bovicola (Damalinia) ovis. *Medical and Veterinary Entomology, 9*(1), 59-65.

Levot, G., & Sales, N. (2008). Resistance to benzoylphenyl urea insecticides in Australian populations of the sheep body louse. *Medical and Veterinary Entomology, 22*(4), 331-334.

Levot, G. (2012). Unstable pyrethroid resistance in sheep body lice Bovicola ovis (Schrank),(Phthiraptera: Trichodectidae) and its implications for lice control on sheep. *Veterinary Parasitology, 185*(2-4), 274-278.

Li, A. Y., Davey, R. B., & George, J. E. (2005). Carbaryl resistance in Mexican strains of the southern cattle tick (Acari: Ixodidae). *Journal of Economic Entomology, 98*(2), 552-556.

Maggi, M. D., Ruffinengo, S. R., Negri, P., & Eguaras, M. J. (2010). Resistance phenomena to amitraz from populations of the ectoparasitic mite *Varroa destructor* of Argentina. *Parasitology Research, 107*, 1189-1192.

Marangi, M., Cafiero, M. A., Capelli, G., Camarda, A., Sparagano, O. A. E., & Giangaspero, A. (2009). Evaluation of the poultry red mite, Dermanyssus gallinae (Acari: Dermanyssidae) susceptibility to some acaricides in field populations from Italy. *Experimental and Applied Acarology, 48*, 11-18.

Martins, J. R., & Furlong, J. (2001). Avermectin resistance of the cattle tick Boophilus microplus in Brazil. *Veterinary Record, 149*(2), 64.

Mckiernan, F., O'Connor, J., Minchin, W., O'Riordan, E., Dillon, A., Harrington, M., & Zintl, A. (2021). A pilot study on the prevalence of lice in Irish beef cattle and the first Irish report of deltamethrin tolerance in Bovicola bovis. *Irish Veterinary Journal, 74*(1), 1-8.

McNair, C. M. (2015). Ectoparasites of medical and veterinary importance: drug resistance and the need for alternative control methods. *Journal of Pharmacy and Pharmacology, 67*(3), 351-363.

Mounsey, K. E., Pasay, C. J., Arlian, L. G., Morgan, M. S., Holt, D. C., Currie, B. J., ... & McCarthy, J. S. (2010). Increased transcription of Glutathione S-transferases in acaricides-exposed scabies mites. *Parasites & Vectors,* 3, 1-9.

Murano, T., Namiki, K., Shiina, K., & Yasukawa, H. (2015). Resistance development of Dermanyssus gallinae against commercial acaricides in poultry farms in Japan. Journal *of the Japan Veterinary Medical Association, 68*(8), 509-514.

National Pesticide Information Center (NPIC) (2017): Piperonyl Butoxide: General fact sheet.

Nordenfors, H., Höglund, J., Tauson, R., & Chirico, J. (2001).

Dermanyssus gallinae in loose-housing systems for laying hens. *Veterinary Parasitology, 102*(1-2), 121-131.

Panini, M., Reguzzi, M. C., Chiesa, O., Cominelli, F., Lupi, D., Moores, G., & Mazzoni, E. (2019). Pyrethroid resistance in Italian populations of the mite Varroa destructor: a focus on the Lombardy region. *Bulletin of Insectology, 72*(2), 227-232.

Perez-Cogollo, L. C., Rodriguez-Vivas, R. I., Ramirez-Cruz, G. T., & Miller, R. J. (2010). First report of the cattle tick Rhipicephalus microplus resistant to ivermectin in Mexico. *Veterinary Parasitology, 168(*1-2), 165-169.

Pessoa, G. C. D. Á., Pinheiro, L. C., Ferraz, M. L., de Mello, B. V., & Diotaiuti, L. (2015). Standardization of laboratory bioassays for the study of Triatoma sordida susceptibility to pyrethroid insecticides. *Parasites & Vectors, 8*, 1-5.

Pohl, P. C., Klafke, G. M., Júnior J.R., Martins J.R., Vaz I.S. & Masuda A. (2012). ABC transporters as a multidrug detoxification mechanism in Rhipicephalus (Boophilus) microplus. *Parasitology Research, 111*, 2345-2351.

Rodriguez-Vivas, R. I., Ojeda-Chi, M. M., Trinidad-Martinez, I., & De León, A. P. (2017). First documentation of ivermectin resistance in Rhipicephalus sanguineus sensu lato (Acari: Ixodidae). *Veterinary Parasitology, 233*, 9-13.

Rust, M. K., Vetter, R., Denholm, I., Blagburn, B., Williamson, M. S., Kopp, S., ... & Tetzner, K. (2014). Susceptibility of cat fleas (Siphonaptera: Pulicidae) to fipronil and imidacloprid using adult and larval bioassays. *Journal of Medical Entomology, 51*(3), 638-643.

Rust, M. K., Vetter, R., Denholm, I., Blagburn, B., Williamson, M. S., Kopp, S., ... & Tetzner, K. (2015). Susceptibility of adult cat fleas (Siphonaptera: Pulicidae) to insecticides and status of insecticide resistance mutations at the Rdl and knockdown resistance loci. *Parasitology Research, 114*, 7-18.

Sagar, S. V., Saini, K., Sharma, A. K., Kumar, S., Kumar, R., Fular, A., ... & Ghosh, S. (2020). Acaricide resistance in Rhipicephalus microplus collected from selected districts of Madhya Pradesh, Uttar Pradesh and Punjab states of India. *Tropical Animal Health and Production, 52*, 611-618.

Schenker, R., Humbert-Droz, E., Moyses, E. W., & Yerly, B. (2001). Efficacy of nitenpyram against a flea strain with resistance to fipronil. *Supplement Compendium on Continuing Education for the Practicing Veterinarian, 23*, 16-19.

Schetters, T., Bishop, R., Crampton, M., Kopáček, P., Lew-Tabor, A., Maritz-Olivier, C., ... & De la Fuente, J. (2016). Cattle tick vaccine researchers join forces in CATVAC.

Schiavone, A., Price, D. R., Pugliese, N., Burgess, S. T., Siddique, I., Circella, E., ... & Camarda, A. (2023). Profiling of Dermanyssus gallinae genes involved in acaricide resistance. *Veterinary Parasitology,* 109957.

Seidy, S., Tavassoli, M., & Malekifard, F. (2022, December). Pyrethroids resistance in *Pulex irritans* and *Ctenocephalides canis* in west and northwest Iran. *Veterinary Research Forum, 13*(4), 529-535.

Shaw, R. D. (1966). Culture of an organophosphorus-resistant strain of Boophilus microplus (Can.) and an assessment of its resistance spectrum. *Bulletin of Entomological Research, 56*(3), 389-405.

Singh, N. K., Singh, H., Singh, N. K., & Rath, S. S. (2016). Multiple mutations in the acetylcholinesterase 3 gene associated with organophosphate resistance in Rhipicephalus (Boophilus) microplus ticks from Punjab, India. *Veterinary Parasitology, 216*, 108-117.

Smith, W. D., Van Den Broek, A., Huntley, J., Pettit, D., Machell, J., Miller, H. R. P., ... & Taylor, M. (2001). Approaches to vaccines for Psoroptes ovis (sheep scab). *Research in Veterinary Science, 70*(1), 87-91.

Smith, W. D., & Pettit, D. M. (2004). Immunization against sheep scab: preliminary identification of fractions of Psoroptes ovis which confer protective effects. *Parasite Immunology, 26*(6-7), 307-314.

Soler, P., Germano, M., & Larroza, M. (2022). First report of in vitro resistance of Psoroptes ovis to ivermectin in Argentina. *Experimental Parasitology, 235*, 108229.

Sparagano, O., George, D. R., Harrington, D., & Giangaspero, A. (2014). Biology, epidemiology, management and risk related to the poultry red mite, Dermanyssus *gallinae*. *Annual Review of Entomology , 59*, 447-466.

Spreafico, M., Eördegh, F. R., Bernardinelli, I., & Colombo, M. (2001). First detection of strains of *Varroa destructor* resistant to coumaphos. Results of laboratory tests and field trials. *Apidologie, 32*(1), 49-55.

Sternberg, E. D., Waite, J. L., & Thomas, M. B. (2014). Evaluating the efficacy of biological and conventional insecticides with the new 'MCD bottle'bioassay. *Malaria Journal, 1*3(1), 1-9.

Strachecka, A., Borsuk, G., Olszewski, K., & Paleolog, J. (2015). A new detection method for a newly revealed mechanism of pyrethroid resistance development in Varroa destructor. *Parasitology Research, 114*, 3999-4004.

Stafford, K. A., & Coles, G. C. C. (2017). Drug resistance in ectoparasites of medical and veterinary importance. *Antimicrobial Drug Resistance: Mechanisms of Drug Resistance, 1*, 735-744.

Sturgess-Osborne, C., Burgess, S., Mitchell, S., & Wall, R. (2019). Multiple resistance to macrocyclic lactones in the sheep scab mite Psoroptes ovis. *Veterinary Parasitology,* 272, 79-82.

Terada, Y., Murayama, N., Ikemura, H., Morita, T., & Nagata, M. (2010). *Sarcoptes scabiei var. canis* refractory to ivermectin treatment in two dogs. *Veterinary Dermatology, 21*(6), 608-612.

Thullner, F., Willadsen, P., & Kemp, D. (2007). Acaricide rotation strategy for managing resistance in the tick Rhipicephalus (Boophilus) microplus (Acarina: Ixodidae): laboratory experiment with a field strain from Costa Rica. *Journal of Medical Entomology, 44*(5), 817-821.

Valsoni, L. M., Freitas, M. G. D., Echeverria, J. T., Borges, D. G. L., Tutija, J., & Borges, F. D. A. (2020). Resistance to all chemical groups of acaricides in a single isolate of Rhipicephalus microplus in Mato Grosso do Sul, Brazil. *International Journal of Acarology, 46*(4), 276-280.

Van Leeuwen, T., Vontas, J., Tsagkarakou, A., Dermauw, W., & Tirry, L. (2010). Acaricide resistance mechanisms in the two-spotted spider mite Tetranychus urticae and other important Acari: a review. *Insect Biochemistry and Molecular Biology, 40*(8), 563-572.

Van Mol, W., De Wilde, N., Casaert, S., Chen, Z., Vanhecke, M., Duchateau, L., & Claerebout, E. (2020). Resistance against macrocyclic lactones in *Psoroptes ovis* in cattle. *Parasites & Vectors, 13*(1), 1-9.

Vargas, M., Montero, C., Sánchez, D., Pérez, D., Valdés, M., Alfonso, A., ... & Farnós, O. (2010). Two initial vaccinations with the Bm86-based Gavacplus vaccine against Rhipicephalus (Boophilus) microplusinduce similar reproductive suppression to three initial vaccinations under production conditions. *BMC Veterinary Research, 6* (1), 1-8.

Vijverberg, H. P., van der Zalm, J. M., & van den Bercken, J. (1982). Similar mode of action of pyrethroids and DDT on sodium channel gating in myelinated nerves. *Nature, 295*, 601-603.

Wang, C., Xu, X., Huang, Y., Yu, H., Li, H., Wan, Q., ... & Pan, B. (2021). Susceptibility of Dermanyssus gallinae from China to acaricides and functional analysis of glutathione S-transferases associated with beta-cypermethrin resistance. *Pesticide Biochemistry and Physiology, 171*, 104724.

Wexler, P., Anderson, B. D., Gad, S. C., & De Peyster, A. (Eds.). (2005). *Encyclopedia of toxicology*. 1, Academic Press.

Wilson, J. A., Heath, A. C. G., Quilter, S., McKay, C., Litchfield, D., & Nottingham, R. (1997). A preliminary investigation into resistance to synthetic pyrethroids by the sheep biting louse (Bovicola ovis) in New Zealand. *New Zealand Veterinary Journal, 45*(1), 8-10.

WHO (1998): Techniques to detect insecticide resistance mechanisms (field and laboratory manual) WHO/CDS/CPC/MAL/98.6.

WHO (2001): Drug resistance in malaria. WHO/CDS/CSR/DRS/2001.4.

WHO (2005): Guidelines for laboratory in field testing of mosquito larvicides.

WHO (2016): Test procedures for insecticide resistance monitoring in malaria vector mosquitoes. 2nd edition, ISBN 978 92 4 151157 5 (NLM classification: WA 240)

Zeman, P. (1987). Encounter the poultry red mite resistance to acaricides in Czechoslovak poultry-farming. *Folia Parasitologica, 34*(4), 369-373.

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Understanding canine aggression: Neurobiological insights for a complex behavior

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ABSTRACT

The relationship between humans and dogs, as the first domesticated animals, exemplifies a significant aspect of human-animal interaction. During domestication, dogs have undergone behavioral changes to establish closer bonds with humans. However, certain dogs face challenges in fully adapting to their new environment, leading to behavioral disorders such as aggression. One of the most prevalent and dangerous behavioral problems in dogs is aggression, which poses risks to both humans and the dogs themselves, sometimes resulting in euthanasia. Canine aggression can arise from various medical and non-medical factors, including physical problems, endocrine system disorders, infectious diseases, central nervous system diseases, hereditary conditions, as well as racial or personal differences. Evaluating aggression based solely on species, breed, and sex characteristics is insufficient. Accurate diagnosis of aggressive behavior requires integrating findings from diverse diagnostic methods, including serum biochemistry, hormone analysis, urinalysis, electroencephalography, radiography, magnetic resonance tests, and behavioral assessments. However, to gain a comprehensive understanding of canine aggression, it is essential to consider the underlying pathophysiological processes and neurobiology. The management of aggressive behavior in dogs necessitates the implementation of diverse treatment strategies aimed at preventing the manifestation of undesirable behaviors. Within the realm of medical interventions, neutering and pharmacotherapy have emerged as prominent approaches. Neutering has shown effectiveness in mitigating aggression among dogs exhibiting aggressive tendencies. On the other hand, pharmacotherapy involves the utilization of complementary and suppressive pharmacological agents that target primary and intermediate components within the mechanisms underlying aggression. These components encompass neurotransmitter/neuromodulator substances, peptides, enzymes, and hormones, all of which contribute to the pathophysiological processes of aggression. Through the modulation of these factors, pharmacotherapy seeks to offer a comprehensive treatment approach for addressing aggressive behavior in dogs. This review aims to investigate the neurobiological basis of aggression in dogs, considering the underlying pathophysiological processes and the role of neurotransmitter/neuromodulator substances, neuropeptides, peptides, enzyme systems, and hormones. Accurate diagnosis and understanding of canine aggression are crucial for the development of effective medical and alternative treatment methods.

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Introduction

The concept of domestication, which involves the process of animals adapting to the settled life of humans, has a long history reaching back to prehistoric times and has played a vital role in forging a closer relationship between humans and the natural world (Siddiq, 2019). Among the many domesticated animals, dogs hold a special place as the first to be domesticated, dating back approximately 14,000 years. The enduring nature of our social interactions with dogs, spanning from ancient times to the present, serves as a fundamental exemplification of the profound bond between humans and animals. Throughout history, dogs have held multifaceted roles in both natural environments

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and human societies. Their companionship and contributions have solidified their status as one of the most prominent illustrations of human-animal interaction. Notably, certain qualities exhibited by dogs, such as loyalty, trainability, obedience, and harmony, have made them valuable assets in the process of image acquisition and socialization of individuals within society (Batmaz, 2021).

The most common behavioral disorder in dogs: Dominant aggression (Dominance-related aggression)

Observable movements in living organisms are known as behavior (Landsberg, 1990). Animal behavior is a complex phenomenon that encompasses survival behaviors developed by animals in response to their natural environment (Odendaal, 1997). Dogs, in particular, have undergone behavioral changes compared to their wolf ancestors due to their adaptability, allowing them to be closer to humans during domestication (Siddiq, 2019). However, dogs have not fully adapted to their new environment biologically within a short period, leading to internal conflicts between their internal structures and the new environment, resulting in stress, aggression, anxiety, and depression-like behaviors (Dodurka, 1999; Dodurka, 2001). Undesirable behavior exhibited by dogs is often labeled as behavioral disorders by humans (Overall, 1997). These behavioral disorders in dogs can manifest due to various factors, including genetic diseases, developmental challenges, breed-specific behaviors, social environment issues, adaptation strategies, and environmental adaptation disorders (Odendaal, 1997). The prevalence of certain behavioral disorders, such as aggression, fear, attention-seeking behaviors, loneliness, anxiety, environmental pollution, neglect of puppies, social withdrawal, destructive behaviors, digging, maternal cannibalism and coprophagia, contributes significantly to the abandonment of dogs in shelters (Feddersen-Petersen, 1990; Jagoe, 1994; Askew, 1996; Odendaal, 1997; Dodurka, 2005; Kwan and Bain, 2013). These behavioral problems not only pose challenges for dog owners but also serve as major reasons for relinquishment to shelters.

Aggression is a prevalent and hazardous behavioral problem in dogs that can lead to harm to humans and euthanasia of dogs (Houpt and Reisner, 1995; Horwitz, 2000). It is most commonly observed in intact male dogs and later in non-neutered female dogs, and neutering has been shown to reduce aggression in both sexes (Beaver, 1992). Aggression in dogs can have medical origins, including physical problems, endocrine system disorders, infectious diseases, central nervous system disorders, and hereditary diseases (Chapman and Voith, 1990; Landsberg, 1990; Dodman et al., 1996a). In addition to disease-related causes, racial or personal differences have also been found to influence aggression in dogs (Zapata et al., 2016).

Research utilizing behavioral tests, such as the Canine Behavioral Assessment and Research Questionnaire (C-BARQ), has revealed that dog aggression varies across species. Dogs were categorized based on aggression towards their owners, unfamiliar individuals, and other dogs. Dogs known for their friendly characteristics tend to display slight aggression towards strangers and other dogs, while dogs with more robust temperaments, including pit bulls, exhibit more pronounced aggression (Duffy et al., 2008). However, a study conducted in 2011 reported that pit bulls adopted from shelters did not show higher aggression compared to other species (MacNeil-Allcock et al., 2011). Another study investigating the degree of aggression based on scar size in pit bulls rescued from dog fighting, a breed often associated with aggression and euthanasia, found that animals with larger scar areas displayed higher levels of aggression. The study also reported that male pit bulls exhibit more aggression than females. Nonetheless, bite marks alone are insufficient to fully understand an animal's aggression, and behavioral tests should also be conducted (Miller et al., 2016). Instead of evaluating canine aggression solely based on species, breed, and sex characteristics, it is important to address the pathophysiological processes and neurobiology underlying aggression. Additionally, supporting these findings with results obtained from behavior observation and tests is essential for accurate diagnosis (Overall, 1997).

Studies utilizing behavioral tests, such as the Canine Behavioral Assessment and Research Questionnaire (C-BARQ), have shed light on the variability of dog aggression. Duffy et al. (2008) conducted a study where aggression in dogs was categorized based on species, including aggression towards the owner, aggression towards unfamiliar individuals, and aggression towards other dogs. The results showed that dogs known for their friendly characteristics exhibited slight aggression towards strangers and other dogs, while dogs with more robust temperaments, such as pit bulls, displayed more pronounced aggression. However, MacNeil-Allcock et al. (2011) reported that pit bulls adopted from shelters did not show higher levels of aggression compared to other species. Another study focused on investigating aggression levels based on scar size in pit bulls, a breed

often associated with aggression and then euthanasia. The study demonstrated that pit bulls with larger scar areas displayed higher aggression levels. Furthermore, the study reported that male pit bulls exhibited more aggression than females. However, it is important to note that bite marks alone are insufficient to fully understand an animal's aggression, and behavioral tests should also be conducted (Miller et al., 2016). Therefore, evaluating canine aggression should not solely rely on species, breed, and sex characteristics, but should also consider the underlying pathophysiological processes and neurobiology of aggression (Overall, 1997).

Aggression in dogs can be classified into different types based on various reasons, including aggression for fear and self-defense, territorial aggression, herd aggression, racial and hereditary factors, aggression during the socialization period, aggression resulting from diseases, dominant aggression, and aggression of unknown origin (Askew, 1996; Feddersen-Petersen, 1990; Jagoe, 1994; Noyan, 2007). Among these types, dominant aggression is the most common and occurs independently of breed, gender, and age characteristics (Reisner et al., 1994; Dodman et al., 1996b; Uchida et al., 1997; Odore et al., 2020). Studies have found that the majority of dogs diagnosed with dominant aggression are young male dogs and purebreds, with bull terriers being one of the breeds associated with a higher incidence of dominant attacks (Blacksaw, 1991; Uchida et al., 1997).

Diagnosing dominant aggression may involve behavioral tests as well as additional methods such as serum biochemistry, hormone analysis, urine analysis, electroencephalography, radiography, and magnetic resonance tests (Landsberg, 1990; Serpell and Hsu, 2005; MacNeil-Allcock et al., 2011). Behavioral tests encompass various criteria for identifying dogs predisposed to dominant aggression. These criteria include prolonged eye contact with the dog, performing painless procedures such as grooming, nail clipping, and ear cleaning, withholding food from the dog, passing by while the dog is eating, adding food to the bowl while eating, attempting to pick up the dog, physically arousing the dog, physical contact such as lifting and hugging, carrying out therapeutic procedures, physical punishment, verbal stimulus as punishment, leash handling including putting on/off and pulling, walking next to the dog in a confined space, testing responsiveness to commands, physical contact of a stranger with loved ones, and a stranger approaching the dog. Dogs that display an aggressive attitude towards at least five of these criteria are classified as predisposed to dominant aggression (Landsberg, 1990; O'Farrell, 1992; Dodman et al., 1996a; Serpell and Hsu, 2005; MacNeil-Allcock et al., 2011).

Breed differences in dog aggression: The assessment of breed differences in dog aggression is often based on sources like bite statistics, behavior clinic reports, and expert opinions. However, relying solely on these sources can be misleading due to biases and racial stereotypes, as they may disproportionately associate higher aggression risks with larger or physically stronger breeds. In order to obtain more reliable information about breed-specific aggression, a study was conducted using the Canine Behavioral Assessment and Research Questionnaire (C-BARQ) to evaluate the behaviors of over 30 breeds of dog in response to various stimuli and situations (Hsu and Serpell, 2003; Duffy et al., 2008). The findings from this study revealed significant variations in aggression among dogs towards strangers, owners, and other dogs. Eight breeds, namely Dachshund, English Springer Spaniel, Golden Retriever, Labrador Retriever, Poodle, Rottweiler, Shetland Sheepdog, and Siberian Husky, showed similar rankings in terms of aggression towards strangers, other dogs, and their owners, respectively. Breeds such as Chihuahuas and Dachshunds scored above average for aggression towards humans and other dogs. Akitas and Pit Bull Terriers exhibited high levels of aggression specifically towards a particular target, such as other dogs. Overall, dogs tend to display aggression primarily towards other dogs, followed by unfamiliar individuals and their owners (Duffy et al., 2008).

Certain breeds showed a propensity for aggressive behavior towards humans. For instance, Dachshunds, Chihuahuas, and Jack Russell Terriers displayed aggressive tendencies towards both strangers and their owners (Liinamo et al., 2007). Australian Cattle Dogs exhibited higher aggression towards foreigners, while American Cocker Spaniels and Beagles showed aggression towards their owners. Notably, more than 20% of Akitas, Jack Russell Terriers, and Pit Bull Terriers exhibited highly aggressive behavior towards unfamiliar dogs. On the other hand, Golden Retrievers, Labrador Retrievers, Bernese Mountain Dogs, Brittany Spaniels, Greyhounds, and Whippets were among the breeds that demonstrated the least aggression towards both humans and other dogs (Duffy et al., 2008).

Legal regulation regarding dogs in the aggressive breed category in Türkiye: The choice of pets often reflects the preferences and personality traits of individuals. Pitbull breeds, characterized by their

muscular and robust bodies, are favored as pets by certain segments of society due to their agility, bravery, protectiveness, and loyalty. However, in other circles, they are considered breeds to be avoided (Stepherd, 2018; Batmaz, 2021). Pitbulls originated in England through the crossing of Terrier and Bulldog dogs. The Pitbull breeds known today include the American Pitbull Terrier, the American Bulldog, the American Staffordshire Terrier, and the Staffordshire Bull Terrier. Over time, Pitbulls have been utilized in various domains. Their warrior-like and aggressive traits made them initially popular in blood sports such as Dog Fighting, Cock Fighting, Gladiator Fights, Hunting, and Bullfighting. They also became symbolic of America during the 1st and 2nd World Wars. While they can be beneficial as "nanny dogs" due to their loyalty, obedience, affection, and protectiveness, Pitbulls have garnered negative attention in society due to their historical involvement in illegal fights and media coverage highlighting Pitbull-related incidents (Gunter et al., 2016; Kogan et al., 2019; Batmaz, 2021). In Turkey, the Animal Protection Law No. 5199, enacted in 2004, prohibits the production, adoption, rehoming, housing, feeding, exchange, display, gifting, and sale of Pitbulls (Resmi Gazete, 01.07.2004). In 2021, the Law on the Amendment of the Animal Protection Law and the Turkish Penal Code was published in the Official Gazette. As per Article 14/1 of this law, individuals engaged in the aforementioned activities are subject to administrative fines. The same article also forbids the transportation of "dangerous" animals registered before January 14, 2022, without a registration certificate, muzzle, and collar, as well as their entry into public areas and children's playgrounds/parks. In case of continued violations, the animals are confiscated, and an administrative fine is imposed, with the animals being taken to the nearest municipality shelter (Resmi Gazete, 14.07.2021).

The role of neuromodulators, neurotransmitters, and neuropeptides in canine aggression: In the treatment of dogs with dominant aggression, various strategies can be employed to prevent the display of undesirable behaviors. Behavioral treatments play a crucial role in reducing and eliminating aggression (Landsberg, 1990; Askew, 1996; Houpt et al., 1996; Uchida et al., 1997; Yeon et al., 1999; Dodman et al., 2006c; Odore et al., 2020). Additionally, medical treatment methods such as neutering and pharmacotherapy are utilized (Askew, 1996; Landsberg et al., 1997). Neutering has been shown to help decrease aggression in dogs with dominant behavior (Askew, 1996; Kuhne, 2012). On the other hand, pharmacotherapy involves the use of pharmacological agents that target neurotransmitter/ neuromodulator substances, peptides, enzymes, and hormones involved in the pathophysiological processes of aggression, aiming to complement and suppress these mechanisms (Dodman et al., 1996a; Reisner et al., 1996; Landsberg et al., 1997; Overall, 1997; Pineda et al., 2014; Notari et al., 2015; Niyyat et al., 2018). To further advance current medical and alternative treatment approaches, it is crucial to elucidate the underlying mechanisms and pathways involved in canine aggression, as well as the roles of neurotransmitter/neuromodulator substances, neuropeptides, peptides, enzyme systems, and hormonal control pathways in these processes.

Serotonin

Serotonin (5-hydroxytryptamine, 5-HT) is a neurotransmitter that plays a vital role in various physiological processes, including the modulation of neuropsychological functions, cardiovascular and respiratory control, gastrointestinal motility, regulation of food intake and energy balance, and urogenital system modulation (Rapport et al. 1948; Roth, 2007). The central and peripheral nervous systems have identified 15 receptors associated with serotonin. Serotonin and its receptors are involved in regulating all brain functions within the central nervous system. Serotonergic neurons, which express serotonin in the raphe nucleus of the brainstem, significantly contribute to the development of behavior in humans and animals through their projections to the cortex, limbic system, midbrain, and hindbrain. The serotonergic system manages behavioral and neuropsychological processes such as mood, perception, reward/punishment, anger, aggression, appetite, memory, and libido. Dysregulation of this system can lead to psychological and neurological disorders (Berger et al., 2009).

Compared to other neurotransmitters, the serotonergic system is the most consistently active neurotransmission system involved in neurobiological mechanisms underlying aggressive behaviors (Miczek et al., 2002 and 2007; Takahashi et al., 2011). Low levels of serotonin/serotonin metabolites and imbalances in the expression of serotonin receptors are key factors contributing to aggression. Additionally, the serotonergic system interacts with other neurotransmitter and neuromodulator substances, amino acids, steroids, and peptides in the central nervous system, further influencing aggression (Takahashi et al., 2012).

Neurotransmitter substances like glutamate and γ-Aminobutyric Acid (GABA), known for their high

functional properties in the brain, directly modulate serotonergic neurons. They exhibit increased expression in regions where serotonin neurons are localized, and GABAergic and Glutamatergic receptors are present in regions where the serotonergic system projects (Takahashi et al., 2012). Corticotropin-releasing factor (CRF) immunoreactive fibers and CRF receptors in the dorsal raphe nucleus also play a crucial role in modulating serotonin neurons related to aggression (Quadros et al., 2009a and 2009b). Arginine vasopressin (AVP), oxytocin, and their respective receptors are responsible for the regulation of serotonergic neurons involved in aggressive behavior. Monoamine oxidase (MAO), the adrenergic system, dopaminergic system, neuropeptide Y (NPY), tryptophan, brain-derived neurotrophic factor (BDNF), and neuronal nitric oxide synthase (NOS) are additional factors that directly or indirectly influence the effectiveness of the serotonergic system in aggressive behaviors (Takahashi et al., 2012).

Studies conducted in humans and various animal species have revealed a relationship between aggressive behavior and serotonin (Kırlı, 2000; Lesch and Merschdorf, 2000; Almeida-Montes et al., 2000). Individuals exhibiting high levels of aggression often have lower levels of serotonin and serotonin metabolites, as well as alterations in the expression of serotonin receptors (Miczek et al., 2002). Research conducted on different dog breeds has reported lower levels of serotonin in serum (Çakıroğlu et al., 2007), cerebrospinal fluid (Reisner et al., 1996), and urine (Wright et al., 2012) in aggressive dogs compared to non-aggressive dogs. Treatment methods that increase serotonin levels in the brain, such as selective serotonin reuptake inhibitors, have shown to reduce aggression in both humans and animals (Bacqué-Cazenave et al., 2020).

Dopamine

Dopamine (3-hydroxy thiamine), a neurotransmitter belonging to the catecholamine family, is synthesized in both the central nervous system and the periphery. Its effects are mediated through specific receptors expressed in both central and peripheral regions (Hansen and Manahan-Vaughan, 2012). The dopaminergic system plays a vital role in modulating various behaviors, including motor control, motivation, reward/punishment, cognitive function, maternal instinct, and reproduction. Imbalances in dopaminergic signaling pathways have been implicated in neurodegenerative disorders. Furthermore, dopamine and its receptors are involved in the pathophysiology and psychopharmacology of neuropsychiatric conditions such as schizophrenia and attention deficit hyperactivity disorder (O-Klein et al., 2019). Although research on the dopaminergic system in aggression is limited compared to the serotonergic system, it has been shown to have a significant impact on aggression development (Rosell and Siever, 2015). There is an inverse relationship between dopamine synthesis capacity, particularly in the midbrain and striatum, and the frequency of aggressive responses. Additionally, midbrain dopamine storage capacity has been found to be negatively correlated with aggression-related behaviors (Hall et al., 2012). Pharmacologically induced increases in dopamine levels have been associated with heightened aggressive behaviors (Almeida et al., 2005). Yu et al. (2014) demonstrated that aggression is augmented by increased activity in dopaminergic neurons, and genetic and pharmacological factors influencing dopamine and serotonin signaling can modulate monoaminergic functions known to impact aggression, potentially altering the risk of aggressive and emotional dysfunctions.

Pharmacotherapeutic agents targeting dopaminergic receptors, such as the dopamine D2 receptor antagonist haloperidol, have long been used to mitigate human aggression. Haloperidol has shown efficacy in treating aggressive behavior in children and adolescents with psychotic disorders and Parkinson's behavior disorders (Glazer and Dickson, 1998; Fitzgerald, 1999; Kennedy et al., 2001; Beauchaine et al., 2000; Diederich et al., 2003; Masi, 2004). Preclinical studies have also implicated dopamine D1, D2, and D3 receptors in the modulation of aggression (Tidey and Miczek, 1992a and 1992b; Sanchez et al., 1993; Miczek et al., 2002 and 2004). In a study on defense-based aggression in cats, D2 receptors were found to play a role, and the stimulation of D2 receptors in the medial preoptic area and anterior hypothalamus, regions associated with defensive behaviors, facilitated the expression of emotional defense behavior (Sweidan et al., 1991).

Oxytocin

Oxytocin, a neuropeptide acting as both a neurotransmitter in the central nervous system and a hormone in the peripheral circulation, is involved in various social behaviors, including maternal-offspring bonding and pair bonding (Churchland and Winkielman, 2012). It is primarily synthesized in the supraoptic and paraventricular nuclei of the hypothalamus and stored in the posterior lobe of the pituitary gland, where it is released into the peripheral circulation to facilitate uterine contractions during

birth process and milk expulsion during breastfeeding (Insel, 2010). In the central nervous system, oxytocin exerts its effects by being released from neurons extending from the paraventricular nucleus to the amygdala, hippocampus, and nucleus accumbens. The oxytocin receptor (OXTR), the sole receptor identified so far, exhibits a sex- and species-specific distribution in both the central and peripheral nervous systems. Variations in OXTR expression and activation contribute to individual differences in the oxytocin system (Buisman-Pijlman et al., 2014).

Oxytocin has been implicated in numerous psychiatric disorders, including autism, schizophrenia, mood disorders, generalized anxiety disorder, social phobia, post-traumatic stress disorder, obsessive-compulsive disorder, attention deficit hyperactivity disorder, aggression, suicidal tendencies, eating disorders, and personality disorders (Ishak et al., 2011). Studies have reported lower plasma oxytocin levels, particularly in females, associated with depression-like behaviors (Frasch et al., 1995; Ozsoy et al., 2009). Furthermore, oxytocin infusion has been shown to increase serotonin release in the raphe nucleus, the central site of action for selective serotonin reuptake inhibitors (Yoshida et al., 2009). Turan et al. (2013) found oxytocin levels to be higher in patients with bipolar disorder during manic episodes compared to depressive episodes and remissions. Acute stress and fear situations activate the oxytocin system, resulting in elevated oxytocin levels in both the central nervous system and peripheral tissues (Engelmann et al., 2004; Neumann, 2007). While chronic anxiety disorder and plasma oxytocin levels were positively correlated in women, an inverse relationship was observed in men (Holt-Lunstad et al., 2011; Weissman et al., 2013). Acute administration of oxytocin has also been shown to reduce anxiety (de Oliveira et al., 2012).

Animal studies with inactivated oxytocin genes/ receptors have demonstrated changes in social and aggressive behaviors (Takayanagi et al., 2005). In humans, lower levels of oxytocin measured in the cerebrospinal fluid have been associated with higher lifetime aggression scores, although no significant association was found with personality disorders (Lee et al., 2009). Polymorphism in the oxytocin receptor gene has been linked to aggressive behaviors observed in childhood (Beitchman et al., 2012), suggesting a potential role for oxytocin in the etiology of aggression.

Gamma-Aminobutyric Acid (GABA)

GABA (gamma-aminobutyric acid) is a neurotransmitter synthesized from its precursor substance, L-glutamate, through decarboxylation mediated by the enzyme glutamate decarboxylase (Wassef et al., 2003). It serves as the primary inhibitory neurotransmitter in the central nervous system of mammals (Gou et al., 2012). GABAergic neurons have widespread projections throughout the brain and exert regulatory effects on various physiological mechanisms. Three types of GABA receptors have been identified: GABAA, GABAB, and GABAC (Wassef et al., 2003).

The involvement of GABA in the pathophysiology of aggression is predominantly associated with its inhibitory function (Miczek et al., 2003). Activation of GABAA receptors is known to decrease aggressive behavior, while positive modulators can exacerbate aggression. Conversely, GABAB receptors are directly linked to increased aggression. Pharmacological activation of GABAB receptors in the dorsal raphe nucleus (DRN) leads to heightened aggressive behavior (Takahashi et al., 2010a). Serotonergic neurons in the DRN play a crucial role in serotonin regulation, and the interplay between GABA and serotonin is critical in aggression development. Numerous studies have demonstrated that high expression of GABAA and GABAB receptors in the DRN regulates serotonin levels (Takahashi et al., 2010a and 2010b). Activation of GABAergic receptors on serotonergic neurons can elevate serotonin levels in the medial prefrontal cortex. Conversely, GABA receptors in the medial raphe nucleus (MRN) do not exert a similar effect on aggression, indicating distinct roles of serotonin neurons in the MRN and DRN in aggression development (Mokler et al., 2009). Furthermore, GABAA receptors located in the lateral septum are involved in maternal aggression in females (Lee and Gammie, 2009). Further research is necessary to fully comprehend the intricate roles of GABA in the development of aggressive behaviors. However, existing studies support GABA receptors as valid therapeutic targets for the regulation of aggressive behavior.

Thyroid hormones

Thyroid hormones (THs) play a crucial role in maintaining energy homeostasis and regulating the metabolic stages necessary for growth and development (Cheng et al., 2010; Brent, 2012). Thyrotropin-releasing hormone (TRH) is produced by neurons in the paraventricular nucleus of the hypothalamus and released from hypothalamic nerve projections known as the median eminence. As a hypophysiotropic hormone, TRH stimulates the synthesis and release of thyroid-stimulating hormone (TSH, Thyrotropin) from the adenohypophysis (Fekete

and Lechan, 2014). Several factors, including kidney failure, fasting, insomnia, depression, cortisol, and growth hormone, can influence TSH secretion (Jackson, 1982; Gary et al., 1996). The target organ of TSH is the thyroid gland, where the synthesis and secretion of thyroid hormones, thyroxine (T4) and triiodothyronine (T3), occur within follicles (van der Spek et al., 2017).

Hyperthyroidism, characterized by excessive secretion of thyroid hormones, is associated with weight loss due to an increased metabolic rate, lower cholesterol levels, heightened lipolysis, and gluconeogenesis. In contrast, hypothyroidism, resulting from the under-secretion of thyroid hormones, leads to weight gain due to a decreased metabolic rate, elevated cholesterol levels, reduced lipolysis, and decreased gluconeogenesis (Motomura and Brent, 1998; Brent, 2012). Thyroid hormones have significant effects on the development of the central nervous system, its functional processes, and behavioral characteristics (Bernal, 2005).

Studies have demonstrated that thyroid hormones regulate oxytocin and arginine vasopressin, essential factors involved in the regulation of behaviors such as sociability, aggression, and maternal behavior (Ferguson et al., 2000; Choleris et al., 2003; Bielsky and Young 2004; Bielsky et al., 2004 and 2005; Crawley et al., 2007; Yu et al., 2016). Decreased thyroid function has been associated with decreased neurotransmitter levels in the brain (Ito et al., 1977; Whybrow and Prange, 1981; Henley and Koehnle 1997; Mano et al., 1998), and it was concluded that thyroid hormones are required for the synthesis of neurotransmitters that are actively involved in behavioral processes and aggression (Bernal and Nunez 1995; Vara et al., 2002). Similarly, thyroid hormone function is closely linked to the dopaminergic and serotonergic systems in the brain (Henley et al., 1991; Henley and Valdic, 1997; Bauer et al., 2002; Strawn et al., 2004).

Hypothyroidism-induced aggression has been reported in dogs in numerous studies (Beaver, 1999; Beaver and Haug, 2003; Mertens and Dodman, 2003). Hypothyroidism is thought to contribute to approximately 1.7% of aggressive behavior, which may occur even in the absence of lethargy, weight gain, and other characteristic clinical signs (Beaver, 1983 and 1999; Juarbe-Diaz, 2002). In dogs, aggression has been associated with increased concentrations of thyroglobulin autoantibody (TgAA) along with T4 and TSH concentrations (Graham et al., 2004). Some dogs with hypothyroidism exhibit a paradoxical response of excitement and aggressive behavior. The aggressive behaviors resemble those displayed in situations of dominance or fear-related aggression and can occur without other typical signs of hypothyroidism (Aronson, 1998; Beaver, 1999). Hypothyroidism in dogs may also manifest as lethargy, mental dullness, intolerance to cold and exercise, decreased libido, cognitive dysfunction, behavioral abnormalities, and aggression (Camps et al., 2019). Treatment with L-thyroxine or levothyroxine supplementation has been reported to alleviate polyneuropathy and aggressive behaviors in a dog exhibiting such symptoms (Indrieri, 1987). Additionally, it has been shown that behavioral expressions displaying aggressive behaviors toward the owner in dogs with hypothyroidism decrease following treatment (Beaver and Haug, 2003).

Conclusion

Studying aggressive behavior presents a significant challenge due to the complex and multifaceted activities of neurotransmitters involved, which are still under investigation. Establishing causal links between these neurotransmitters and aggression is highly challenging, as their interactions often involve other neurotransmitters, neuromodulators, neuropeptides, or hormones. While there are numerous instances where different neurotransmitters are implicated in aggressive behaviors, understanding the precise mechanisms by which these neurotransmitters function in the majority of animal models used for studying aggression remains unclear. Understanding how individuals of a particular breed respond to their competitors is crucial before socializing them and interacting with humans and highlights the continuing importance of studying aggressive behavior in dogs. Traditional therapeutic approaches have sometimes hindered progress in understanding the multifactorial mechanisms underlying aggression. However, the application of innovative techniques, such as epigenetic research methods, holds promise for gaining deeper insights into how environmental changes influence and promote aggressive behavior. By investigating changes in methylation patterns associated with aggression and exploring genes related to neurotransmitter receptors or transporters, we can significantly contribute to elucidating the causes of heightened aggression and identifying novel targets for aggression prevention and treatment.

Gaining knowledge about the functioning of each neurotransmitter and their interplay in situations of increased aggressive behavior triggered by various factors will facilitate the development of interventions for pathological aggression. By delving into the intricate mechanisms and relationships among neurotransmitters, we can better understand how aggressive behavior manifests and explore potential avenues for managing and mitigating aggression.

References

Almeida, R. M. M., Ferrari, P. F., Parmigiani, S., Miczek, K. A. (2005). Escalated aggressive behavior: Dopamine, serotonin and GABA. *European Journal of Pharmacology*, *526*, 51–64.

Almeida-Montes, L. G., Valles-Sanchez, V., Moreno-Aguilar, J. (2000). Relation of serum cholesterol, lipid, serotonin and tryptofhan levels to severity of depression and to suicide attempts. *Journal of Psychiatry and Neuroscience, 25* (4), 371-377.

Aronson, L. (1998). *Systemic causes of aggression and their treatment. In Psychopharmacology of Animal Behavior Disorders. Eds* N. H. Dodman, L. Shuster. Oxford, Blackwell Science, 88-92.

Askew, H. R. (1996). *Treatment of Behavior Problems in Dogs and Cats*. Blackwell Science, Oxford.

Bacqué-Cazenave, J., Bharatiya, R., Barrière, G., Delbecque, J. P., Bouguiyoud, N., Giovanni, G., Cattaert, D., Deurwaerdère, P. (2020). Serotonin in Animal Cognition and Behavior. *International Journal of Molecular Sciences, 21*(5), 1649.

Batmaz, T. (2021). *Toplumsal Cinsiyet Bağlamında Evcil Hayvan Besleme Davranışı Olarak Pitbull Tercihi*, 9. Türkiye Lisansüstü Çalışmalar Kongresi Bildiriler Kitabı – IV, 325-347.

Bauer, M., Heinz, A., Whybrow, P. C. (2002). Thyroid hormones, serotonin, and mood: of synergy and significance in the adult brain. *Molecular Psychiatry, 7*, 140-156.

Beauchaine, T. P., Gartner, J., Hagen, B. (2000). Comorbid depression and heart rate variability as predictors of aggressive and hyperactive symptom responsiveness during inpatient treatment of conduct-disordered. ADHD boys. *Aggressive Behavior, 26*, 425–441.

Beaver, B. (1992). *Canine behavior, aguide for veterinarians, 1th edition*, W. B. Saunders Co, Philadelpia, X+355.

Beaver, B. V. (1983). Clinical classification of canine aggression. *Applied Animal Ethology, 10*, 35–43.

Beaver, B. V. (1999). *Canine social behavior. In: Beaver, B.V. (Ed.), Canine Behavior: A Guide for Veterinarians*. W.B. Saunders, Philadelphia, PA, USA, 151–175.

Beaver, B. V. & Haug, L. I. (2003). Canine behaviors associated with hypothyroidism. *Journal of the American Animal Hospital Association, 39*, 431–434.

Beitchman, J. H., Zai, C. C., Muir, K., Berall, L., Nowrouzi, B., Choi, E. (2012). Childhood aggression, callous-unemotional traits and oxytocin genes. *European Child & Adolescent Psychiatry, 21*, 125-132.

Berger, M., Gray, J. A., Roth, B. L. (2009). The expanded biology of serotonin. *Annual Review of Medicine, 60*, 355-366.

Bernal, J. (2005). Thyroid hormones and brain development. Vitamins and hormones, 71, 95–122.

Bernal, J. and Nunez, J. (1995). Thyroid hormones and brain development. *European Journal of Endocrinology*, 133 (4), 390–398.

Bielsky, I. F. and Young, L. J. (2004). Oxytocin, vasopressin, and social recognition in mammals. *Peptides, 25*, 1565–1574.

Bielsky, I. F., Hu, S. B., Ren, X., Terwilliger, E. F., Young, L. J. (2005). The V1a vasopressin receptor is necessary and sufficient for normal social recognition: A gene replacement study. *Neuron, 47*, 503–513.

Bielsky, I. F., Hu, S. B., Szegda, K. L., Westphal, H., Young, L. J. (2004). Profound impairment in social recognition and reduction in anxiety-like behavior in vasopressin V1a receptor knockout mice. *Neuropsychopharmacology, 29,* 483–493.

Blacksaw, J. K. (1991). An overwiew of types of agressive behaviour in dogs and methods of treatment. *Applied Animal Behaviour Science, 30*, 351-361.

Brent, G. A. (2012). Mechanisms of thyroid hormone action. *Journal of Clinical Investigation, 122*, 3035–3043.

Buisman-Pijlman, F. T., Sumracki, N. M., Gordon, J. J., Hull, P. R., Carter, C. S., Tops, M. (2014). Individual differences underlying susceptibility to addiction: Role for the endogenous oxytocin system. *Pharmacology Biochemistry and Behavior, 119*, 22-38.

Çakıroğlu, D., Meral, Y., Sancak, A., Çiftçi, G. (2007). Relationship between the serum concentrations of serotonin and lipids and aggression in dogs. *The Veterinary Record, 161*(2), 59-61.

Camps, T., Amat, M., Manteca, X. (2019). A review of medical conditions and behavioral problems in dogs and cats. *Animals, 9*(12), 1133.

Chapman, B. L. and Voith,V. L. (1990). Behavioral problems in old dogs 26 cases (1984-1987). *Journal of the American Veterinary Medical Association*.

Cheng, S. Y., Leonard, J. L., Davis, P. J. (2010). Molecular aspects of thyroid hormone actions. *Endocrine Reviews*, *31*, 139 –170.

Choleris, E., Gustafsson, J. A., Korach, K. S., Muglia, L. J., Pfaff, D. W., Ogawa, S. (2003). An estrogen-dependent four-gene micronet regulating social recognition: A study with oxytocin and estrogen receptor-alpha and -beta knockout mice. *Proceedings of the National Academy of Sciences , 100*, 6192–6197.

Churchland, P. S. and Winkielman, P. (2012). Modulating social behavior with oxytocin: how does it work? what does it mean? *Hormones and Behavior, 61*, 392-399.

Crawley, J. N., Chen, T., Puri, A., Washburn, R., Sullivan, T. L., Hill, J. M., Young, N. B., Nadler, J. J., Moy, S. S., Young, L. J., Caldwell, H. K., Young, WS. (2007). Social approach behaviors in oxytocin knockout mice: Comparison of two independent lines tested in different laboratory environments. *Neuropeptides, 41*, 145–163.

de Oliveira, D. C., Zuardi, A. W., Graeff, F. G., Queiroz, R. H., Crippa, J. A. (2012). Anxiolytic-like effect of oxytocin in the simulated public speaking test. *Journal of Psychopharmacology, 26*, 497–504.

Diederich, N. J., Moore, C. G., Leurgans, S. E., Chmura, T. A., Goetz, C. G. (2003). Parkinson disease with old-age onset: a comparative study with subjects with middle-age onset. *Archives of neurology, 60*, 529–533.

Dodman, H. D., Donnelly, R., Shuster, L., Mertens, P., Rand, W., Miczek, K. (1996a). Use of fluoxetine to treat dominance aggression in dogs. *Journal of America Veterinary Medicine Association, 209*, 1585-1587.

Dodman, N. H., Moon, R., Zelin, M. (1996c). Influence of owner personality type on expression and treatment outcome of dominance aggression in dogs. *Journal of America Veterinary Medicine Association, 209*, 1107-1109.

Dodman, N. H., Reisner, I., Shuster, L., Rand, W., Luescher, U. A., Robinson, I. & Houpt, K. A. (1996b). Effect of dietary protein content on behavior in dogs. *Journal of America Veterinary Medicine Association, 208*, 376-379.

Dodurka, H. T. (1999). *Köpek Psikolojisi, 1.* Baskı, Teknik Yayınları, İstanbul, XII+201.

Dodurka, H. T. (2001). Köpeklerde görülen davranış sorunları ve bunlara etki etmesi. *Yüksek lisans Tezi,* Sosyal Bilimler Estitüsü.

Dodurka, H. T. (2005). *Köpeklerde Davranış Sorunları.* 1. Baskı, Remzi Kitabevi, İstanbul.

Duffy, D. L., Hsu, Y., Serpell, J. A. (2008). Breed differences in canine aggression. *Applied Animal Behaviour Science, 114*, 441–460.

Engelmann, M., Landgraf, R., Wotjak, C. T. (2004). The hypothalamicneurohypophysial system regulates the hypothalamic-pituitaryadrenal axis under stress: an old concept revisited. Frontiers in Endocrinology, 25, 132-149.

Feddersen-Petersen, D. (1990). Behavior of dogs. *Dtsch Tierarztl Wochenschr, 97*(6), 231-236.

Fekete, C. and Lechan, R. M. (2014). Central Regulation of Hypothalamic-Pituitary-Thyroid Axis under Physiological and Pathophysiological Conditions. *Endocrine Reviews, 35*, 159–194.

Ferguson, J. N., Young, L. J., Hearn, E. F., Matzuk, M. M., Insel, T. R., Winslow, J. T. (2000). Social amnesia in mice lacking the oxytocin gene. *Nature Genetics, 25*, 284–288.

Fitzgerald, P. (1999). Long-acting antipsychotic medication, restraint and treatment in the management of acute psychosis. *Australian & New Zealand Journal of Psychiatry, 33*, 660–666.

Frasch, A., Zetzsche, T., Steiger, A., Jirikowski, G. F. (1995). Reduction of plasma oxytocin levels in patients suffering from major depression. *Advances in Experimental Medicine and Biology, 395*, 257-258.

Gary, K. A., Winokur, A., Douglas, S. D., Kapoor, S., Zaugg, L., Dinges, D. F. (1996). Total sleep deprivation and the thyroid axis: effects of sleep and waking activity. Aviation, *Space, and Environmental Medicine, 67*, 513–519.

Glazer, W. M. and Dickson, R. A. (1998). Clozapine reduces violence and persistent aggression in schizophrenia. *Journal of Clinical Psychiatry, 59*(Suppl 3), 8–14.

Gou, Z. H., Wang, X., Wang, W. (2012). Evolution of neurotransmitter gamma-aminobutyric acid, glutamate and their receptors. *Zoological Research, 33*, E75-E81.

Graham, P. A., Lundquist, R. B., Refsal, K. R., Nachreiner, R. F. (2004). *Reported clinical signs in 8317 cases of canine hypothyroidism and 2647 cases of subclinical thyroiditis*. In: 2004 Scientific Proceedings of the British Small Animal Veterinary Association Congress, Birmingham, UK, 529.

Gunter, L. M., Barber, R. T., Wynne, C. D. L. (2016). What's in a Name? Effect of Breed Perceptions & Labeling on Attractiveness, Adoptions & Length of Stay for Pit-Bull-Type Dogs, *PLoS One, 11*(3), e0146857.

Hall, J. E., Simon, T. R., Lee, R. D., Mercy, J. A. (2012). Implications of direct protective factors for public health research and prevention strategies to reduce youth violence. *American Journal of Preventive Medicine, 43*(2 Suppl 1), S76–S83.

Hansen, N. and Manahan-Vaughan, D. (2012). Dopamine D1/ D5 receptors mediate informational saliency that promotes persistent hippocampal long-term plasticity. *Cerebral Cortex, 24,* 845-858.

Henley, W. N. and Koehnle, T. J. (1997). Thyroid hormones and the treatment of depression: an examination of basic hormonal actions in the mature mammalian brain. *Synapse, 27(*1), 36–44.

Henley, W. N. and Valdic, F. (1997). Hypothyroid-induced changes in autonomic control have a central serotonergic component. *American Journal of Physiology-Heart and Circulatory Physiology, 272*, 894–903.

Henley, W. N., Chen, S., Klettner, C., Bellush, L., Notestine, M. A. (1991). Hypothyroidism increases serotonin turnover and sympathetic activity in the adult rat. *Canadian Journal of Physiology and Pharmacology, 69*, 205–210.

Holt-Lunstad, J., Birmingham, W., Light, K. C. (2011). The influence of depressive symptomatology and perceived stress on plasma and salivary oxytocin before, during and after a support enhancement intervention. *Psychoneuroendocrinology, 36*, 1249-1256.

Horwitz, D. F. (2000). Differences and similarities between behavioral and internal medicine. *Journal of America Veterinary Medicine Association, 217*, 1372-1376

Houpt, K. A. and Reisner, I. R. (1995). *Behavioral disoders,* volume 1, 4th edition. Editors: Etine, S. J. and Feldman, E. C. Veterinary Internal Medicine. W. B. Saunders Co. Philadelphia, 179-187.

Houpt, K. A., Honig, S. U., Reisner, I. R. (1996). Breaking the human-Companion animal bond. *Journal of America Veterinary Medicine Association, 208*, 1653-1658.

Hsu, Y. and Serpell, J. A., (2003). Development and validation of a questionnaire for measuring behavior and temperament traits in pet dogs. Journal of America Veterinary Medicine Association, *223*, 1293–1300.

Indrieri, R. J., Whalen, L. R., Cardinet, G. H., Holliday, T. A. (1987). Neuromuscular abnormalities associated with hypothyroidism and lymphocytic thyroiditis in three dogs. *Journal of the American Veterinary Medical Association*, *190*(5), 544–548.

Insel, T. R. (2010). The challenge of translation in social neuroscience: a review of oxytocin, vasopressin, and affiliative behavior. *Neuron, 65*, 768-779.

Ishak, W. W., Kahloon, M., Fakhry, H. (2011). Oxytocin role in enhancing well-being: a literature review. *Journal of Affective Disorders, 130*, 1-9.

Ito, J. M., Valcana, T., Timiras, P. S. (1977). Effect of hypo- and hyperthyroidism on regional monoamine metabolism in the adult rat brain. *Neuroendocrinology, 24*(1), 55–64.

Jackson, I. M. D. (1982). Thyrotropin-releasing hormone. *The New England Journal of Medicine, 306*, 145–155.

Jagoe, J. A. (1994). *Behaviour problems in the domestic dog: a retrospective and prospective study to identify factors influencing their development* (Doctoral thesis). https://doi.org/10.17863/CAM.31066Phd thesis.

Juarbe-Diaz, S. V. (2002). *Behavioral imposters of medical disorders in small animals*, In: Proceedings of the Forum of the American College of Veterinary Internal Medicine.

Kennedy, J. S., Bymaster, F. P., Schuh, L., Calligaro, D. O., Nomikos, G., Felder, C. C., Bernauer, M., Kinon, B. J., Baker, R. W., Hay, D., Roth, H., Dossenbach, M., Kaiser, C., Beasley, C. M., Holcombe, J. H., Effron, M. B., Breier, A. (2001). A current review of olanzapine's safety in the geriatric patient: from pre-clinical pharmacology to clinical data. *International Journal of Geriatric Psychiatry*, *16*, 33–61.

Kırlı, S. (2000). *Depresyonun Biyolojik Oluşumu ve Farmakolojik Tedavisi*, F. Özcan Matbaacılık San. Ve Tic. Ltd. Şti., Bursa, 31-97.

Kogan, L. R., Schoenfeld-Tacher, R. M., Hellyer, P. W., Oxley, J. A., Rishniw, M. (2019). Small Animal Veterinarians' Perceptions, Experiences, and Views of Common Dog Breeds, Dog Aggression, and Breed-Specific Laws in the United States. *International Journal of Environmental Research and Public Health, 16*(21), 4081.

Kuhne, F. (2012). Castration of dogs from the standpoint of behaviour therapy. *Tierarztl Prax Ausg K Kleintiere Heimtiere, 40*(2), 140-145.

Kwan, J. Y. and Bain, M. J. (2013). Owner attachment and problem behaviors related to relinquishment and training techniques of dogs. *Journal of applied animal welfare science, 16*(2), 168-183.

Landsberg, G. M. (1990). Diagnosing dominance agresyon. *Canadian Veterinary Journal, 31*, 45-46.

Landsberg, G. M., Hunthausen, W. L., Ackerman, L. J. (1997). *Handbook of behavior problems of the dog and cat*. Saunders.

Lee, G. and Gammie, S. C. (2009). GABAA receptor signaling in the lateral septum regulates maternal aggression in mice. *Behavioral Neuroscience, 123*(6), 1169-1177.

Lee, R., Ferris, C., Van de Kar, L. D., Coccaro, E. F. (2009). Cerebrospinal fluid oxytocin, life history of aggression, and personality disorder. *Psychoneuroendocrinology, 34*, 1567-1573.

Lesch, K. L. and Merschdorf, U. (2000). Impulsivity, aggression, and serotonin: a molecular psychobiological perspective. *Behavioral Science and Law*, *18*, 581-604.

Liinamo, A., van den Berg, L., Leegwater, P. A. J., Schilder, M.B.H., van Arendonk, J.A.M., van Oost, B.A., (2007). Genetic variation in aggression-related traits in golden retriever dogs. *Applied Animal Behaviour Science. 104*, 95 –106.

MacNeil-Allcock, A., Clarke, N. M., Ledger, R. A., Fraser, D. (2011). Aggression, behaviour, and animal care among pit bulls and other dogs adopted from an animal shelter. *Animal Welfare, 20*, 463-468.

Mano, T., Sakamoto, H., Fujita, K., Makino, M., Kakizawa, H., Nagata, M., Kotake, M., Hamada, M., Uchimura, K., Hayakawa, N., Hayashi, R., Nakai, A., Itoh, M., Kuzuya, H., Nagasaka, A. (1998). Effects of thyroid hormone on catecholamine and its metabolite concentrations in rat cardiac muscle and cerebral cortex. *Thyroid, 8*, 353–358.

Masi, G. (2004). Pharmacotherapy of pervasive developmental disorders in children and adolescents. CNS *Drugs, 18*, 1031–1052.

Mertens, P. and Dodman, N. (2003). Animal behavior case of the month. *Journal of the American Veterinary Medical Association, 223*, 1436-1442.

Miczek, K. A., de Almeida, R. M. M., Kravitz, E. A., Rissman, E. F., de Boer, S. F., Raine, A. (2007). Neurobiology of escalated aggression and violence. *Journal of Neuroscience, 27*, 11803–11806.

Miczek, K. A., Faccidomo, S., de Almeida, R. M., Bannai, M., Fish, E. W., de Bold, J. F. (2004). Escalated aggressive behavior: new pharmacotherapeutic approaches and opportunities. *Annals of the New York Academy of Sciences journal, 1036*, 336–355.

Miczek, K. A., Fish, E. W., de Bold, J. F. (2003). Neurosteroids, GABAA receptors, and escalated aggressive behavior. *Hormones and Behavior, 44*, 242-257.

Miczek, K. A., Fish, E. W., de Bold, J. F., de Almeida, R. M. M. (2002). Social and neural determinants of aggressive behavior: pharmacotherapeutic targets at serotonin, dopamine and c-aminobutyric acid systems. *Psychopharmacology, 163*, 434–458.

Miller, K. A., Touroo, R., Spain, C. V., Jones, K., Reid, P., Lockwood, R. (2016). Relationship Between Scarring and Dog Aggression in Pit Bull-Type Dogs Involved in Organized Dogfighting. *Animals, 6*, 72.

Mokler, D. J., Dugal, J. R., Hoffman, J. M., Morgane, P. J. (2009). Functional interrelations between nucleus raphé dorsalis and nucleus raphé medianus: a dual probe microdialysis study of glutamate-stimulated serotonin release. *Brain Research Bulletin, 78*, 132-138.

Motomura, K. and Brent, G. A. (1998). Mechanisms of thyroid hormone action. Implications for the clinical manifestation of thyrotoxicosis. *Endocrinology & Metabolism Clinics of North America, 27*, 1–23.

Neumann, I. D. (2007). Stimuli and consequences of dendritic release of oxytocin within the brain. *Biochemical Society Transactions, 35*, 1252-1257.

Niyyat, M. R., Azizzadeh, M., Khoshnegah, J. (2018). Effect of Supplementation with Omega-3 Fatty Acids, Magnesium, and Zinc on Canine Behavioral Disorders: Results of a Pilot Study. *Topics in Companion Animal Medicine, 33*(4), 150-155.

Notari, L., Burman, O., Mills, D. (2015). Behavioural changes in dogs treated with corticosteroids. *Physiology & behavior, 151*, 609-616.

Noyan, A. (2007). *Yaşamda ve Hekimlikte Fizyoloji*, Palme Yayınları.

O'Farrell, V. (1992). *Manual of canine behviour 2th edition,* Cheltenhan Glos British Small Animal Veterinary Assoiation, XII+132.

Odendaal, J. S. J. (1997). A Diagnostic Classification of Problem Behavior in Dogs and Cats. Veterinary Clinics of *North America: Small Animal Practice, 27*, 427-442.

Odore, R., Rendini, D., Badino, P., Gardini, G., Cagnotti, G., Meucci, V., Intorre, L., Bellino, C., D'Angelo, A. (2020). Behavioral Therapy and Fluoxetine Treatment in Aggressive Dogs: A Case Study. *Animals (Basel), 10*(5), 832.

O-Klein, M., Battagello, D. S., Cardoso, A. R., Hauser, D. N., Bittencourt, J. C., Correa, R. G. (2019). Dopamine: Functions, Signaling, and Association with Neurological Diseases. *Cellular and Molecular Neurobiology, 39*(1), 31-59.

Overall, K. L. (1997). Pharmacologic treatments for behavior problems. *Veterinary Clinics of North America: Small Animal Practice, 27*, 637-665.

Ozsoy, S., Esel, E., Kula, M. (2009). Serum oxytocin levels in patients with depression and the effects of gender and antidepressant treatment. *Psychiatry Research, 169*, 249-252.

Pineda, S., Anzola, B., Olivares, A., Ibáñez, M. (2014). Fluoxetine combined with clorazepate dipotassium and behaviour modification for treatment of anxiety-related disorders in dogs. *The Veterinary Journal, 199*(3), 387-391.

Quadros, I. M., Miguel, T. T., de Bold, J. F., Miczek, K. A. (2009a). *Opposing action of CRF1 vs. CRF2 receptors in the dorsal raphé: modulation of alcohol-heightened aggression*. Neuroscience meeting planner. Society for neuroscience, Chicago, Program No. 445.5/T8.

Quadros, I. M., Takahashi, A., Miczek, K. A. (2009b). *Serotonin and aggression.* In: Müller CP, Jacobs BL (eds) Handbook of the behavioral neurobiology of serotonin. Academic Press, 687–714.

Rapport, M. M., Green, A. A., Page, I. H. (1948). Serum vasoconstrictor, serotonin; isolation and characterization. *Journal of Biological Chemistry, 176*, 243–251. Reisner, I. R., Erb, H. N., Houpt, K. A. (1994). Risk factors for behavior -related euthanasia among dominant-aggressive dogs: 110 cases (1989-1992). *Journal of America Veterinary Medicine Association, 205*, 855-863.

Reisner, I. R., Mann, J. J., Stanley, M., Huang, Y., Houpta, K. A. (1996). Comparison of cerebrospinal fluid monoamine metabolite levels in dominant-aggressive and non-aggressive dogs. *Brain Research, 714* (1-2), 57-64.

Resmi Gazete, 01.07.2004; https://www.resmigazete.gov.tr/eskiler/2004/07/20040701.htm

Resmi Gazete, 14.07.2021; https://www.resmigazete.gov.tr/eskiler/2021/07/20210714-9.htm

Rosell, D. R. and Siever, L. J. (2015). The neurobiology of aggression and violence. *CNS Spectrums, 20,* 254–279.

Roth, B. L., (2007). editor. The Serotonin Receptors: From Molecular Pharmacology to Human Therapeutics. Totowa, NJ: Humana.

Sanchez, C., Arnt, J., Hyttel, J., Moltzen, E. K. (1993). The role of serotonergic mechanisms in inhibition of isolation-induced aggression in male mice. *Psychopharmacology, 110*, 53–59.

Serpell, J. A. and Hsu, Y. A. (2005). Effects of breed, sex, and neuter status on trainability in dogs. *Anthrozoös, 18*(3), 196-207.

Siddiq, A. B. (2019). Tarih öncesi toplumlarda insan-hayvan ilişkisi ve Orta Anadolu çanak çömleksiz neolitik dönem faunası. 1. Baskı. Konya: Çizgi Kitabevi.

Stepherd, R. C. (2018). *Pit Bull:* Journal. Independently published, ISBN-10: 1720108668.

Strawn, J. R., Ekhator, N. N., D'Souza, B. B., Geracioti Jr, T. D. (2004). Pituitary-thyroid state correlates with central dopaminergic and serotonergic activity in healthy humans. *Neuropsychobiology, 49*, 84–87.

Sweidan, S., Edinger, H., Siegel, A. (1991). D2 dopamine receptor-mediated mechanisms in the medial preoptic-anterior hypothalamus regulate effective defense behavior in the cat. *Brain Research, 549*, 127–137.

Takahashi, A., Kwa, C., de Bold, J. F., Miczek, K. A. (2010a). GABAA receptors in the dorsal raphé nucleus of mice: escalation of aggression after alcohol consumption. *Psychopharmacology, 211*, 467-477.

Takahashi, A., Quadros, I. M., de Almeida, R. M., Miczek, K. A. (2011). Brain serotonin receptors and transporters: initiation vs. termination of escalated aggression. *Psychopharmacology, 213*, 183–212.

Takahashi, A., Quadros, I. M., de Almeida, R. M., Miczek, K. A. (2012). Behavioral and pharmacogenetics of aggressive behavior. *Current Topics in Behavioral Neurosciences, 12*, 73-138.

Takahashi, A., Shimamoto, A., Boyson, C. O., De Bold, J. F., Miczek, K. A. (2010b). GABAB receptor modulation of serotonin neurons in the dorsal raphé nucleus and escalation of aggression in mice. *Journal of Neuroscience, 30*(35), 11771-11780.

Takayanagi, Y., Yoshida, M., Bielsky, I. F., Ross, H. E., Kawamata, M., Onaka, T. (2005). Pervasive social deficits, but normal parturition, in oxytocin receptor-deficient mice. *Proceedings of the National Academy of Sciences of the United States of America* , *102*, 16096-16101.

Tidey, J. W. and Miczek, K. (1992a). Effects of SKF38393 and quinpirole on patterns of aggressive, motor and schedule-controlled behaviors in mice. *Behavioural Pharmacology,*

Tidey, J. W. and Miczek, K. A. (1992b). Morphine withdrawal aggression: modification with D1 and D2 receptor agonists. *Psychopharmacology, 108*, 177–184.

Turan, T., Uysal, C., Asdemir, A., Kılıç, E. (2013). May oxytocin be a trait marker for bipolar disorder? *Psychoneuroendocrinology, 38*, 2890-2896.

Uchida, Y., Dodman, N., Denapoli, J., Aronson, L. (1997). Characterization and treatmant of 20 canine dominance aggression cases. *Journal of Veterinary Medical Science, 59*(5), 397-399.

van der Spek, A. H., Fliers, E., Boelen, A. (2017). The classic pathways of thyroid hormone metabolism. *Molecular and Cellular Endocrinology, 458*, 29-38.

Vara, H., Martinez, B., Santos, A., Colino, A. (2002). Thyroid hormone regulates neurotransmitter release in neonatal rat hippocampus. *Neuroscience, 110*(1), 19–28.

Wassef, A, Baker, H., Kochan, L. D. (2003). GABA and schizophrenia: a review of basic science and clinical studies. *Journal of Clinical Psychopharmacology, 23*(6), 601-640.

Weisman, O., Zagoory-Sharon, O., Schneiderman, I., Gordon, I., Feldman, R. (2013). Plasma oxytocin distributions in a large cohort of women and men and their gender-specific associations with anxiety, *Psychoneuroendocrinology, 38*, (5), 694-701.

Whybrow, P. C. and Prange, A. J. (1981). A hypothesis of thyroid-catecholaminereceptor interaction: its relevance to affective illness. *Archives of General Psychiatry, 38*(1), 106–113.

Wright, H. F., Mills, D. S., Pollux, P. M. J. (2012). Behavioural and physiological correlates of impulsivity in the domestic dog (Canis familiaris). *Physiology and Behaviour, 105*(3), 676-682.

Yeon, S. C., Erb, H. N., Houpt, K. A. (1999). A retrospective study of canine house soiling: *Diagnosis and treatment, 35*, 101-106.

Yoshida, M., Takayanagi, Y., Inoue, K., Kimura, T., Young, L. J., Onaka, T. (2009). Evidence that oxytocin exerts anxiolytic effects via oxytocin receptor expressed in serotonergic neurons in mice. *Journal of Neuroscience, 29*, 2259-2271.

Yu, C. J., Zhang, S. W., Tai, F. D. (2016). Effects of nucleus accumbens oxytocin and its antagonist on social approach behavior. *Behavioral Pharmacology, 27*, 672–680.

Yu, Q., Teixeira, C. M., Mahadevia, D., Huang, Y., Balsam, D., Mann, J. J., Gingrich, J. A., Ansorge, M. S. (2014). Dopamine and serotonin signaling during two sensitive developmental periods differentially impact adult aggressive and affective behaviors in mice. *Molecular Psychiatry, 19*(6), 688-698.

Zapata, I., Serpell, J. A., Alvarez, C. E. (2016). Genetic mapping of canine fear and aggression. *BMC Genomics*, 17, 572.

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Colostrum induced passive immune transfer in lambs

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ABSTRACT

During last decades, the production and consumption of small ruminant milk have been increased. As a result of this, sheep and goat farming have been developing and scientists are focused on clinical and feeding strategy researches on these animals. By the evolutionary challenges and adaptations, colostrum has a crucial role in immune complementation for litter. As a result of these challenges and adaptations neonatal life is more important especially in ruminants due to its lifetime effect and the future of livestock. The passive immune transfer is the main mechanism explained by biological evolution between the dam and lamb which is influenced by certain factors related to both dam and litter. Today the importance of passive immune transfer is well known for the future of livestock economy and animal welfare. In the literature, researchers are focused on correlation between colostrum quality (especially immunoglobulin concentration) and blood serum levels of immunoglobulin levels in newborns. The aims of the present review are to discuss data of recent studies and how passive immune transfer occurred in lambs as well as effecting factors and to supply new ideas to researchers.

Keywords: colostrum, passive immunity, sheep, lamb mortality.

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Introduction

The domestication of the sheep is considered approximately ten thousand years ago during the Neolithic age in Central Asia (Zohary et al., 1998). Since that age sheep farming has become an important food and animal-by product resource for human beings. Milk and dairy products of small ruminants are quite important for proper human nutrition where cow milk is not readily available or affordable (Haenlein, 2001).

The placental structure of the sheep is epitheliochorial and due to that structure, maternal antibodies are considered not transferred in utero to the offspring (Agenbag et al., 2021). Thus, colostrum induced passive immune transfer (PIT) is crucial for the whole life of the lamb. Alongside a strong immunostimulant activity of the colostrum, it is a nutrient rich source for the litter. Newborn lambs are born quite limited energy reserves thus they need immediate access to intake colostrum which has enough amount and quality (Nowak and Poindron, 2006). Colostrum has immunological and nutritional composition (Moreno-Indias et al., 2012; Övet, 2023). It also has high magnesium concentration that plays an essential role in peristaltic activation of newborns. Alongside that peristaltic activity, colostrum promotes the removal of meconium and helps avoiding the bacterial colonization in the gastrointestinal tract (Barza et al., 1993). All these properties of colostrum make it a unique life source for the newborn.

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The fetus is well-adapted to the relatively hypoxemic intrauterine environment. The transition from intra- to extrauterine life requires rapid, complex and well-orchestrated steps to ensure neonatal survival (Morton and Brodsky, 2016) and that challenge has combined some influences such as the behavior of the litter and mother after birth (Nowak et al., 2000). According to literature data, passive immunity in ruminant newborns not only ensures prevention against diseases but also accelerates growth performance (Dewell et al., 2006; Yalçın and Temizel, 2010; Gokce et al., 2013a). Neonatal lamb mortality has no one specifical cause (dos Santos et al., 2023); it has a multifactorial issue. Besides its major function of digestion and absorption of nutrients, gastrointestinal tract provides immunological defense against pathogens, endotoxins and antigenic substances (Turner, 2009). In newborn ruminants jejunum is a major intestinal region for IgG (immunoglobulin G) absorption (Nordi et al., 2012; Yang et al., 2019).

Why PIT is Crucial?

Although in some mammals (i.e., human, rabbit, mouse), PIT is completely occurred via placenta during intrauterine life (DuBourdieu, 2019) in ungulates, it is occurred limited or considered not to be occurred (Silva et al., 2022). Due to placental structure, transfer of maternal antibodies occurs via colostrum not only in lambs but also in other neonatal ruminants in 24 hours post partum; thus PIT is related to colostrum quality and its amount intake by the litter. Mechanism of PIT is based on absorption of maternal immunoglobulins via consumed colostrum. That absorption decreases rapidly in 24h postpartum (Loste et al., 2008). Meanwhile digestion of proteins and amino acid catabolism are slightly (Constable et al., 2016).

Failure of PIT causes major economical losses in livestock; it is an important economical concern for producers. Thus PIT is crucial for producers to prevent neonatal mortality and morbidity by monitoring the immune status of lambs (Pekcan et al. 2013; Elitok, 2018). The nature of PIT is an adaptive natural immunity (Figure 1).

Lamb mortality is a key factor influencing the productivity of ewes and the profitability of livestock (Shiels et al., 2021). Mortality rates are variable by different circumstances (such as management, gestational diseases, common infections, failure of PIT and others) and during last decades the average mortality rate of newborn lambs remained relatively constant by 15% around the world. This rate could be to be higher (up to 30%) in small-scale sheep farming systems in developing countries (dos Santos et al., 2023). According to studies, failure of PIT's incidence ranges between 3.4% and 20% and; in Kars province in

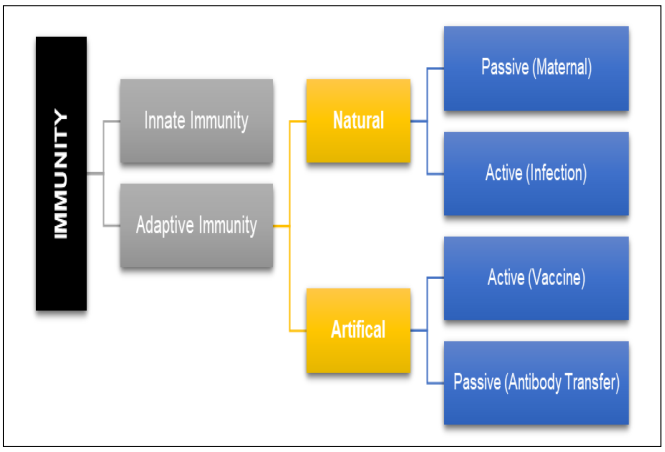

Figure 1. Summarized diagram of immunity.

Turkey mortality rates vary between 45% and 50% and during the first 2 weeks of neonatal life (Erdogan, 2009). Failure of PIT in neonatal lambs has a significant consequence on neonatal mortality and newborn losses of infectious causes are positively correlated with low concentrations of serum Ig (Sallam, 2019; Ibrahim et al., 2020) Several factors cause failure of PIT as follows: **1.** Insufficient concentration of Ig in the colostrum. **2.** Lack of specific pathogen exposure or an inability to respond. **3.** Insufficient intake of colostrum by the litter. **4.** Insufficient production of colostrum by the dam. **5.** Lack of transmural Ig transfer from the neonatal intestine to blood.

Hence, failure of PIT has been related to multiple conditions of lambs, including respiratory disease, diarrhea, septicemia, and commonly omphalophlebitis (Herndorn et al., 2011; Demis et al., 2020). All these conditions and lack of colostrum intake during the first weeks of neonatal life, would affect the litter's whole life (Agenbag et al., 2021). The amount of colostrum is important but also management during the suckling and weaning period; such as stress produced by dam separation, milk quality and suckling frequency, can affect the final immune status of the lambs (Hernández-Castellano et al., 2015).

The gastrointestinal tract of newborn lamb is considered sterile and once it's exposed to microorganisms after birth, development and maturation of the intestinal mucosal immune system start (Wesemann et al., 2013). Although it's not well-explained yet, it is known that the mechanism of passive immune transfer from dam to litter occurs by the high permeability of the intestinal tract of litter to macromolecules to pass through especially immunoglobulins in ruminants. This permeability is highest at the first 6 hours of birth and it decreases in 24 hours (Castro-Alonso et al., 2008; Hernández-Castellano et al., 2014a,b). Pinocytosis of enterocytes also has a role in that maternal antibody absorption by the newborn (Brujeni et al., 2010). PIT is a complex of

reactions by acting together with Toll-Like Receptors, mucins, antimicrobial peptides, and claudins in intestinal defense during the PIT in newborn lambs (Zhu et al., 2020). On the other hand, Fc receptor mediated pathways are key mechanisms in IgG metabolism (Tizard, 2017). However, that high permeability increases also the risk for pathogens enter to the circulation (Fischer et al., 2019).

Effecting factors of the PIT

Factors up to dam: Dam's health is one of the most important factors to produce high-quality colostrum. The healthy udder gland is key to producing high-quality milk in dairy ruminants (Castro et al., 2019). Nutrition is a major contributing factor to the quantity and quality of colostrum (Banchero et al., 2015; McGovern et al., 2015). Sufficient energy to the dam is ingesting and whether that meets its gestational requirements (Banchero et al., 2006; Muñoz et al., 2008). Viola et al. (2022) have indicated that an ewe's diet in the last period of gestation can effect colostral IgG concentration; for instance hazelnut skin in ewe's diet effects positively colostral IgG concentration. Under that condition, colostrum quality is associated directly to the dam's nutrition. In the late gestation, sheep supplemented with oat grain had higher colostral protein and IgG and high IgG concentrations in the blood serum of their lambs (Castellaro et al., 2022).

According to some recent studies, age of dam has no significant effect on growth performance in neonatal lambs (Talore, 2009; Taye et al., 2010; Abegaz et al., 2011; Gokce et al., 2013a). Although data on the effect of parity on colostrum quality in sheep and goats is not numerous, some studies reported that primiparous ewes have higher colostral protein and IgG concentrations (Higaki et al., 2013; Tabatabaei et al., 2013). But in contrast, Sjoberg and Van Saun (2021) reported that parity has no effect on colostral IgG levels. In a previous study, parity influenced characteristics of colostrum in multiparous dams; lambs born from primiparous dams have lower protein, glucose and plasma IgG concentrations than lambs born from multiparous dams (Chniter et al., 2013). Gokce et al. (2012) reported that risk of neonatal mortality and morbidity are higher in dams at first parity than the dams that have higher parities because ewes show mismothering at first lambing. Physiological mechanisms in first pregnancy might play a role in increasing stress in primiparous ewes since; they are still growing and need to partition nutrients to sustain their growth physiology and their fetuses (Chniter et al., 2015). Eventually, parity is one of the important effecting factor on colostrum quality and lamb morbidity-mortality.

In cows, the use of probiotics and prebiotics leads to higher levels of colostral immunoglobulin (Sol Morales et al., 2000; Strusinska et al., 2004). In the sow, dietary probiotics improve colostrum quality and growth performance in piglets (Wu et al., 2023). There is insufficient data on the effects of using probiotics on colostrum quality in sheep nutrition. Nouri et al. (2023) have demonstrated that prepartum and postpartum feed restriction in fat-tailed dairy sheep does not affect colostral IgG or lamb serum IgG concentration. Vaccination has important effects on colostrum quality and PIT in newborns; higher serum antibodies in ewes would effect antibody concentrations in colostrum (Burezq and Khalil, 2022).

Factors up to litter: Not only lambs but also kids and calves need to access colostrum in sufficient amount and quality. In the literature it is controversial the relationship between litter size and colostrum quality. While some studies (Mandal et al., 2007; Turkson and Sualisu, 2005; Yapi et al., 1990) have shown that there is a significant relationship between single and multiple-born lambs, other studies discussed multiple births may increase the risk of neonatal mortality (Holmoy et al., 2012). Alves et al. (2015) have shown that lambs demonstrated the failure of PIT once their a serum IgG concentration lower than 15 mg/mL at the 36th hour postpartum.. Similarly lambs have lower serum total protein (TP) concentration at 24th-hour postpartum show higher morbidity-mortality rates (Gokce and Atakisi, 2019). Management applications and animal characteristics (e.g. singleton or twin, birth weight, gestational diseases in ewes) are also associated with PIT (Gokce et al., 2013b).

Evaluation methods

Nowadays, various methods have been developed and still used to evaluate PIT in newborn ruminants. These methods are mainly divided into direct and indirect methods (Table 1). According to literature data, the most accurate method for evaluating colostrum quality is radial immunodiffusion (RID); Enzyme-Linked ImmunoSorbent Assay (ELISA) is also a reliable method (Lee et al., 2008; Cuttance et al., 2019). However, RID is an expensive laboratory method and requires time for results. Although it is not well-accurate, in farm-practice the best method is brix refractometry to evaluate colostrum quality because it is fastest and easiest method (Agenbag et al., 2023). Another method is Split trehalase immunoglobulin G assay (STIGA) that used in bovine colostrum (Drikic et al., 2018). Besides, the radial gel immunodiffusion

immunodiffusion technique can be used to determine serum and colostral IgG concentration (Castellaro et al., 2022). Total immunoglobulin levels of blood serum and colostrum can be evaluated by Zinc Sulfate Turbidity Test (Vatankhah, 2013). Transmission Infrared Spectroscopy (TIR) is also another direct method to measure colostral IgG concentration (Elsohaby et al., 2016).

Table 1. Common direct and indirect methods to evaluate colostrum quality.

Type	Method
Direct	RID
	ELISA
	STIGA
	TIR
Indirect	Refractometer
	Colostrometer
	Zinc Sulfate Turbidity Test
	Sodium Sulfite Turbidity Test

Capillary electrophoresis (CE) is also utilizable to evaluate colostral IgG concentration and it might be reliable method to evaluate total Ig concentration in sheep colostrum (Lopreiato et al., 2017). CE is suitable for a reliable estimate of IgG in lamb serum (Morittu et al., 2020).

Colostral TP and Ig concentration

The majority of total colostral protein is originated by immunoglobulins, especially IgG in ruminant colostrum. Management, gestational diseases, mastitis, age and parity are factors that affect colostrum quality (Swarnkar et al., 2019). Sufficient amount and quality of colostrum are important factors for the PIT. According to the ELISA method, values between 29.55 and 53.41 are considered high-quality (Alves et al., 2015; Constantin and Sipos, 2021). Brix refractometry can be used in farm practice to evaluate colostral protein and values are changeable (Table 2).

Table 2. Brix values of sheep colostrum in different studies.

Brix Values Range (%)	Breed	Reference
14.4 – 17.1	Awassi	Berge et al. (2018)
13.0 – 23.5	Crossbreed	Constantin and Sipos (2021)
8.6 – 40.0	Santa Inês	de Sousa et al. (2018)
16.8 – 22.6	Lacaune	Torres-Rovira et al. (2017)
15.4 – 40.0	Unknown	Kessler et al. (2021)
21.6 – 44.7	Merino	Agenbag et al. (2023)
16.8 – 27.0	Unknown	Todaro et al. (2023)

Immunoglobulin levels in lamb blood serum

There are different methods to estimate serum Ig levels. Detection of IgG levels by ELISA (Yenilmez et al., 2021) is one of the common methods. Healthy newborn lambs (in 21 days after birth) have significantly higher serum IgG levels than before they consume colostrum; and also their dams have higher colostral TP levels (Gokce and Atakisi, 2019). Laser-induced breakdown spectroscopy method also can be used evaluation of proteins in sheep colostrum (Abdel-Salam et al., 2019). That method is based on spectroscopic detection and analysis of atomic, ionic and molecular emission of a laser produced plasma; it can be used for in-situ and real time measurements (Harmon and Senesi, 2021). Another evaluation method is the Zinc Sulphate Turbidity Test (ZST) which creates turbidity which is proportional to the quantity of gamma globulin in the sample and can be quantified in a calorimeter at 525 nm/Spectrophotometer 460 nm. This method was used for the first time in the 70s to determine gamma globulin levels in calves. According to ZST, neonatal lambs with have total serum level below 12 are considered to indicate failure of PIT (Demis et al., 2020). Enzymatic colorimetric kits can be used to estimate serum TP and albumin concentrations (Alves et al., 2015).

Table 3. IgG, fat and protein concentrations in different breeds of sheep colostrum (Alves et al., 2015; Kessler et al., 2019).

Breed	*Component*		
	Fat (%)	*Protein (%)*	*IgG (mg/ml)*
Merino Land	7.44	22.49	44.2
Brown-Headed Meat	13.64	20.30	35.0
Swiss Charollais	8.05	17.55	28.9
Lacaune Dairy	4.04	14.07	20.2
Santa Inês	7.43	8.24	15.7

Conclusion

According to FAO 2022 report consumers especially in high-income countries, are more interested about what they eat and how their food is produced, processed and transported than undeveloped countries (FAO, 2022). Sheep farmers produce consumable products (meat and milk) and animal-by products (wool and skins) for national and/or international markets (Morris, 2017). These economical changes and feeding preferences lead farmers, governments and researchers to focus on small ruminant practices. Suckling lambs intake non-immunological factors such as nutrients, vitamins,

minerals, hormones, and growth factors alongside colostral IgG (Massimini et al., 2006). Because newborn lambs bore quite limited energy reserves, they need immediate access to intake colostrum that has enough amount and quality (Nowak and Poindron, 2006). Today, most veterinarians use field-based methods in livestock routinely which leads them to make medical decisions on newborns. There are two main reasons to detect PIT in practice: accurate diagnosis and treatment of newborns and ensure better management (Massimini et al., 2006; Pekcan et al., 2013; Elitok, 2018). Immunological differences between species or breeds lead to different strategies on farm-wide or country-wide. There are immunological differences between sheep species; for instance Bighorn Sheep (Ovis canadensis) lambs are more susceptible to Mannheimia haemolytica infections than the other breeds (Herndorn et al., 2011). Although there have been attempts to reduce lamb mortality in recent years (from 1970 to 2014), it hasn't changed significantly and has remained at an average of 15% in many countries (dos Santos et al., 2023). Gokce and Atakisi (2018) have shown that neonatal losses occurred mainly first week of life (84.6% rate). Eventually in nature, newborn mortality is an inevitable case

The major keys of PIT are colostral Ig concentration and absorption by the litter. In the literature, the role of colostral immunoglobulin concentration in passive immune transfer to newborn kids has demonstrated (Castro et al., 2005; Rodríguez et al. 2009). Gokce et al. (2013a) have shown that neonatal morbidity and mortality risks are higher in lambs who have low birth weight than in medium or high birth weight lambs in Kars province in Turkey. The lambing season may affect mortality rates, but some studies in the literature (Mukasa-Mugerwa et al., 2000; Tibbo et al., 2003; Berhan and Van Arendonk, 2006; Swarnkar et al., 2019) claim that season has a significant effect on mortality and some studies (Turkson and Sualisu, 2005; Mandal et al., 2007; Piwczynski et al., 2012) have indicated that birth season has insignificant effect. The influence of gender on neonatal mortality is controversial. While some studies (Vatankhah and Talebi, 2009; Ahmed et al., 2010; Abdelqader et al., 2017) have indicated higher mortality in male lambs compared to female lambs, Turkson and Sualisu (2005) reported higher mortality in female lambs. In a study on the Shaul breed (Brujeni et al., 2010), PIT wasn't affected by sex, litter size, parity and birth weight. Yenilmez et al. (2021) have showed that twin born affects TP and globuline levels in blood serum, but it does not affect IgG levels. Failure of PIT in lambs has a significant effect on neonatal mortality and losses due to infectious causes are positively correlated with low concentrations of serum immunoglobulins (Sallam, 2019; Ibrahim et al., 2020). Lamb's serum IgG levels at post partum 24th hour are between 21.51 and 81.25 mg/mL (Castellaro et al., 2022). Hunter et al. (1977) reported that these concentrations could be in a range of 0 to 102 mg/mL in post partum 24th hour. Increased 24th hour serum immunoglobulin levels have a significant relationship with growth performance in lambs (Gokce et al.,2013a). Eventually, newborn lambs should consume at least 30 g of IgG in the first 24 h postpartum to ensure adequate PIT (Alves et al., 2015).

In conclusion, the importance of small ruminant farming has been increasing especially in developed, high-income countries. Thus in consideration of economical losses, management and animal welfare have importance and lead us to evaluate PIT and new strategies on that aspect. On the other hand, lambs need to utilize enough maternal IgG via colostrum as well as consume high-quality colostrum.

References

Abdelqader, A., Irshaid, R., Tabbaa, M. J., Abuajamieh, M., Titi, H., & Al-Fataftah, A. R. (2017). Factors influencing Awassi lambs survivorship under fields conditions. *Livestock Science, 199*, 1-6.

Abdel-Salam, Z. A., Abdel-Salam, S. A. M., Abdel-Mageed, I. I., & Harith, M. A. (2019). Evaluation of proteins in sheep colostrum via laser-induced breakdown spectroscopy and multivariate analysis. *Journal of Advanced Research, 15*, 19-25.

Abegaz, S., Hegde, B. P., & Taye, M. (2011). Growth and physical body characteristics of Gumuz sheep under traditional management systems in Amhara Regional State, Ethiopia. Livestock *Research for Rural Development, 23*(5), 10.

Agenbag, B., Swinbourne, A. M., Petrovski, K., & van Wettere, W. H. (2021). Lambs need colostrum: A review. Livestock *Science, 25*1, 104624.

Agenbag, B., Swinbourne, A. M., Petrovski, K., & van Wettere, W. H. (2023). Validation of a handheld refractometer to assess Merino ewe colostrum and transition milk quality. *Journal of Dairy Science, 106*(2), 1394-1402.

Ahmed, A., Egwu, G. O., Garba, H. S., & Magaji, A. A. (2010). Studies on risk factors of mortality in lambs in Sokoto, Nigeria. *Nigerian Veterinary Journal, 31*(1), 56-65.

Alves, A. C., Alves, N. G., Ascari, I. J., Junqueira, F. B., Coutinho, A. S., Lima, R. R., ... & Abreu, L. R. (2015). Colostrum composition of Santa Inês sheep and passive transfer of immunity to lambs. *Journal of Dairy science, 98* (6), 3706-3716.

Banchero, G. E., Clariget, R. P., Bencini, R., Lindsay, D. R., Milton, J. T., & Martin, G. B. (2006). Endocrine and metabolic factors involved in the effect of nutrition on the production of colostrum in female sheep. Reproduction nutrition development, 46(4), 447-460.

Banchero, G. E., Milton, J. T. B., Lindsay, D. R., Martin, G. B., & Quintans, G. (2015). Colostrum production in ewes: a review of regulation mechanisms and of energy supply. *Animal, 9*(5), 831-837.

Barza, H.; Marinescu, M.; Blaga, L. (1993) Disorders of foals 0 –10 days of age. Part I. *Revista Romana de Medicina Veterinara, 3*, 9-20.

Berge, A. C., Hassid, G., Leibovich, H., Solomon, D., & Haines, D. M. (2018). A field trial evaluating the health and performance of lambs fed a bovine colostrum replacement. *Journal of animal research and nutrition, 3*, 1-6

Berhan, A., & Van Arendonk, J. (2006). Reproductive performance and mortality rate in Menz and Horro sheep following controlled breeding in Ethiopia. *Small Ruminant Research, 63*(3), 297-303.

Brujeni, G. N., Jani, S. S., Alidadi, N., Tabatabaei, S., Sharifi, H., & Mohri, M. (2010). Passive immune transfer in fat-tailed sheep: Evaluation with different methods. *Small Ruminant Research, 90*(1-3), 146-149.

Burezq, H. A., & Khalil, F. (2022). Improved vaccination protocol to enhance immunity in lambs of Kuwait farms. *Iraqi Journal of Veterinary Sciences, 36*(2), 539-548.

Castellaro, G., Ochoa, I., Borie, C., & Parraguez, V. H. (2022). Effects of Strategic Supplementation with Lupinus angustifolius and Avena sativa Grains on Colostrum Quality and Passive Immunological Transfer to Newborn Lambs. *Animals, 12*(22), 3159.

Castro, I., Alba, C., Aparicio, M., Arroyo, R., Jiménez, L., Fernández, L., ... & Rodríguez, J. M. (2019). Metataxonomic and immunological analysis of milk from ewes with or without a history of mastitis. Journal *of dairy science, 102*(10), 9298-9311.

Castro, N., Capote, J., Alvarez, S., & Argüello, A. (2005). Effects of lyophilized colostrum and different colostrum feeding regimens on passive transfer of immunoglobulin G in Majorera goat kids. *Journal of Dairy Science, 88*(10), 3650-3654.

Castro-Alonso, A., Castro, N., Capote, J., Morales-delaNuez, A., Moreno-Indias, I., Sánchez-Macias, D., ... & Argüello, A. (2008). Apoptosis regulates passive immune transfer in newborn kids. *Journal of Dairy Science, 91*(5), 2086-2088.

Chniter, M., Salhi, I., Harrabi, H., Khorchani, T., Lainé, A. L., Nowak, R., & Hammadi, M. (2016). Physiological changes in the peri-partum period and colostral IgG transfer in prolific D'man sheep: effects of parity and litter size. *Tropical Animal Health and Production, 48*, 387-394.

Constable, P. D., Hinchcliff, K. W., Done, S. H., & Grünberg, W. (2016). *Veterinary medicine: a textbook of the diseases of cattle, horses, sheep, pigs and goats*. Elsevier Health Sciences.

Constantin, N. T., & Sipos, A. (2021). Passive transfer of immunoglobulıns from ewe to lamb. *Scientific Works. Series C, Veterinary Medicine, 67*(1).

Cuttance, E. L., Regnerus, C., & Laven, R. A. (2019). A review of diagnostic tests for diagnosing failure of transfer of passive immunity in dairy calves in New Zealand. *New Zealand Veterinary Journal, 67*(6), 277-286.

de Sousa, I. V. P., Silva, C. B., & Ribeiro, C. V. (2018). Influence of the birth order on the total solids concentration of frozen colostrum from Santa Inês ewes. In 55a Reunião Anual da Sociedade Brasileira de Zootecnia, 28° Congresso Brasileiro de Zootecnia, Goiânia, Brasil, 27 a 30 de agosto de 2018. Sociedade Brasileira de Zootecnia-SBZ, Associação Brasileira dos Zootecnistas.

Demis, C., Aydefruhim, D., Wondifra, Y., Ayele, F., Alemnew, E., & Asfaw, T. (2020). Maternal Immunoglobulin in the Serum of Newborn Lambs and Its Relation With Neonatal Mortality. ,*Journal of Animal and Feed Research, 10*(3), 119-124.

Dewell, R. D., Hungerford, L. L., Keen, J. E., Laegreid, W. W., Griffin, D. D., Rupp, G. P., & Grotelueschen, D. M. (2006). Association of neonatal serum immunoglobulin G1 concentration with health and performance in beef calves. *Journal of American Veterinary Medical Association, 228*(6), 914-921.

dos Santos, JDC., Saraiva, EP., Pimenta Filho, EC., Neta, GCX., Morais, LKC., Teti, HS., & Fidelis, SS. (2023). Risk factors for neonatal mortality in sheep farming systems in tropical semi-arid regions. *Journal of Agricultural Science*, 1-12.

Drikic, M., Windeyer, C., Olsen, S., Fu, Y., Doepel, L., & De Buck, J. (2018). Determining the IgG concentrations in bovine colostrum and calf sera with a novel enzymatic assay. Journal of animal science and biotechnology, 9, 1-9.

DuBourdieu, D. (2019). Colostrum antibodies, egg antibodies and monoclonal antibodies providing passive immunity for animals. Nutraceuticals in Veterinary Medicine, 245-257.

Elitok, B. (2018). Indicators of passive immunity failure in neonatal calves. Oncol Res Rev, 1(3), 1-2.

Elsohaby, I., McClure, J. T., Hou, S., Riley, C. B., Shaw, R. A., & Keefe, G. P. (2016). A novel method for the quantification of bovine colostral immunoglobulin G using infrared spectroscopy. International Dairy Journal, 52, 35-41.

Erdoğan, H. M. (2009). An epidemiological study on neonatal lamb health. Kafkas Üniversitesi Veteriner Fakültesi Dergisi, 15(2). 225-236.

Fischer, A. J., Villot, C., van Niekerk, J. K., Yohe, T. T., Renaud, D. L., & Steele, M. A. (2019). Invited Review: Nutritional regulation of gut function in dairy calves: From colostrum to weaning. *Applied Animal Science, 35*(5), 498-510.

Gokce, E., & Atakisi, O. (2019). Interrelationships of serum and colostral IgG (passive immunity) with total protein concentrations and health status in lambs. Kafkas *Universitesi Veteriner Fakultesi Dergisi, 25*(4), 387-396.

Gokce, E., AH, K., Atakisi, O., & HM, E. (2013b). Risk factors associated with passive immunity, health, birth weight and growth performance in lambs: III. The relationship among passive immunity, birth weight gender, birth type, parity, dam. *Kafkas Üniversitesi Veteriner Fakültesi Dergisi, 19*(5), 741-747.

Gokce, E., Atakisi, O., Kirmizigul, A. H., Erdogan, H. M. (2013a). Risk factors associated with passive

Immunity, Health, Birth Weight and Growth Performance in Lambs: II. Effects of Passive Immunity and Some Risk Factors on Growth Performance During the First 12 Weeks of Life. Kafkas *Üniversitesi Veteriner Fakültesi Dergisi, 19*(4). 619-627.

Haenlein, G. F. W. (2001). Past, present, and future perspectives of small ruminant dairy research. *Journal of dairy science, 84*(9), 2097-2115.

Harmon, R. S., & Senesi, G. S. (2021). Laser-induced breakdown spectroscopy–a geochemical tool for the 21st century. *Applied Geochemistry, 128*, 104929.

Hernández-Castellano, L. E., Almeida, A. M., Ventosa, M., Coelho, A. V., Castro, N., & Argüello, A. (2014b). The effect of colostrum intake on blood plasma proteome profile in newborn lambs: low abundance proteins. *BMC Veterinary Research, 10*, 1-9.

Hernández-Castellano, L. E., Suárez-Trujillo, A., Martell-Jaizme, D., Cugno, G., Argüello, A., & Castro, N. (2015). The effect of colostrum period management on BW and immune system in lambs: from birth to weaning. *Animal, 9*(10), 1672-1679.

Hernandez-Castellano, L., M Almeida, A., Castro, N., & Arguello, A. (2014a). The colostrum proteome, ruminant nutrition and immunity: a review. *Current Protein and Peptide Science, 15*(1), 64-74.

Herndon, C. N., Shanthalingam, S., Knowles, D. P., Call, D. R., & Srikumaran, S. (2011). Comparison of passively transferred antibodies in bighorn and domestic lambs reveals one factor in differential susceptibility of these species to Mannheimia haemolytica-induced pneumonia. *Clinical and Vaccine Immunology, 18*(7), 1133 -1138.

Higaki, S., Nagano, M., Katagiri, S., & Takahashi, Y. (2013). Effects of parity and litter size on the energy contents and immunoglobulin G concentrations of Awassi ewe colostrum. *Turkish Journal of Veterinary & Animal Sciences, 37*(1), 109-112.

Holmøy, I. H., Kielland, C., Stubsjøen, S. M., Hektoen, L., & Waage, S. (2012). Housing conditions and management practices associated with neonatal lamb mortality in sheep flocks in Norway. *Preventive Veterinary Medicine, 107*(3-4), 231-241.

Hunter, A. G., Reneau, J. K., & Williams, J. B. (1977). Factors affecting IgG concentration in day-old lambs. *Journal of Animal Science, 45*(5), 1146-1151.

Ibrahim, N. H., Badawy, M. T., Zakzouk, I. A., & Younis, F. E. (2020). Kids' survivability as affected by their body weight, blood biochemical indices and maternal and kids' behavior in baladi and shami goats under semi-arid condition. *World's Veterinary Journal, 10*(1), 105-117.

Kessler, E. C., Bruckmaier, R. M., & Gross, J. J. (2019). Immunoglobulin G content and colostrum composition of different goat and sheep breeds in Switzerland and Germany. Journal of dairy science, 102(6), 5542-5549.

Kessler, E. C., Bruckmaier, R. M., & Gross, J. J. (2021). Comparative estimation of colostrum quality by Brix refractometry in bovine, caprine, and ovine colostrum. Journal of dairy science, 104(2), 2438-2444.

Lee, S. H., Jaekal, J., Bae, C. S., Chung, B. H., Yun, S. C., Gwak, M. J., ... & Lee, D. H. (2008). Enzyme-linked immunosorbent assay, single radial immunodiffusion, and indirect methods for the detection of failure of transfer of passive immunity in dairy calves. Journal of veterinary internal medicine, 22(1), 212-218.

Lopreiato, V., Ceniti, C., Trimboli, F., Fratto, E., Marotta, M., Britti, D., & Morittu, V. M. (2017). Evaluation of the capillary electrophoresis method for measurement of immunoglobulin concentration in ewe colostrum. Journal of Dairy Science, 100(8), 6465-6469.

Loste, A., Ramos, J. J., Fernández, A., Ferrer, L. M., Lacasta, D., Verde, M. T., ... & Ortín, A. (2008). Effect of colostrum treated by heat on immunological parameters in newborn lambs. Livestock Science, 117(2-3), 176-183.

Mandal, A., Prasad, H., Kumar, A., Roy, R., & Sharma, N. (2007). Factors associated with lamb mortalities in Muzaffarnagari sheep. Small Ruminant Research, 71(1-3), 273-279.

Massimini, G., Britti, D., Peli, A., Cinotti, S. (2006) Effect of Passive Transfer Status on Preweaning Growth Performance in Dairy Lambs. Journal of the American Veterinary Medical Association, 229, 111-115

McGovern, F. M., Campion, F. P., Lott, S., & Boland, T. M. (2015). Altering ewe nutrition in late gestation: I. The impact on pre-and postpartum ewe performance. Journal of animal science, 93(10), 4860-4872.

Moreno-Indias, I., Sánchez-Macías, D., Castro, N., Morales-delaNuez, A., Hernández-Castellano, L. E., Capote, J., & Argüello, A. (2012). Chemical composition and immune status of dairy goat colostrum fractions during the first 10 h after partum. Small Ruminant Research, 103(2-3), 220-224.

Morittu, V. M., Lopreiato, V., Ceniti, C., Spina, A. A., Minuti, A., Trevisi, E., ... & Trimboli, F. (2020). Capillary electrophoresis as a rapid test for the quantification of immunoglobulin G in serum of newborn lambs. Journal of dairy science, 103(7), 6583-6587.

Morris, S. T. (2017). Overview of sheep production systems. In Advances in sheep welfare (pp. 19-35). Woodhead Publishing.

Morton, S. U., & Brodsky, D. (2016). Fetal physiology and the transition to extrauterine life. Clinics in perinatology, 43 (3), 395-407.

Mukasa-Mugerwa, E., Lahlou-Kassi, A., Anindo, D., Rege, J. E. O., Tembely, S., Tibbo, M., & Baker, R. L. (2000). Between and within breed variation in lamb survival and the risk factors associated with major causes of mortality in indigenous Horro and Menz sheep in Ethiopia. Small Ruminant Research, 37(1-2), 1-12.

Muñoz, C., Carson, A. F., McCoy, M. A., Dawson, L. E. R., O'Connell, N. E., & Gordon, A. W. (2008). Nutritional status of adult ewes during early and mid-pregnancy. 1. Effects of plane of nutrition on ewe reproduction and offspring performance to weaning. Animal, 2(1), 52-63.

Nordi, W. M., Moretti, D. B., Lima, A. L., Pauletti, P., Susin, I., & Machado-Neto, R. (2012). Intestinal IgG uptake by small intestine of goat kid fed goat or lyophilized bovine colostrum. *Livestock Science, 144*(3), 205-210.

Nouri, M., Zarrin, M., Ahmadpour, A., Castro, N., González-Cabrera, M., & Hernández-Castellano, L. E. (2023). Feed restriction around parturition does not affect colostrum immunoglobulin G concentration in dairy fat-tailed sheep but does affect performance and blood metabolites in newborn lambs. *Journal of Dairy Science, 106*(4), 2980-2988.

Nowak, R., & Poindron, P. (2006). From birth to colostrum: early steps leading to lamb survival. *Reproduction Nutrition Development, 46*(4), 431-446.

Nowak, R., Porter, R. H., Lévy, F., Orgeur, P., & Schaal, B. (2000). Role of mother-young interactions in the survival of offspring in domestic mammals. *Reviews of Reproduction, 5*(3), 153-163.

Övet, C. (2023). Cytokines and growth factors in goat colostrum: a short review. *Journal of Bahri Dagdas Animal Research, 12*(1), 89-97.

Pekcan, M., Fidanci, U. R., Yuceer, B., & Ozbeyaz, C. (2013). Estimation of passive immunity in newborn calves with routine clinical chemistry measurements. *Ankara Üniversitesi Veteriner Fakültesi Dergisi, 60*(2), 85-88.

Piwczyński, D., Sitkowska, B., & Wiśniewska, E. (2012). Application of classification trees and logistic regression to determine factors responsible for lamb mortality. *Small Ruminant Research, 103*(2-3), 225-231.

Rodríguez, C., Castro, N., Capote, J., Morales-delaNuez, A., Moreno-Indias, I., Sánchez-Macías, D., & Argüello, A. (2009). Effect of colostrum immunoglobulin concentration on immunity in Majorera goat kids. *Journal of Dairy Science, 92*(4), 1696-1701.

Sallam, A. M. (2019). Risk factors and genetic analysis of pre-weaning mortality in Barki lambs. *Livestock Science, 230*, 103818.

Shiels, D., Loughrey, J., Dwyer, C. M., Hanrahan, K., Mee, J. F., & Keady, T. W. (2021). A survey of farm management practices relating to the risk factors, prevalence, and causes of lamb mortality in Ireland. *Animals, 12*(1), 30.

Silva, S. R., Sacarrão-Birrento, L., Almeida, M., Ribeiro, D. M., Guedes, C., González Montaña, J. R., Pereira, A. F., Zaralis, K., Geraldo, A., Tzamaloukas, O., Cabrera, M. G., Castro, N., Argüello, A., Hernández-Castellano, L. E., Alonso-Diez, Á. J., Martín, M. J., Cal-Pereyra, L. G., Stilwell, G., & de Almeida, A. M. (2022). Extensive sheep and goat production: The role of novel technologies towards sustainability and animal welfare. *Animals, 12*(7), 885.

Sjoberg, A., & Van Saun, R. J. (2021). *Use of brix refractometer in assessing sheep colostrum.* In American Association of Bovine Practitioners Conference Proceedings (pp. 273-273).

Sol Morales, M., Palmquist, D.L., Weiss, W.P. (2000): Milk fat composition on Holstein and Jersey cows with control or depleted copper status and fed whole soybeans or tallow. *Journal of Dairy Science 83*(9), 2112-2119.

Strusińska, D., Mierzejewska, J., Skok, A. (2004): Concentration of mineral components β-carotene, vitamins A and E in cow colostrum and milk when using mineralvitamin supplements. *Medycyna Weterynaryjna 60*(2), 202-206.

Swarnkar, C. P., Gowane, G. R., Prince, L. L. L., & Sonawane, G. G. (2019). Risk factor analysis for neonatal lamb mortality in Malpura sheep. *Journal of Animal Sciences 89* (6), 640-644.

Tabatabaei, S., Nikbakht, G., Vatankhah, M., Sharifi, H., & Alidadi, N. (2013). Variation in colostral immunoglobulin G concentration in fat tailed sheep and evaluation of methods for estimation of colostral immunoglobulin content. *Acta Veterinaria Brno, 82*(3), 271-275.

Talore, G. D. (2009). On-farm performance evaluation of indigenous sheep and goats in Alaba, Southern Ethiopia, *Doctoral dissertation*, Hawassa University.

Taye, M., Abebe, G., Gizaw, S., Lemma, S., Mekoya, A., & Tibbo, M. (2010). Growth performances of Washera sheep under smallholder management systems in Yilmanadensa and Quarit districts, Ethiopia. *Tropical Animal Health and Production, 42*, 659-667.

Tibbo, M., Mukasa-Mugerwa, E., Woldemeskel, M., & Rege, J. E. O. (2003). Risk factors for mortality associated with respiratory disease among Menz and Horro sheep in Ethiopia. *The veterinary journal, 165*(3), 276-287.

Tizard, I. R. (2017). *Veterinary Immunology-E-Book*. Elsevier Health Sciences.

Todaro, M., Maniaci, G., Gannuscio, R., Pampinella, D., & Scatassa, M. L. (2023). Chemometric approaches to analyse the composition of a ewe's colostrum. *Animals, 13*(6), 983.

Torres-Rovira, L., Pesantez-Pacheco, J. L., Hernandez, F., Elvira-Partida, L., Perez-Solana, M. L., Gonzalez-Martin, J. V., ... & Astiz, S. (2017). Identification of factors affecting colostrum quality of dairy Lacaune ewes assessed with the Brix refractometer. *Journal of Dairy Research, 84*(4), 440-443.

Turkson, P. K., & Sualisu, M. (2005). Risk factors for lamb mortality in Sahelian sheep on a breeding station in Ghana. *Tropical Animal Health and Production, 37*, 49-64.

Turner, J. R. (2009). Intestinal mucosal barrier function in health and disease. *Nature Reviews Immunology, 9* (11), 799-809.

Vatankhah, M. (2013). Relationship Between Immunoglobulin Concentrations in The Ewe's Serum and Colostrum, and Lamb's Serum in Lori-Bakhtiari Sheep. *Iranian Journal of Applied Animal Science, 3*(3), 539-544.

Vatankhah, M., & Talebi, M. A. (2009). Genetic and non-genetic factors affecting mortality in Lori-Bakhtiari lambs. *Asian-Australasian Journal of Animal Sciences, 22* (4), 459-464.

Viola, I., Tizzani, P., Perona, G., Lussiana, C., Mimosi, A., Ponzio, P., & Cornale, P. (2022). Hazelnut skin in ewes' diet: Effects on colostrum immunoglobulin g and passive transfer of immunity to the lambs. *Animals, 12*(22), 3220.

Wesemann, D. R., Portuguese, A. J., Meyers, R. M., Gallagher, M. P., Cluff-Jones, K., Magee, J. M., ... & Alt, F. W. (2013). Microbial colonization influences early B-lineage development in the gut lamina propria. *Nature, 501* (7465), 112-115.

Wu, H., Xu, C., Wang, J., Hu, C., Ji, F., Xie, J., ... & Lv, R. (2023). Effects of Dietary Probiotics and Acidifiers on the Production Performance, Colostrum Components, Serum Antioxidant Activity and Hormone Levels, and Gene Expression in Mammary Tissue of Lactating Sows. *Animals, 13*(9), 1536.

Yalçın, E., & Temizel, E. M. (2010). Sütten Kesme Öncesi Dönemde Oğlakların Büyüme Performansına Pasif Transfer Durumunun Etkisi. *Uludağ Üniversitesi Veteriner Fakültesi Dergisi, 29*(1), 23-26.

Yang, Y., Zhao, X., Huang, D., Wang, J., Qi, Y., Jiang, L., Zhao, H. & Cheng, G. (2019). Changes in intestinal proteins induced by colostrum uptake in neonatal calves: Analysis by two-dimensional gel electrophoresis-based proteomics analysis. *Animal Production Science, 59*(8), 1483-1490.

Yenilmez, K., Arslan, S., Kılıç, S., & Atalay, H. (2021). The effect of twinship on mineral matter, immunoglobulin G and lamb birth weight in late pregnant ewes and their newborn lambs. *Van Veterinary Journal, 32*(2), 62-68.

Zhu, H. L., Zhao, X. W., Han, R. W., Du, Q. J., Qi, Y. X., Jiang, H. N., Huang, D. W. & Yang, Y. X. (2021). Changes in bacterial community and expression of genes involved in intestinal innate immunity in the jejunum of newborn lambs during the first 24 hours of life. *Journal of Dairy Science, 104*(8), 9263-9275.

Zohary, D., Tchernov, E., & Horwitz, L. K. (1998). The role of unconscious selection in the domestication of sheep and goats. *Journal of Zoology, 245*(2), 129-135.

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Canine brucellosis: A bibliometric analysis based on scopus and web of science databases

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ABSTRACT

Brucellosis is a zoonotic infection that poses a threat to both human and animal health. The aim of this study is to investigate the publication trends in research on canine brucellosis and to determine the changes and general characteristics of the disease throughout history using bibliometric analysis. For this reason, we conducted a bibliometric analysis of 316 articles published in the Scopus and Web of Science (WoS) databases from 1980 to 2022. The analysis was performed using the RStudio-Bibliometrix package and the Biblioshiny application. The publications included contributions from a total of 1241 authors. In addition, a total of 804 keywords plus and 398 author keywords were identified. The earliest study among the top 10 most cited articles dates back to 1985, while the most recent one was published in 2011. The majority of the publications were published in the years 2012 (n=17) and 2014 (n=17). The United States of America (USA) (n=55), Brazil (n=40), and Argentina (n=25) were identified as the countries with the highest number of published articles. Notably, Switzerland ranked first in terms of the average number of citations per article category (n=55.50), despite only two publications being attributed to this country. Keid L. (n=15), Lucero N. (n=14), Carmichael L. (n=13), and Kim J. (n=13) emerged as the most prolific contributors. Lucero N. obtained the highest number of total citations (TC=408). In the initial years of publication, the disease was predominantly characterized by keywords such as "brucellosis," "infection," "abortus," and "*Brucella canis*". However, keywords related to diagnosis and treatment, such as "diagnosis," "serodiagnosis," "vaccine," and "agar gel," gained significance and became increasingly relevant. The themes and sub-themes identified through this study can provide a basis for further investigation and scientific inquiry, thus contributing to the development of new research questions and opportunities for future studies.

Keywords: bibliometrix; biblioshiny; *Brucella canis*; brucellosis; bibliometric analysis

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Introduction

Canine brucellosis is a zoonotic disease caused by the bacteria *Brucella canis*, which can infect both dogs and humans. The disease is transmitted through direct contact with infected dogs, particularly by the contact with contaminated aborted fetuses or secretions. While symptoms of intermittent fever, chills, sweating, back pain or joint pain in humans (Holst et al., 2012; Lucero et al., 2005), among dogs; infertility, abortion, and orchitis are frequently observed (Keid et al., 2009; Wanke, 2004).

B. canis agent, which was identified by Carmichael in 1966, has been diagnosed and reported in many countries around the world (Carmichael, 1966; Hollett, 2006; Wanke, 2004). Various approaches have been

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used to understand the disease and determine optimum diagnostic strategies (Daly et al., 2020; Hinić et al., 2008; Holst et al., 2012; Keid et al., 2009; López-Goñi et al., 2011; Saytekin et al., 2018). These approaches can be combined and analyzed using bibliometric analysis methods.

Although bibliometric analyses employ various methods, certain indices such as citations, authors, and keywords (Leung et al., 2017) are commonly used to measure productivity, while others such as the most influential journals or articles and the most productive countries (Silva et al., 2022) are used to assess impact. In this article, we used the bibliometric method, a statistical approach that allows for quantitative and qualitative analysis of written sources (Ekundayo and Okoh, 2018; Ellegaard and Wallin, 2015). Through this analysis, we aimed to facilitate the correct allocation of resources and policy formation for public health (Geaney et al., 2015).

While several publications have conducted bibliometric analyses, systematic reviews, and meta-analyses on brucellosis (Bakri et al., 2018; Dastani et al., 2022; Jamil et al., 2022; Mizrakçi et al., 2021; Shi et al., 2021), none have specifically focused on B. canis. Therefore, this study seeks to fill this gap in the related literature by focusing on the research specifically on canine brucellosis, using the bibliometric approach proposed by Cobo et al. (2011a). By visualizing the thematic development of the field, this approach allows for a deeper understanding of the existing literature. This study seeks to address the following research questions: 1. What is the distribution of the articles written on canine brucellosis by years and by journal? 2. What are the top 10 most highly-cited articles written on canine brucellosis between 1980 and 2022? 3. What are the most frequently used keywords in canine brucellosis-related research and how have they changed over years? 4. What is the thematic evolution of research articles on canine brucellosis between 1980 and 2022? 5. What is the productivity level of the most productive authors and countries?

Material and Methods

This current study used bibliometric data retrieved from Scopus and WoS databases as they are considered to be the major online data sources to be used for bibliometric analysis. Scopus allows researchers to search both forward and backwards in time, making it easy to access a wide range of data and references (Abbas et al., 2022; Cobo et al., 2011b). Meanwhile, WoS is a reliable and comprehensive database that hosts a wide range of high-impact scientific studies (Ekundayo and Okoh, 2018; Martín-Martín et al., 2018).

The purpose of the bibliometric analysis methodology in this study was to analyze publications, citations, and journals through the contents obtained from data sources. By using the this method, appropriate answers to purpose-oriented questions can be found through analysis (Aria and Cuccurullo, 2017; Cobo et al., 2011a; Rodríguez-Soler et al., 2020).

These bibliographic data are processed in a prepared workflow methodology: Study strategy, data collection, data analysis, visualization, Interpretation (Aria and Cuccurullo, 2017; Cobo et al., 2011b; Derviş, 2020).

Study strategy

Data for bibliometric analysis were obtained by using word combinations of "*Brucella canis*", "canine brucellosis", and "*B. canis*". These words were the most widely used ones in scientific literature and in the publications that were investigating canine brucellosis. The word “babesia” was excluded from the results as it refers to a blood parasite transmitted by ticks called "*Babesia canis* (*B. canis*)", which can also infect dogs (Schnittger et al., 2012). The data was retrieved from Scopus and WoS on 17/12/2022 by selecting only articles as the document type. The search flow of the study is presented in Appendix A and Appendix B. Data analysis was performed using Bibliometrix R-Tool (Aria and Cuccurullo, 2017).

Data collection

Data were retrieved from the Scopus database as Bibtex and from the WoS database as Plain text format. The Bibliometrix package (Aria and Cuccurullo, 2017) was used to combine the retrieved data and to eliminate duplicate publications using mathematical analysis methods. To ensure accuracy, articles published before 1980 and grey literature with incomplete or missing information (such as authors or keywords) were excluded from the study. No language restrictions were applied. The data were processed using the data collection flow diagram utilized by Rocio Rodriguez-Soler et al. (2020). This diagram consisted of five sub-steps (Figure 1) to obtain the final data set.

We first removed duplicate publications after the data collection process. 417 articles were retrieved. Out of these, 101 articles were excluded from the study as they did not fit the research purpose. This excluded set of articles consisted of 96 publications that were published before 1980 and 5 publications that did not include authors, keywords, or author keywords.

Data analysis

The R program (Version 4.2.2) was used to examine

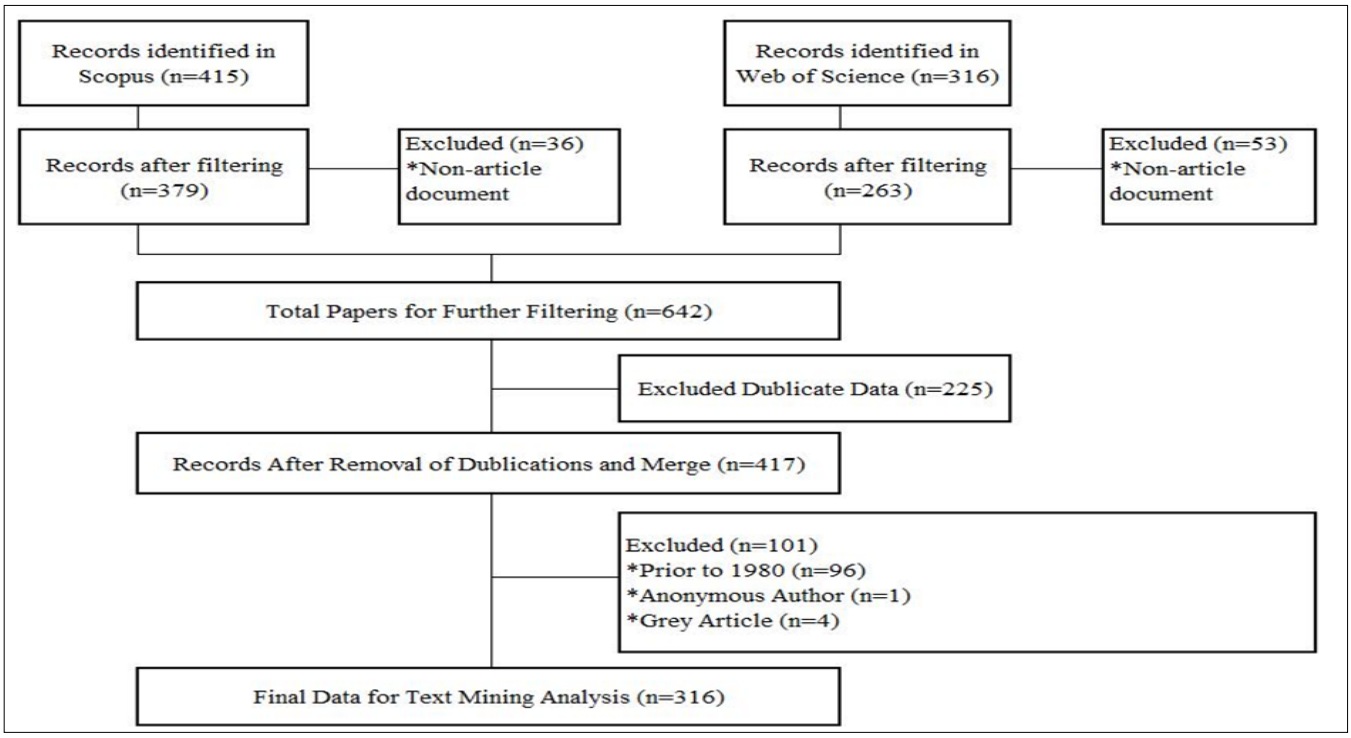

Figure 1. Data collection flow diagram of articles downloaded from Scopus and Wos

the contents of the articles bibliometrically. For data analysis, the program's Bibliometrix package was used. This package provides numerous tools for quantitative research (Aria and Cuccurullo, 2017).

Visualization

The results of the analysis were made more accessible and easy to understand by using the Biblioshiny interface, which is a user-friendly software based on Java (Rusliana et al., 2022). By utilizing the Biblioshiny interface, the analyzed results were presented in a clear and concise manner, making it easier for readers to comprehend the findings.

Interpretation

Using the data gathered in this study, various aspects of canine brucellosis research were analyzed. This included key information about the topic, yearly scientific output, important sources, highly cited articles, commonly used keywords, thematic trends, productivity of top authors over time, the most cited countries, and details regarding each country's scientific output.

Results

Main information

The Bibliometrix package was used to create a table of performance indicators (Aria and Cuccurullo, 2017), which served as the first step of the analysis. Table 1 presents the characteristics of publications on canine brucellosis between 1980 and 2022. A total of 316 articles were identified, yielding 4085 references from 161 sources. The average number of citations per article was 12.16, indicating that most articles received a low number of citations (Niknejad et al., 2021; Wang and Zhang, 2022). The study identified 1241 authors and 398 author keywords, with an average of 5.41 author collaborations per article (Co-Authors per Article). 14 authors also contributed to 18 single-author publications, and all other authors worked on multi-author articles. Biblioshiny estimated the average number of articles published per year to be 17.8.

Table 1. The analysis was conducted on 316 articles, revealing valuable insights and capturing significant research trends

Description	Results
Articles	316
Timespan	1980-2022
Keywords Plus	804
Author's Keywords	398
Sources	161
Article Average Age	17,8
Average Citations per Articles	12,16
References	4085
Authors	1241
Authors of Single-Authored Articles	14
Authors Collaboration	
Single-Authored Articles	18
Co-Authors per Articles	5,41
International Co-Authorships %	11,39

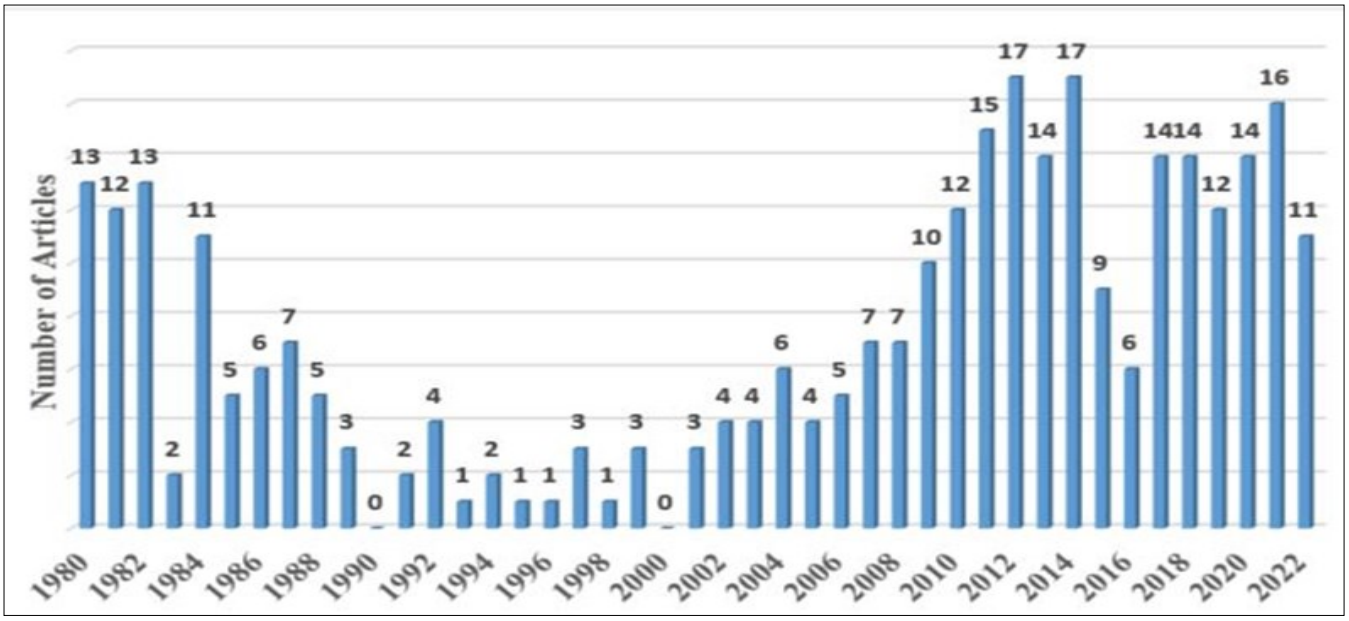

Figure 2. Annual scientific production from 1980 to 2022. Source: Data extracted from Biblioshiny

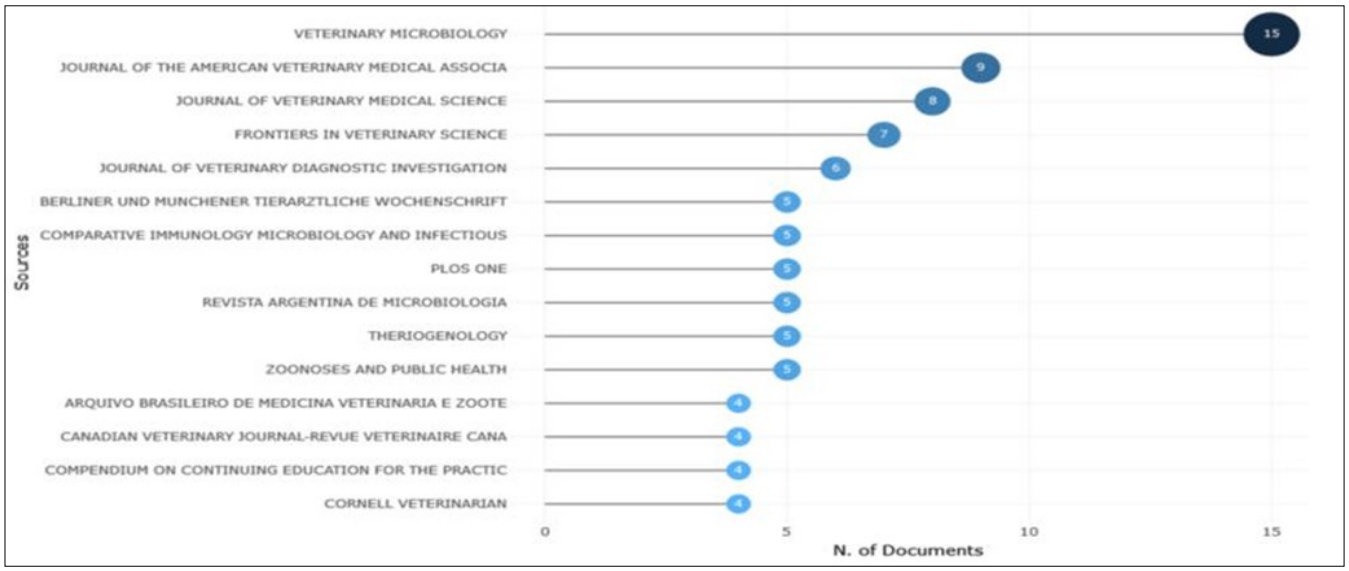

Figure 3. Most relevant sources that publish articles on canine brucellosis**.** N. of Documents**=** Number of Documents**.** Source: Data extracted from Biblioshiny

Annual scientific production

Figure 2 displays the years of publication for the literature analyzed. As can clearly be seen in Figure 2 below, while there was no consistent pattern in the number of publications between 1980 and 2022, a significant increase in the number of articles was observed since 2011, with a total of 15 articles published that year. Furthermore, varying numbers of publications were produced in subsequent years, with the highest number of articles published in 2012 (n=17) and 2014 (n=17). In this study, the 316 publications included were published in a variety of languages: English (n=257, 81.33%) was the most common language, followed by Spanish (n=18, 5.70%), Portuguese (n=14, 4.43%), German (n=10, 3.16%), Chinese (n=6, 1.90%), Turkish (n=6, 1.90%), Italian (n=2, 0.63%), Korean (n=1, 0.32%), Hungarian (n=1, 0.32%), and Russian (n=1, 0.32%).

Most relevant sources

Figure 3 displays the 15 most relevant sources in canine brucellosis studies out of a total of 161 published sources. The top five journals in this field are Veterinary Microbiology, Journal of The American Veterinary Medical Association, Journal of Veterinary Medical Science, Frontiers in Veterinary Science, and Journal of Veterinary Diagnostic Investigation. Veterinary Microbiology published the highest number of articles (n=15, 4.7%), followed by Journal of the American Veterinary Medical Association (n=9, 2.8%), Journal of Veterinary Medical Science (n=8, 2.5%), Frontiers in Veterinary Science (n=7, 2.2%), and Journal of Veterinary Diagnostic Investigation (n=6, 1.9%). The remaining sources produced 5 (1.6%) and 4 (1.3%) publications, respectively.

Table 2. The top 10 most cited articles in the field of canine brucellosis

R	Title of Article	Autor(s)	Year	LC	GC	TCpY
1	Canine Brucellosis	Wanke M	2004	66	121	6,05
2	Canine Brucellosis: outbreaks and compliance	Hollett RB	2006	56	107	5,94
3	Novel identification and differentiation of *Brucella melitensis, B. abortus, B. suis, B. ovis, B. canis*, and *B. neotomae* suitable for both conventional and real-time PCR systems	Hinić V, Brodard I, Thomann A, Cvetnić Z, Makaya PV, Frey J, Abril C.	2008	4	103	6,44
4	Human *Brucella canis* outbreak linked to infection in dogs.	Lucero NE, Corazza R, Almuzara MN, Reynes E, Escobar GI, Boeri E, Ayala SM.	2010	45	88	6,29
5	New Bruce-ladder multiplex PCR assay for the biovar typing of *Brucella suis* and the discrimination of *Brucella suis* and *Brucella canis*	López-Goñi I, García-Yoldi D, Marín CM, de Miguel MJ, Barquero-Calvo E, Guzmán-Verri C, Albert D, Garin-Bastuji B.	2011	11	80	6,15
6	Treatment of *Brucella canis* and *Brucella abortus* in vitro and in vivo by stable plurilamellar vesicle-encapsulated aminoglycosides	Fountain MW, Weiss SJ, Fountain AG, Shen A, Lenk RP.	1985	0	78	2
7	Diagnosis of human brucellosis caused by *Brucella canis*.	Lucero NE, Escobar GI, Ayala SM, Jacob N.	2005	35	63	3,32
8	Diskospondylitis associated with *Brucella canis* infection in dogs: 14 cases (1980-1991)	Kerwin SC, Lewis DD, Hribernik TN, Partington B, Hosgood G, Eilts BE.	1992	17	61	1,91
9	Canine brucellosis: a diagnostician's dilemma	Carmichael LE, Shin SJ.	1996	37	59	2,11
10	Comparison of agar gel immunodiffusion test, rapid slide agglutination test, microbiological culture and PCR for the diagnosis of canine brucellosis	Keid LB, Soares RM, Vasconcellos SA, Megid J, Salgado VR, Richtzenhain LJ.	2009	31	54	3,6

R= Rank; **LC=** Local Citations within the 316 articles; **GC=** Global Citations; **TCpY=** Total Citations per Year, Source: Data extracted from Biblioshiny

Most cited articles

Table 2 below displays the top 10 most cited articles written on canine brucellosis and it includes details such as the author(s), year of publication, local citations, global citations, and total citations per year. Local citations indicate how many times an article was cited among the 316 articles included in this research, while global citations represent the number of citations of the article in Scopus and WoS databases (Niknejad et al., 2021). The results of the analysis indicate that there are differences between the local citation and global citation values, which suggests that the topic of this research has also drawn the attention of researchers from other fields.

The authors with the highest global citation counts were Wanke M. (GC=121), Hollet RB. (GC=107), and Hinić V. et al. (GC=13). Wanke M. was also the most cited author (LC=66) among the authors of the 316 articles in this study. The article with the highest total citations per year (TCpY) was "Novel identification and differentiation of Brucella melitensis, B. abortus, B. suis, B. ovis, B. canis, and B. neotomae suitable for both conventional and real-time PCR systems" published by Hinić et al. in 2008.

Most frequent words

Word Cloud is a data visualization technique used to represent text data of the published articles' keywords, in which the size of each word indicates its frequency or importance (Javed Ali et al., 2022; Nasir et al., 2020; Wang and Zhang, 2022). The term "Author's keywords" commonly denotes the topical and contextual inclinations of researchers in a given study, whereas "keywords plus" expresses the terms, methods, and techniques used in a particular study (Patil, 2020; Zhang et al., 2016). In our study, we limited the number of keywords to 50 and randomly chose the colours for both keyword plus (Figure 4) and authors' keywords (Figure 5). The most frequently used keyword plus in the articles was "brucellosis" (n=113), followed by "dogs" (n=90), "brucella" (n=85), "animal" (n=79), "article" (n=63), "infection" (n=61), "diagnosis" (n=60), "dog" (n=55), "male" (n=49), and "*Brucella canis*" (n=48). "*Brucella canis*" (n=113) was the most frequently used authors' keyword, followed by "brucellosis" (n=49), "canine brucellosis" (n=40), "zoonosis" (n=26), "dog" (n=21), "serology" (n=19), and "dogs" (n=19).

Figure 4. Word cloud showing the most used keywords plus in publications. Source: Data extracted from Biblioshiny.

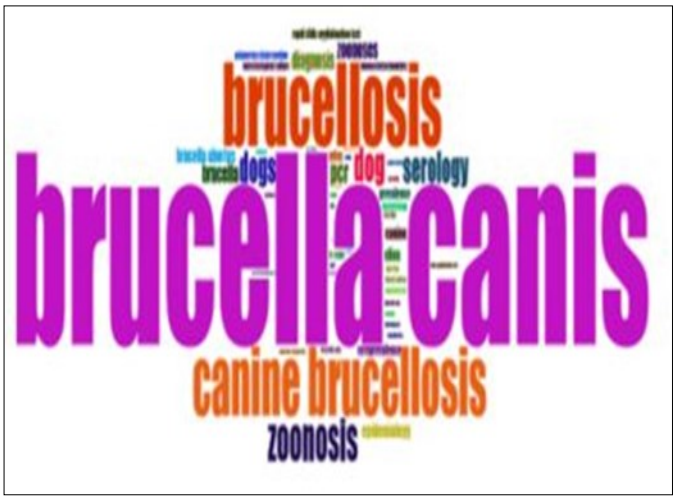

Figure 5. Word cloud showing the most used autor's keywords in publications. Source: Data extracted from Biblioshiny

Thematic evolution

Thematic evolution analysis is a useful tool for understanding how the focus of research in a specific field has changed over time (Hernandez-Cruz, 2021; Rusliana et al., 2022). To perform a comprehensive analysis of the evolution of research on canine brucellosis over time, the study was divided into three distinct segments based on year ranges: 1980-1999, 2001-2015, and 2016-2022 (Figure 6.). The first segment, spanning from 1980 to 1999, mainly focused on describing the disease using words such as "infection", "abortus", "brucellosis", and "Brucella canis". However, in the second and third segments (2001-2015 and 2016-2022), there was a shift towards research on the diagnosis and prevention of the disease. The most commonly used words during these periods were "abortus", "dogs", "infection", "outbreak", "brucellosis", "diagnosis", "abortus infection", "*brucella canis*", "serodiagnosis", "animals", "kennel", "vaccine", and "agar gel". Nonetheless, the words "infection" and "abortus" remained the two dominant subjects and remained at the focal point throughout all periods.

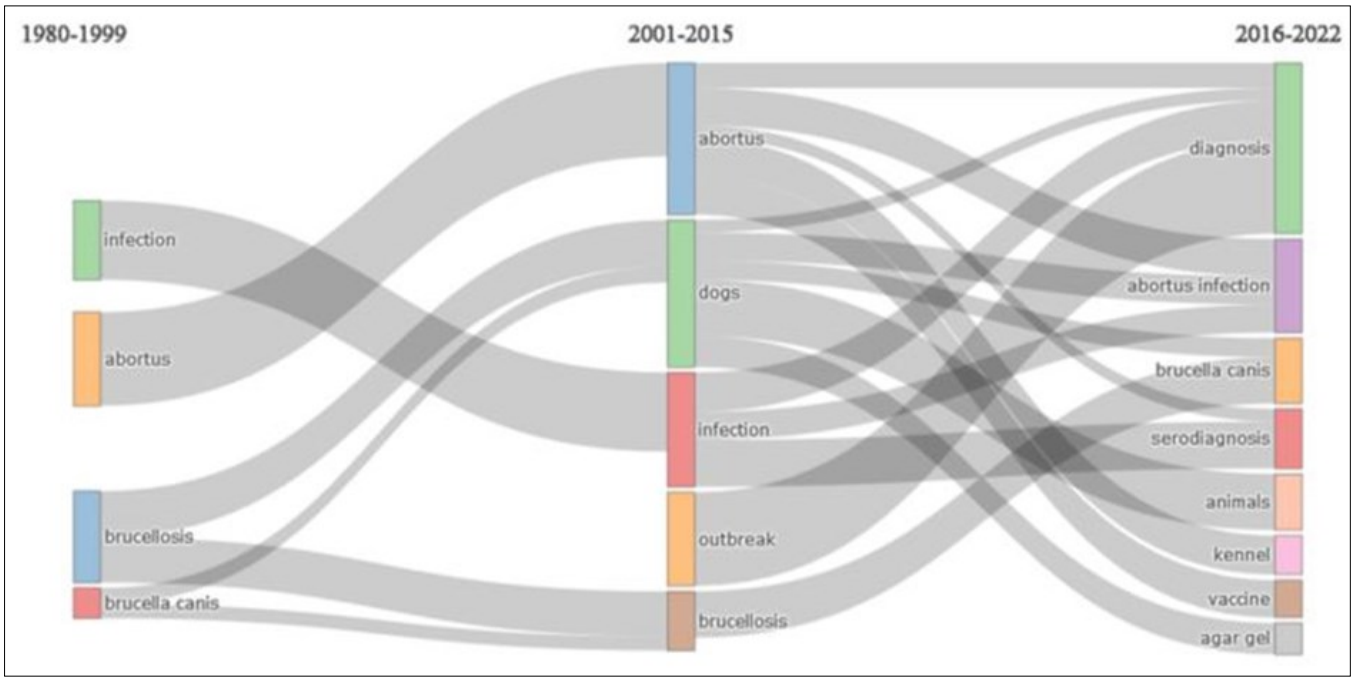

Figure 6. Thematic evolution of words from 1980 to 2022. Source: Data extracted from Biblioshiny

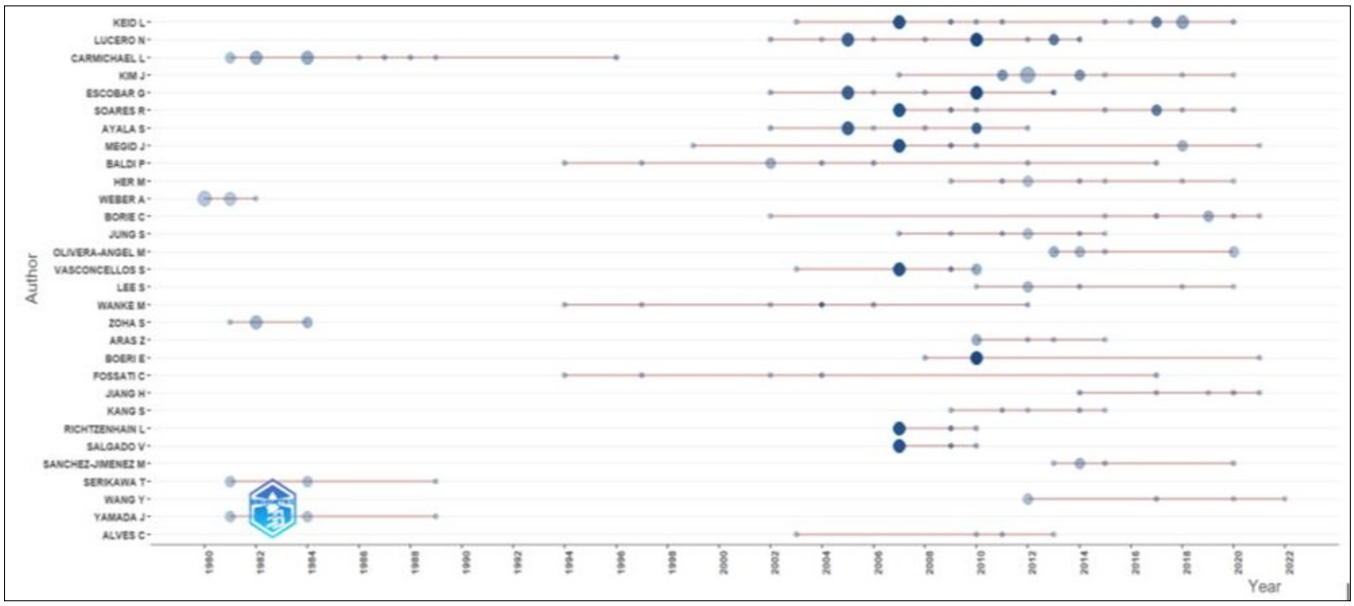

Figure 7. Production trends of the top 30 authors over the time period spanning from 1980 to 2022. Source: Data extracted from Biblioshiny

Top authors production over the time

Figure 7 displays the quality of articles produced by the 30 most productive authors between 1980 and 2022. The figure depicts the total number of citations received per year as indicated by the darkness of the circle, while the size of the circle corresponds to the number of publications. This visualization technique helps determine the most influential authors and their contributions to the research field (Wang and Zhang, 2022). The 30 most productive authors contributed to a total of 222 (70.25%) articles in the field of canine brucellosis. Among these authors, Keid L. (n=15), Lucero N. (n=14), Carmichael L. (n=13), Kim J. (n=13), Boeri E. (n=12), Escobar G. (n=10), and Soares R. (n=10) are the most prolific writers. Figure 7 further illustrates that Carmichael L. (n=13), Weber A. (n=7), Zoha S. (n=6), Serikawa T. (n=5), and Yamada J. (n=5) are considered pioneers in the field of canine brucellosis. Table 3 summarizes the publication numbers of the top 10 most productive authors, the total number of citations received by all their publications, and the year of the most cited publication. Keid L. has the highest number of publications (n=15), while Lucero N. (TC=408), Escobar G. (TC=353), and Carmichael L. (TC=337) are the top three authors with the highest total citation count.

Number of articles and citations by countries

Table 4 presents the top 10 countries with publications in the field of "Canine Brucellosis," along with the number of publications, total citation count, and average number of citations per article. The United States leads the pack with the highest number of publications (n=55), followed by Brazil (n=40) and Argentina (n=25). In terms of total citation count, the USA (n=942), Argentina (n=694), and Brazil (n=453) are

Table 3. The study assessed the productivity of the top 10 authors, analyzing their contributions and output within the field

Authors	Articles	TC	MCPY
Keid L.	15	310	2007
Lucero N.	14	408	2005
Carmichael L.	13	337	1984
Kim J.	13	105	2011
Boeri E.	12	175	2010
Escobar G.	10	353	2005
Soares R.	10	264	2007
Ayala S.	9	290	2005
Megid J.	9	228	2007
Baldi P.	8	183	2004

TC= Total Citations, **MCPY**= Most Cited Publication Year.
Source: Data extracted from Biblioshiny

Table 4. The study identified the top 10 highly cited countries, highlighting their significant contributions to the field.

R	Country	Articles	TC	AAC
1	USA	55	942	17.13
2	Brazil	40	453	11.32
3	Argentina	25	694	27.76
4	Colombia	15	98	6.53
5	China	14	69	4.93
6	Turkey	14	96	6.86
7	Korea	13	84	6.46
8	Chile	9	42	4.67
9	Japan	9	92	10.22
10	Germany	8	52	6.50

R= Rank; TC= Total Citations; AAC= Average Article Citations
Source: Data extracted from Biblioshiny

Appendix A

No	Search query (Scopus)	Result
2	(TITLE("*brucella canis*") OR TITLE("canine brucellosis") OR TITLE("*B. canis*") AND NOT TITLE(babesia)) AND (LIMIT-TO (DOCTYPE,"ar"))	379
1	(TITLE("*brucella canis*") OR TITLE("canine brucellosis") OR TITLE("*B. canis*") AND NOT TITLE(babesia))	415

Appendix B

No	Search query (Web of Science)	Result
2	**"***Brucella canis*" (Title) OR "canine brucellosis" (Title) OR "*B. canis*" (Title) NOT babesia (Title) and Article (Document Types)	263
1	"*Brucella canis*" (Title) OR "canine brucellosis" (Title) OR "*B. canis*" (Title) NOT babesia (Title)	316

at the forefront. Interestingly, Switzerland, with only two articles, has the highest average number of citations per article (n=55.50), which is significantly higher than the other countries on the list. Other countries with the highest number of citations per article are Spain (n= 36.25), Finland (n= 31.00), Argentina (n= 27.76), Sweden (n= 27.00), and Hungary (n= 19.50).

Discussion

In bibliometric analyses, it is observed that English is the most common language for articles (Abbas et al., 2022; Cuccurullo et al., 2016; Wang and Zhang, 2022). This finding arises due to the higher citation rate of English articles (Bakri et al., 2018). The determination that 81.33% of the publication language in our study is English supports this finding.

Bibliometric analyses reveal quantitative data. Due to its ability to provide a robust and versatile statistical software environment, RStudio is widely utilized in the processing of such data (Rodríguez-Soler et al., 2020). which is why we also chose to use it. Our research focuses on canine brucellosis and addresses the significance of this zoonotic disease, as well as the ongoing challenges associated with its eradication. Although no specific article has conducted a bibliometric analysis solely on canine brucellosis, previous studies have examined bibliometric analyses of brucellosis in general, including highly cited articles, institutions, and journals in the field (Bakri et al., 2018; Dastani et al., 2022; Mizrakçi et al., 2021).

Comprehensive studies on various aspects of Brucella species have consistently shown an increase in research publications since 2000. Mizrakçi et al. (2021) identified 2020 as the year with the highest number of publications (n=254), while Destani et al. (2022) found a continuous rise in brucellosis publications in Iran, reaching a peak in 2019 and 2020. Bakri et al. (2018) reported that the highest number of papers was in 2002.

Focusing specifically on canine brucellosis, our study reveals a rising trend in the number of publications since 2011, with the highest number of articles (n=17) published in 2012 and 2014. This indicates a growing interest and recognition of the importance of studying this zoonotic infection.

Mizrakci et al. (2021) identified a total of 8,903 articles on brucellosis spanning the years 1930 to 2021, while Destani et al. (2022) extracted 816 scientific publications on brucellosis conducted by Iranian researchers until 2020. In our study, which specifically focuses on canine brucellosis, we found 316 articles published between 1980 and 2022. Bakri et al. (2018) focused on the 50 most cited articles in the field of Brucella studies. While none of the top 50 articles specifically addressed canine brucellosis, the article identifies that within the topic of Brucella outbreaks, the article "Canine Brucellosis: Outbreaks and Compliance" by R. Bruce Hollet (2006) had the highest number of citations. In our own findings, Wanke M's article "Canine Brucellosis" (2004) ranked first, with "Canine Brucellosis: Outbreaks and Compliance" being the second most cited publication. The disparity in citation counts is believed to be attributed to the topics addressed in the content of the article.

The prominence of Veterinary Microbiology as the leading journal in canine brucellosis research is a noteworthy finding. Our study, along with Bakri et al. (2018)'s findings, emphasizes the significant role of this journal in publishing research on canine brucellosis. Additionally, the breadth of topics covered

by the articles demonstrates the multidimensional nature of canine brucellosis research. Diagnostic methods received significant attention, highlighting the importance of accurate detection techniques. Similarly, Destani et al. (2022) found that Iranian scientific publications on brucellosis primarily revolved around three main topics: prevalence, diagnosis, and treatment. Bakri et al. (2018) conducted an analysis that categorized their findings into different topics, such as genome sequencing, new species identification, molecular diagnostic tests, vaccination, pathogenicity, and case series. Our word cloud analysis of keywords further reinforces the evolving research focus and priorities in the field.

Conclusion

The bibliometric findings of this study can serve as a valuable reference for future research on canine brucellosis. Future studies could further explore the topic using terms such as "diagnosis" and "brucellosis", which were not analysed in depth within this study. Additionally, comprehensive studies could be designed using databases other than Scopus and WoS to provide a more comprehensive understanding of this important topic.

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Conflict of interest

The authors declared that there is no conflict of interest.

References

Abbas, A. F., Jusoh, A., Mas'od, A., Alsharif, A. H., & Ali, J. (2022). Bibliometrix analysis of information sharing in social media. *Cogent Business & Management, 9* (1), 2016556.

Ali, J., Jusoh, A., Idris, N., Airij, A. G., & Chandio, R. (2022). Wearable devices in healthcare services. bibliometrix analysis by using R package. international *Journal of Online & Biomedical Engineering*, 18(8). 61-86.

Aria, M., & Cuccurullo, C. (2017). Bibliometrix: An R-tool for comprehensive science mapping analysis. *Journal of Informetrics, 11*(4), 959–975.

Bakri, F. G., AlQadiri, H. M., & Adwan, M. H. (2018). The highest cited papers in brucellosis: Identification using two databases and review of the papers' major findings. *BioMed Research International*, 1–10.

Carmichael, L. E. (1966). Abortion in 200 beagles. *Journal of the American Veterinary Medical Association, 149*, 1126.

Cobo, M. J., López-Herrera, A. G., Herrera-Viedma, E., & Herrera, F. (2011a). An approach for detecting, quantifying, and visualizing the evolution of a research field: A practical application to the fuzzy sets theory field. *Journal of Informetrics, 5*(1), 146–166.

Cobo, M. J., López-Herrera, A. G., Herrera-Viedma, E., & Herrera, F. (2011b). Science mapping software tools: Review, analysis, and cooperative study among tools. *Journal of the American Society for Information Science and Technology, 62*(7), 1382–1402.

Cuccurullo, C., Aria, M., & Sarto, F. (2016). Foundations and trends in performance management. A twenty-five years bibliometric analysis in business and public administration domains. *Scientometrics, 108*(2), 595 –611.

Daly, R., Willis, K. C., Wood, J., Brown, K., Brown, D., Beguin-Strong, T., Smith, R., & Ruesch, H. (2020). Seroprevalence of *Brucella canis* in dogs rescued from South Dakota Indian reservations, 2015–2019. *Preventive Veterinary Medicine, 184*, 105157.

Dastani, M., Mardaneh, J., & Moshari, J. (2022). Mapping the scientific structure of Iranian brucellosis researches using the co-authorship and co-occurrence network analysis. *Iranian Journal of Medical Microbiology, 16*(4), 336-339.

Derviş, H. (2020). Bibliometric analysis using bibliometrix an R package. *Journal of Scientometric Research, 8*(3), 156–160.

Ekundayo, T. C., & Okoh, A. I. (2018). A global bibliometric analysis of plesiomonas-related research (1990 – 2017). *PloS one, 13*(11), e0207655.

Ellegaard, O., & Wallin, J. A. (2015). The bibliometric analysis of scholarly production: How great is the impact? *Scientometrics, 105*(3), 1809–1831.

Geaney, F., Scutaru, C., Kelly, C., Glynn, R. W., & Perry, I. J. (2015). Type 2 diabetes research yield, 1951-2012: Bibliometrics analysis and density-equalizing mapping. *PloS one, 10*(7), e0133009.

Hernandez-Cruz, N. (2021). Mapping the thematic evolution in communication over the first two decades from the 21st century: A longitudinal approach. *Iberoamerican Journal of Science Measurement and Communication, 1*(3), 1–10.

Hinić, V., Brodard, I., Thomann, A., Cvetnić, Ž., Makaya, P. V., Frey, J., & Abril, C. (2008). Novel identification and differentiation of *Brucella melitensis*, *B. abortus*, *B. suis*, *B. ovis*, *B. canis*, and *B. neotomae* suitable for both conventional and real-time PCR systems. *Journal of Microbiological Methods, 75*(2), 375–378.

Hollett, R. B. (2006). Canine brucellosis: Outbreaks and

compliance. *Theriogenology, 66*(3), 575–587.

Holst, B. S., Löfqvist, K., Ernholm, L., Eld, K., Cedersmyg, M., & Hallgren, G. (2012). The first case of Brucella canis in Sweden: Background, case report and recommendations from a northern European perspective. *Acta Veterinaria Scandinavica, 54*(1), 18.

Jamil, T., Akar, K., Erdenlig, S., Murugaiyan, J., Sandalakis, V., Boukouvala, E., Psaroulaki, A., Melzer, F., Neubauer, H., & Wareth, G. (2022). Spatio-Temporal Distribution of Brucellosis in European Terrestrial and Marine Wildlife Species and Its Regional Implications. *Microorganisms, 10*(10), 1970.

Keid, L. B., Soares, R. M., Vasconcellos, S. A., Megid, J., Salgado, V. R., & Richtzenhain, L. J. (2009). Comparison of agar gel immunodiffusion test, rapid slide agglutination test, microbiological culture and PCR for the diagnosis of canine brucellosis. Research *in Veterinary Science, 86*(1), 22–26.

Leung, X. Y., Sun, J., & Bai, B. (2017). Bibliometrics of social media research: A co-citation and co-word analysis. International *Journal of Hospitality Management, 66*, 35–45.

López-Goñi, I., García-Yoldi, D., Marín, C. M., de Miguel, M. J., Barquero-Calvo, E., Guzmán-Verri, C., Albert, D., & Garin-Bastuji, B. (2011). New Bruce-ladder multiplex PCR assay for the biovar typing of Brucella suis and the discrimination of Brucella suis and Brucella canis. *Veterinary Microbiology, 154*(1–2), 152–155.

Lucero, N. E., Jacob, N. O., Ayala, S. M., Escobar, G. I., Tuccillo, P., & Jacques, I. (2005). Unusual clinical presentation of brucellosis caused by *Brucella canis*. *Journal of Medical Microbiology, 54*(5), 505–508.

Martín-Martín, A., Orduna-Malea, E., Thelwall, M., & Delgado López-Cózar, E. (2018). Google scholar, web of science, and scopus: A systematic comparison of citations in 252 subject categories. *Journal of Informetrics, 12*(4), 1160–1177.

Mizrakci, S., Demiray, E. K.D., & Akyüz, H. Ö. (2021). Bruselloz konulu yayınların global analizi ve Türkiye'den yapılan yayınlarla karşılaştırılması. *Journal of Biotechnology and Strategic Health Research, 5*(3), 229-235.

Nasir, A., Shaukat, K., Hameed, I. A., Luo, S., Alam, T. M., & Iqbal, F. (2020). A bibliometric analysis of corona pandemic in social sciences: A review of influential aspects and conceptual structure. *IEEE Access, 8*, 133377–133402.

Niknejad, N., Ismail, W., Bahari, M., Hendradi, R., & Salleh, A. Z. (2021). Mapping the research trends on blockchain technology in food and agriculture industry: A bibliometric analysis. *Environmental Technology & Innovation, 21*, 101272.

Patil, S. B. (2020). Global library & information science research seen through prism of biblioshiny. *Studies in Indian Place Names, 40*(49), 157–170.

Rodríguez-Soler, R., Uribe-Toril, J., & De Pablo Valenciano, J. (2020). Worldwide trends in the scientific production on rural depopulation, a bibliometric analysis using bibliometrix R-tool. *Land Use Policy, 97*, 104787.

Rusliana, N., Komaludin, A., & Firmansyah, M. F. (2022). A scientometric analysis of urban economic development: R bibliometrix biblioshiny application. *Journal Ekonomi Pembangunan, 11*(2), 80–94.

Saytekin, A. M., Karagül, M. S., Eroğlu, B., Yildiz Öz, G., Yilmazbaş MeciToğlu, G., & Yilmaz, Ö. (2018). The first-time isolation of *Brucella canis* from two aborted bitches in a kennel in Turkey. *Turkish Journal of Veterinary and Animal Sciences, 42*(6), 665–668.

Schnittger, L., Rodriguez, A. E., Florin-Christensen, M., & Morrison, D. A. (2012). Babesia: A world emerging. *Infection, Genetics and Evolution, 12*(8), 1788–1809.

Shi, J.-F., Gong, Q.-L., Zhao, B., Ma, B.-Y., Chen, Z.-Y., Yang, Y., Sun, Y.-H., Wang, Q., Leng, X., Zong, Y., Li, J. -M., & Du, R. (2021). Seroprevalence of brucellosis in buffalo worldwide and associated risk factors: A Systematic review and meta-Analysis. *Frontiers in Veterinary Science,* 8, 649252.

Silva, M. do S. T., Oliveira, V. M. de, & Correia, S. É. N. (2022). Scientific mapping in scopus with biblioshiny: A bibliometric analysis of organizational tensions. *Contextus – Revista Contemporânea de Economia e Gestão, 20*, 54–71.

Wang, J., & Zhang, S. (2022). Cross-Cultural Learning: A visualized bibliometric analysis based on bibliometrix from 2002 to 2021. *Mobile Information Systems,* 1–11.

Wanke, M. M. (2004). Canine brucellosis. *Animal Reproduction Science, 82–83*, 195–207.

Zhang, J., Yu, Q., Zheng, F., Long, C., Lu, Z., & Duan, Z. (2016). Comparing keywords plus of WOS and author keywords: A case study of patient adherence research: Comparing Keywords Plus of WOS and Author Keywords. *Journal of the Association for Information Science and Technology, 67*(4), 967–972.

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Can diet impress horse behaviour?

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ABSTRACT

Along with domestication, various changes in nutrition and digestion have occurred in horses as well as in all species. Our review aims to compile existing studies examining the role of nutrition and digestion in the emergence of undesirable behaviours in domestic horses. The success achieved in species with high adaptability has not been sustained by the change in breeding purposes over the years. Welfare, defined as the physical and mental health of animals. The responses of animals to various stimuli are defined as behaviour. Since the behaviour of animals plays a primary role in monitoring welfare, studies on animal behaviour have intensified. The development of undesirable behaviours complicates the care and management practices and negatively affects the welfare of the animals in the following periods, resulting in negative consequences for both the animal and its environment. Stereotypes, which are among the unwanted behaviours and exhibited in the form of purposeless, repetitive movements, are one of the biggest behavioural problems of horse breeding. Oral stereotypes impede food intake, locomotor stereotypes impede mobility and are instrumental in the emergence of chronic and more complex health problems. Other undesirable behaviours may also harm the horse's keeper and other horses kept together in the same box, resulting in serious injury. It is known that nutrition and nutrients affect the development of undesirable behaviours in horses. It is known that anticipatory behaviour creates a basis for repetitive behaviours as a result of inhibition when feeding time and nutrient content are out of preference or insufficient. The effect of nutrition and nutrient content on the emergence of stereotypes and undesirable behaviours has not been fully addressed and the subject remains up-to-date. The purpose of this paper is to provide a brief discussion of studies conducted to assess the effect of nutritional imbalances on stereotypic behaviour in horses. The PRISMA 2020 guidelines were applied to the review using systematic review rules

Keywords: animal nutrition, behaviour, feed, stereotypes, horse.

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Introduction

Horses have played an important role in human history for many years. After the industrial revolution, horse breeding evolved for sports and hobby purposes. Also, horses breed for meat and labour, but their share is small. In present conditions, it is important to have well-developed behavioural characteristics as well as physiological health in horse breeding, which is carried out with special care and feeding. In order to maintain continuity of growth and development and vital activities within physiological limits, nutrition and nutrient composition are of great importance. Diet varies according to species, breed, age, physiological phase and individual preferences are also important. Sensory factors such as taste and smell are known to vary between individuals. It is also thought that diets that are disliked or not enjoyed sensually can cause restlessness. Housing problems are one of the main causes of stress in horses and are the main cause of undesirable behaviour. However, the fact that similar behavioural problems are observed in equestrian horses reared in good conditions and away from the stress of transport suggests that factors other than housing may also cause behavioural problems.

Behaviour of animals is used to provide environmental

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control and to develop a rapid adaptation (Kappeler, 2010). Behaviours can be examined under many titles such as social behaviours, species/breed-specific behaviours, and reproductive behaviours. Behaviours that are outside of normal behaviours, aimless and repeated are defined as stereotyped behaviours. It is known that poor welfare and poor management conditions are effective in the occurrence of undesirable behaviours and stereotypes in horses (Waters et al. 2010; Sarrafchi and Blokhuis, 2013).

Repetitive behaviours are frequently observed in individuals or group stabled horses, especially due to lack of space and nutrients (Johnson et al. 2010). The most common of these behaviours are crib biting and pawing, which can occur at a very young age as a result of nutritional deficiencies and stress. Crib-biting behaviour can be caused oral cavity injuries and colic pains that may develop due to air swallowing, can be economic damage to the stable equipment also negatively affect animal health and welfare (Eser and Erat, 2022). This situation also leads to poor performance in horses used for equestrian.

Feeding times are defined as periods of over excitement and frustration because the arrival of feed is a very exciting and gratifying event for the horses as same as other animals (Mills and Clarke, 2002). Many concentration feeds' delectable flavour and unusually high energy content can operate as super stimulants, raising anticipation before feeding (Goodwin et al. 2005). This anticipation can be shown through stereotypic behaviours like pawing and circling, repetitive behaviour like kicking the stable door, and behaviour showing enthusiasm and arousal (van den Berg et al. 2016a; Eser and Erat 2022; Hockenhull and Creighton, 2023).

While examining the effects of nutrients on horses, studies were conducted on the reactivity, temperament and preferences of horses, but the direct effects of nutrients were not mentioned. Although it is known that preference orientations are not only affected by organoleptic characteristics, few studies showing that they are affected by some behavioural patterns.

This study aims to evaluate the studies examining the effects of feeding and nutrient contents on horse behaviour as a systematic review.

The literature review procedure (review, inclusion criteria, and data acquisition) was prepared according to the PRISMA-2020 guidelines (Page et al. 2020). The relevant diagram is given in Figure 1.

In the study, the literature was searched in both Turkish and English languages using the keywords “horse”, “stereotypes in horses”, “nutrition in horses”, and “nutrition and stereotype relationship” in the databases of Elsevier, Wiley and Google Scholar. Since there are not many studies on the subject, the year range is not specified. The researches obtained from the databases included in the study consisted of 3 compilations, 2 printed book chapters, 1 pre-rehearsal article, 3 conference proceedings and all the remaining 41 were original research articles. No ethical permission is required for the study.

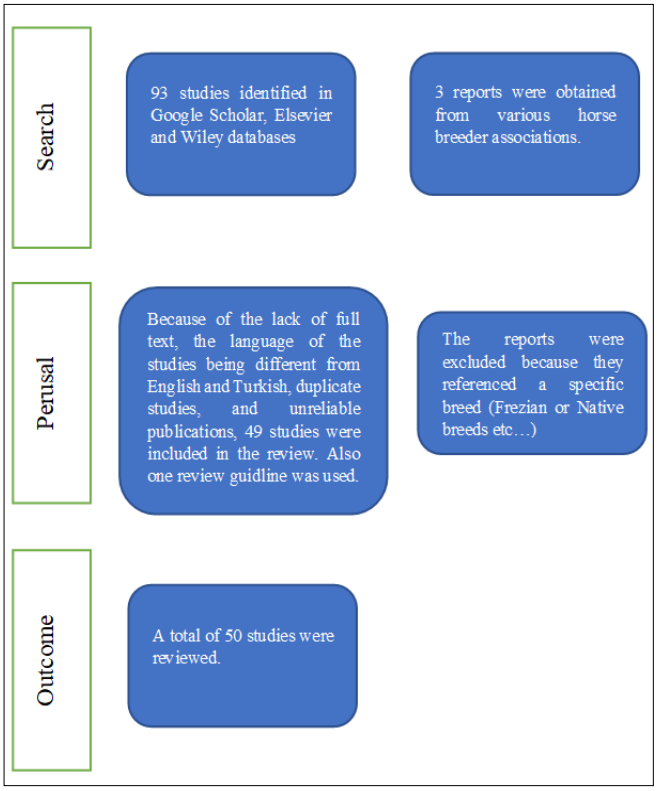

Figure 1. The PRISMA procedure followed in obtaining and using the literature reviewed in the study (Page et al. 2020).

Feeding Behaviour and Food Preferences in Horses

Feeding behaviours studies in herbivores have focused heavily on ruminants. However there are limited studies on food preferences in horses according to nutritient content (van den Berg et al. 2016b).

It has been observed that herbivores with grazing behaviours increase their bite size as the differences in grass height discrepancies grow. Foraging models indicate that herbivores should prefer long grass to short grass. Furthermore, the feeding region can be identified by intermediate biomass for optimal digestible nutrient intake, although there may be a trade-off between forage quality and quantity. The foraging models propose that herbivores should select tall grass over short grass. However, the feeding area can be characterized by intermediate biomass to optimize the uptake of digestible nutrients due to the trade-off between forage quality and quantity

(Catanese et al., 2013). This behaviour has been observed in studies on sheep (Naujeck et al., 2005) and cattle (Ungar et al., 2001), but few studies have investigated the feed preferences of horses (Naujeck et al., 2005; Ellis et al., 2005; Hanis et al., 2020a).

Horses get their food in small chunks and graze at short intervals without going too far from the area. Some studies reported that horses prefer foods with lower indigestible carbohydrate content (Naujeck et al. 2005; Ellis et al. 2005; Hanis et al. 2020b). In addition, feeds with high energy content are preferred; in the absence of this type of feed in the environment that the energy need is met by increasing the amount and frequency of food intake (van den Berg et al. 2016c).

Physiologically, horses have evolved to consume structural plants that take significantly longer to chew than grains or processed feeds. Since chewing is the main stimulus for saliva production in the horse, the amount of saliva produced can also be compromised when feeding concentrates, which can affect subsequent digestion as well as buffering in the colon. The inclusion of dried shredded fodder or straw is becoming increasingly common. However, there are few studies on the effects of straw length or inclusion ratio on feeding behaviour (Ellis et al. 2005).

Horses graze for 12 to 18 hours a day in their natural habitat, but the amount of food and time they consume it in the shelter is often limited (Ellis, 2010 and 2012; Harris et al. 2017; Raspa et al. 2017). According to Burla et al. (2016), agonistic behaviour was generally reported to be more intense at feeding times in group bred horses. Additionally, horses fed at timed feeding intervals exhibited more locomotor activity prior to feeding than horses fed at untimed intervals, although no difference was seen during feeding. Regardless of the feeding arrangement, it is strongly advised from an ethological perspective to supply not just grass but also hay. In another study, Cooper et al. (2005) revealed that the frequency of feeding in horses causes an overall increase in stereotypic behaviorsThe study concludes that splitting up concentrated rations for barn horses into smaller meals may be an effective strategy to eliminate oral stereotypes, but pre-feeding stereotypes may still exist and practise may make stereotyped behaviours more frequent. Horses in visual contact but not fed.

Regardless of the preferred flavour, Cairns et al (2002) reported that horses preferred higher energy diets to lower energy diets. However, it has been hypothesised that ruminal fermentation, particularly when multiple feeds are provided at the same time, may make it difficult for horses to associate the chosen feed with the post-digestion results. Redgate et al. (2014), compared the effect of selecting three roughages in a simultaneous preference study when horses consumed a single type of feed rich in water-soluble carbohydrates, lipids or protein. In the study, it was observed that feeds rich in water-soluble carbohydrates or protein were preferred, and it was concluded that the macronutrients in the diets triggered a response in the horses and activated the feedback mechanisms.

Nutrient Composition and Behaviour Relationship in Horses

Animal behaviour and its evaluation by various methods has become a popular topic in the scientific community in recent decades. As the general definition of behaviour, it is seen that there are many factors that have an effect. Although it is known that the most influential factor on behaviour is breeding and husbandry conditions and care, recent studies have tried to reveal that nutrient contents also lead to the development of undesirable behaviours through various physico-chemical pathways or habits (Gillham et al. 1994; Holland et al. 1996a; b; McBride and Hemmings, 2009; Ellis, 2010 and 2012; Hemmings and Hale, 2013; Roberts et al. 2015; Moore-Colyer et al. 2016; Hanis et al. 2020b). Since nutrient intake is also driven by preferences, we cannot make a strong generalization about the effect of nutrient content on behaviour due to the influence of individual factors. Therefore, a conclusive statement similar to housing and care conditions has yet to be clarified.

Although there are intensive studies on roughage in animal nutrition, there is no comprehensive explanation. In general, there is limited research in other species on the carbohydrate and roughage components of rations, which are essential in ruminant diets. On the other hand, in studies with increasing descriptive features, roughages are mentioned as components that are not easily degraded with the enzymes, and their effects on physical satiety are continuing to be demonstrated (De Leeuw et al. 2008). In addition to physiological satiety, roughage should also be considered in terms of the animal busy with mastication, reducing stress and preventing the development of abnormal behaviours (Paintal, 1954; Rolls and Rolls, 1997; Cummings and Overduin, 2007; De Leeuw et al. 2008).

It is known that there is a connection between stereotypic behaviours and endogenous opioids in many species, especially horses (Gillham et al. 1994). According to Albright et al. (2017), one of the most effective crib-biting triggers, mediated by the opioid

and dopaminergic systems, is a highly appetising meal. Extremely appetising taste is still a potential contributing component to the concentrated diet, despite the possibility of gastric and post-gastric repercussions.

The effects of roughage and plain oat consumption on the crib-biting behaviour were examined and it was determined that they were not at the same level of effect in the studies. Studies have determined that as the preferability of the diet increases, endogenous opioids are released and the opioid, dopaminergic and glutaminergic neural systems are fed by feedback mechanisms (Albright et al. 2017). It has been observed that in horses fed alfalfa pellet, the crib-biting stereotype begins within 10 minutes after feed intake, similar results are seen in horses fed with cereal grains with high carbohydrate content, and the stereotype is exhibited for 30 minutes (Gillham et al. 1994).

To prevent obesity and weight gain in horses, hay intake is restricted. Ellis (2010), reported that horses exhibit food intake behaviour of 12.5+2.5 hours a day and this is necessary to protect both digestive system health and mental health. It is known that both physiological and mental balance is impaired in case of restriction of time or food intake (Ellis, 2012). A parallel study found that in Thoroughbred horses receiving less than 6.8 kg of fibre per day, stereotypic behaviour and feed limitation were positively correlated. After weaning, foals fed concentrated feed showed a 4 times higher incidence of crib-biting behaviour than other foals (Waters et al. 2002). According to Roberts et al. (2015), concentrated feed-induced increases have been associated with dopamine neurotransmission and more intricate changes in neuroactive molecules including leptin and ghrelin (Hemmann et al. 2013; Hanis et al. 2020b). On the contrary side, bait reduces the intensity of crib-biting (Hemmings and Hale, 2013), but the feeding time has no impact on locomotor activity in terms of typical or stereotypic behaviour over the course of a 24-hour period (Piccione et al. 2013). For this reason, it is recommended to progressively introduce roughage to horses displaying crib-biting behaviour in order to prevent the onset of the behaviour and lower the frequency of stereotyped behaviour.

It is thought that dopamine released during the performance of oral stereotypes such as biting the wood and wind sucking the perpetuation of the stereotypes. For this reason, giving adequate feed and some substance for chewing or occupy the oral cavity in indoor horses is a great solution to prevent the formation of behavioural disorders (Moore-Colyer et al. 2016).

Foals supplied with a diet of fat and fibre were less stressed than those fed a diet of starch and sugar, according to Nicol et al. (2005). Horses' reduced responsiveness can be ascribed to high blood sugar levels following meals when they consume a lot of starch (Bulmer et al. 2015). If we evaluate the effects of macronutrients on horses diet choises, horses prefer protein and hydrolyzed carbs over fats, according to Redgate et al. (2014). The energy content has no bearing on the horses' preferred feed, according to van den Berg et al. (2016c). Similar to wild horses, it has been reported that they have complicated dietary habits and consume a variety of plant species that are high in fibre and low in calories. Additionally, it was proposed by van den Berg et al. (2016a; b) that organoleptic signals including taste and smell might affect nutritional choice. For instance, it has been said that silage preferred by horses than hay or straw. (Müller and Uden, 2007). According to a recent study, feed preferences in ponies are influenced by lateralization, smell, and excitability (Vinassa et al. 2020). Mares given hay continuously have better bodily conditions and higher reproductive rates than mares fed the same amount of roughage exclusively at night, according to reports. This is also effects to their well-being and behavioural patterns (Benhajali et al. 2013).

Limited studies have been conducted on the impact of nutrition on normal behaviour, reactivity, or stress reaction in horses, despite mounting evidence linking starch-rich diets to abnormal behaviour. Although commercial feed supplements that promise to lower general reactivity are frequently employed, there hasn't been much evidence of them having a detrimental impact on reactivity. There is, however, little proof that dietary lipids influence reactivity. Holland et al (1996a) reported that, horses were fed test diets with additional fat, observed lower sensitivity to pressure, auditory, and visual stimuli as well as decreased locomotion. Foals fed a diet high in fat and fibre had lower pre-weaning cortisol levels than foals fed a diet high in starch and sugar, according to Holland et al. (1996b). In the study, which also examined at the weaning behaviour of foals, it was discovered that, at least in part of the observed weaning groups, foals that got fat and fibre supplements demonstrated increased grazing behaviour and seemed less agitated.

Non-structural carbohydrates are prevalent in meals made of grains, which are frequently offered two or three times per day. However, high grain consumption is not compatible with the

gastrointestinal system of horses, and according to George and Harris (2007), this feeding method is linked to the emergence of gastrointestinal issues, including gastric ulcer disease and colic linked to hindgut dysfunction. Additionally, research on horses indicates that low-fibre diets put animals at a higher risk of exhibiting abnormal behaviours like stereotypes, potentially as a result of shortened eating intervals and visceral disturbance (George and Harris, 2007).

Hanis et al. (2020), examined the frequency of locomotor or oral stereotyped and directed behaviours as well as their connection to regular management and feeding practises. Horses that are utilised for riding, equestrian, polo, endurance, and patrolling were chosen for the study and are housed in a variety of ways. Oral stereotypic behaviour was found to be most prevalent in the study. It has been found that the number of straws used, the concentration level, and the amount of time spent working all have a substantial impact on oral stereotypic behaviour. Another finding of the study was that the quantity and frequency of feedings had an impact on both locomotor stereotyped and redirected behaviours. The study's findings indicated that consistent management and feeding techniques can influence the frequency of various abnormal behaviours in the majority of working horses.

In a study that investigated the relationship between high-fibre diets and high-starch diets and behaviour of horses, behavioural activities connected to feeding a diet rich in fibre produced better results than providing a diet high in Fstarch. Giving horses a diet high in fibre is beneficial for their welfare and profitable from an economic standpoint, allowing the animals to express their feeding behaviour more fully. Animals that are excited or triggered have been observed to consume less feed with high energy content. It has been proven that fiber-based diets can be utilised to stop horses from acting out in undesirable behaviours in this way (Raspa et al. 2022).

The display of oral stereotypic behaviour has been shown to depend on meal times. Horses with the crib-biting had a significant plasma ghrelin peak before a concentrated feed, according to Hemmann et al. (2012 and 2013). After feeding, plasma leptin levels in crib-biting horses were considerably lower than those in healthy horses. These results imply that more study is required to determine how nutrition affects ghrelin, leptin, and abnormal oral behaviour. Another study found that among the horse population under investigation, directed behaviour and oral stereotypes were the most common abnormal oral behaviours (Hanis et al. 2021). Both behaviours were found to be strongly related to nutritional qualities like dietary fibre and low straw content (Hanis et al. 2020 and 2021). In other study suggests that, persistent observation of abnormal oral behaviours following meals may point to a lack of postprandial satiety (Hanis et al. 2023).

Conclusion

It is a well-known that poor care conditions and management lead to stress and low welfare in all animal species. The absence of suitable housing and management, which are key factors contributing to inadequate care, results in animal distress and undesirable behaviours. However, the lack of sheltering space restricts the normal behaviour, and increases the development of abnormal behaviours and stereotypes. Reproduction and feeding conditions, which are the leading environmental factors in horses, are of great importance on behaviours as well as quantitative characteristics. Inadequate and unhealthy feeding, which is one of the care deficiencies, causes both physical and physiological problems in animals and negatively affects welfare. Although there are many studies on animal welfare care conditions and shelter characteristics, the effect of nutrition and nutritional characteristics on welfare indicators has not been fully explained. Studies have shown that feeding time and frequency can affect behavioural patterns in animals. It has also been reported that diet can lead to various repetitive behaviuors in animals and trigger abnormal behaviours, often referred to as stereotypes. The feed preferences, which are thought to have developed in the evolutionary process depending on the digestive physiology of the horses, were determined as the feeds containing carbohydrates with high digestibility. Considering the energy intake, it has been obtained as a result of the studies that the energy is kept in balance by increasing the amount of feed. Considering the nutrient content, it was found that malnutrition in animals caused oral stereotypes; it was concluded that deficiencies in the ration structure, depending on the rearing purpose and activity, may trigger other abnormal behaviours. In horses, preoccupation has been identified as a method of coping with the stress of individual housing and has been reported to account for a large proportion of chewing behaviour. It has been reported that horses whose chewing behaviour is restricted by feeding low roughage diets become mentally fatigued and increase undesirable behaviours. Although there are many issues to investigate regarding the effect of diet composition on behaviour, the number of available

studies is relatively small. The limited number of studies on the identification of secondary factors in the development of stereotypic behaviour in horses has meant that the subject under discussion in this review has not been supported by sufficient literature. Although many titles on the effect of diet composition on behavioural patterns need to be examined, the number of available studies is very few. Considering the developments in the field of animal nutrition and behaviour, it is clear that more multidisciplinary studies investigating the effects of diet and nutrient content on equine behaviour are needed.

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Author contributions: Erva ESER writing-original draft and conceptualization, Şevket EVCİ performed writing, review and editing, conceptualization.

References

Albright, J., Sun, X., & Houpt, K. (2017). Does cribbing behaviour in horses vary with dietary taste or direct gastric stimuli? *Applied Animal Behaviour Science. 189*, 36 -40.

Benhajali, H., Ezzaouia, M., Lunel, C., Charfi, F., & Hausberger, M. (2013). Temporal feeding pattern may influence reproduction efficiency, the example of breeding mares. *Plos One, 8*(9), e73858.

Bulmer, L., McBride, S., Williams, K., & Murray, J.A. (2015). The effects of a high-starch or high fibre diet on equine reactivity and handling behaviour. *Applied Animal Behaviour Science, 165*, 95-102.

Burla, J. B., Ostertag, A., Patt, A., Bachmann, I., & Hillmann, E. (2016). Effects of feeding management and group composition on agonistic behaviour of group-housed horses. *Applied Animal Behaviour Science, 176*,32-42.

Cairns, M. C., Cooper, J. J., Davidson, H. P. B., & Mills, D. S. (2002). Association in horses oforosensory characteristics of foods with their post-ingestive consequences. *Animal Science, 75*, 257–265.

Catanese, F., Obelar, M., Villalba, J. J., & Distel, R. A. (2013). The importance of diet choice on stress-related responses by lambs? *Applied Animal Behaviour Science, 148*(1-2), 37-45.

Cooper, J.J., Mcall, N., Johnson, S., & Davidson, H.P.D. (2005). The short-term effects of increasing meal frequency on stereotypic behaviour of stabled horses. *Applied Animal Behaviour Science, 90*(3-4), 351-364.

Cummings, D. E., & Overduin, J. (2007) Gastrointestinal regulation of food intake. Journal *of Clinical Investigation. 117*, 13–23.

De Leeuw, J. A., Bolhuis, J. E., Bosch, G., & Gerrits, W. J. J. (2008). Effects of dietary fibre on behaviour and satiety in pigs Proceedings of the Nutrition Society Proceedings of the Nutrition Society, March 2008: 334-342.

Ellis, A. D. (2010). *Biological basis of behaviour and feed intake. In: Ellis, A. D., Long- land, A. C., Coenen, M. (Eds.*), The Impaction of Nutritionon the Health and Welfare of Horses. Wageningen Academic Publishers, Wageningen, The Netherlands, pp.53–74, EAAP Publication No.128.

Ellis, A. D. (2012). *Effect of forage presentation on feed intake behaviour in stabled horses. In: Saastamoinen M.M.J., Fradinho., A.S., Santos., N. (Eds.),* Forages and Grazing in Horse Nutrition. Wageningen Academic Publishers, Wageningen, The Netherlands, pp.53–74.

Ellis, A. D., Thomas, S., Arkell, K., & Harris, P. (2005). *Adding chopped straw to concentrate feed: The effect of inclusion rate and particle length on intake behaviour of horses*. Equine Nutrition Conferance, Hannover, Germany.

Eser, E., & Erat, S. (2022). *A systematic review: Stereotypic behaviours of horses and their effects on horse welfare*. 7th International Congress on Veterinary and Animal Sciences 20-22 October 2022, Online.

George, R. J., & Harris P. A. (2007). *How to minimize gastrointestinal disease associated with carbonhydrate nutrition in horses. Processors Annual Conve*ntion, American Association of Equine Practioners Annual Convention, 53: 178-185.

Gillham, S. B., Dodman, N. H., Shuster, L., Kream, R., & Rand W. (1994). The effect of diet on cribbing behaviour and plasma b-endorphin in horses. *Applied Animal Behaviour Science, 41*(3-4),147-153.

Goodwin, D., Davidson, H. P. B., & Harris, P. (2005). Sensory varieties in concentrate diet for stabled horses: effects on behaviour and selection. *Applied Animal Behaviour Science, 90*(3-4), 337-349.

Hanis, F., Chung, E. L. T., Kamalluddin, M. H., & Idrus, Z. (2021). Do nutritient composition of feedtufffs affect the proportion of oral stereotypies and redirected behaviours among horse working groups? *Journal of Veterinary Behaviour, 46*, 7-14.

Hanis, F., Chung, E. L. T., Kamalludin, M. H., & Idrus, Z. (2020b). Discovering the relationship between dietary nutrients on cortisol and ghrelin hormones in horses exhibiting oral stereotypic behaviour: *A Review Journal of Veterinary Behaviour, 39*, 90-98.

Hanis, F., Chung, E. L. T., Kamaluddin, M. H., & Idrus, Z. (2020a). The influence of stable management and feeding practices on the abnormal behaviours among stabled horses in Malaysia. Journal *of Equine Veterinary Science, 94*, 103230.

Hanis, F., Chung, E. L. T., Kamulluddin, M. H., & Idrus Z. (2023). Effect of feed modification on the behaviour, blood profile, and telomere in horses exhibiting abnormal oral behaviours. *Journal of Veterinary Behaviour. 60*, 28-36.

Harris, P. A., Ellis, A. D., Fradinho, M. J., Jansson, A., Julliand, V., Luthersson, N., Santos, A. S., & Vervuert, I. (2017). Review: Feeding conserved forage to horses: recent advances and recommendations. *Animal, 11*(6), 958-967.

Hemmann, K., Koho, N.M., Vainio, O.M., & Raekallio, M.R. (2013). Effects of feed on plasma leptin and ghrelin concentrations in crib-biting horses. *Veterinary Journal, 198*,122-126.

Hemmann, K., Raekallio, M., Kanerva, M., Hänninen, L., Pastell, M., Palviainen, M., & Vainio, O. (2012). Circadian variation in ghrelin and certain stress hormones in crib-biting horses. *Veterinary Journal, 193*, 97-102.

Hemmings, A., & Hale C. (2013). From *gut to brain. In: Proceedings of Lesaffre Feed Additives Satellite Symposium*. World Veterinary Congress, Czech Republic.

Hockenhull, J., & Creighton, E. (2023). Pre-feeding behaviour in UK leisure horses and associated feeding routine risk factors. *Animal Welfare, 23*(3), 297-308.

Holland, J. L., Kronfeld, D. S., & Meacham, T. N. (1996b). Behaviour of horses is affected by soy lecithin and corn oil in the diet. *Journal of Animal Science, 74*(6), 1252-1255.

Holland, J. L., Kronfeld, D. S., Hoffman, R. M., Greiwe-Crandell, K. M., Boyd, T. L., Cooper, W. L., & Harris, P. A. (1996a). Weaning stress is affected by nutrition and weaning methods. *Pferdeheilkunde, 12*, 257-260.

Johnson, K.G., Tyrell, J., Rowe, J.B., & Pethick, D.W. (2010). Behavioural changes in stabled horses given nontherapeutic levels of virginiamycin. *Equine Veterinary Journal, 30*(2),139-143.

Kappeler, P. (2010). *Animal Behaviour: Evolution and Mechanisms*. Berlin, Germany, Springer,

McBride, S., & Hemmings, A. (2009). A neurological perspective of equine stereotypy. *Journal of Equine Veterinary Science 29*, 10-16.

Mills, D. S., & Clarke, A. F. (2002). *Housing, management and welfare. In: Waran, N. (Ed.), The Welfare of Horses*. Dordrecht, Netherlands, Kluwer Academic Publishers,

Moore-Colyer, M. J. S., Hemmings, A., & Hewer, N. (2016). A preliminasry investigation into the effect of ad libitum or restricted hay with or without Horslyx on the intake and switching behaviour of normal and crib biting horses. *Livestock Science, 186*,59-62.

Müller, C.E., & Uden, P. (2007). Preference of horses for grass conserved as hay, haylage or silage. *Animal Feed Science and Technology, 132*(1-2),66-78.

Naujeck, A., Hill, J., & Gibbs, M. J. (2005). Inluence of sward height on diet selection by horses. *Applied Animal Behaviour Science. 90*(1), 49-63.

Nicol, C. J., Badnell Waters, A. J., Bice, R., Kelland, A., Wilson, A. D., & Harris P. A. (2005). The effects of diet and weaning method on the behaviour of young horses. *Applied Animal Behaviour Science. 95*(3-4), 205-2011.

Page, M. J., McKenzie, J. E., Bossuyt, P. M., Boutron, I., Hoffmann, T. C., Mulrow, C. D., Shamseer, L., Tetzlaff, J. M., Akl, E. A., Brennan, S. E., Chou, R., Glanville, J., Grimshaw, J. M., Hróbjartsson, A., Lalu, M. M., Li, T., Loder, E. W., Mayo-Wilson, E., McDonald, S., McGuinness, L. A., … Moher, D. (2021). The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. *BMJ (Clinical research ed.)*, 372, n71.

Paintal, A. S. (1954) A study of gastric stretch receptors. Theirrole in the peripheral mechanism of satiation of hunger andthirst. *Journal of Physiology,126*, 255–270.

Piccione, G., Giannetto, C., Marafioti, S., Panzera, M., Assenza, A., & Fazio, F. (2013). Influence of time of food administration on daily rhythm of total locomotor activity in ponies. *Journal of Veterinary Behavior, 8*, 40–45.

Raspa, F., Tarantola, M., Muca, E., Bergero, D., Soglia, D., Cavallini, D., Vervuert, I., Bordin, C., De Palo, P., & Valle E. (2022). Does feeding management make a difference to behavioural activities and welfare of horses reared for meat production? *Animals,12*, 1740.

Redgate, S.E., Cooper, J.J., Hall, S., Eady, P., & Harris P.A. (2014). Dietary experience modifies horses feeding behaviour and selection patterns of three macronutrient rich diets. *Journal of Animal Science, 92*,1524-1530.

Roberts, K., Hemmings, A., Moore-Colyer, M. J. S., & Hale C. (2015). Cognitive differences in horses performing locomotory versus oral stereotypic behaviour. *Applied Animal Behaviour Science, 168*, 37–44.

Rolls, E. T., & Rolls, J. H. (1997) Olfactory sensory-specificsatiety in humans. *Physiology & Behavior 61*, 461–473.29.

Sarrafchi, A., & Blokhuis, H. J. (2013). Equine stereotypic behaviours: Causation, occurence, and prevention. *Journal of Veterinary Behaviour. 8*(5), 386-394.

Ungar, E.D., Ravid, N., & Bruckental, I. (2001). Bite dimensions for cattle grazing herbage at low levels of depletion. *Grass and Forage Science, 56*(1), 35-45.

van den Berg, M., Giagos, V., Lee, C., Brown, W.Y., & Hinch, G.N. (2016b). Acceptance of novel food by horses: The influence of food cues and nutritient composition. *Applied Animal Behaviour Science. 183*, 59-67.

van den Berg, M., Giagos, V., Lee, C., Brown, W.Y., Cawdell-Smith, A.J., & Hinch, G.N. (2016a). The influence of odour, taste and nutritients on feeding behaviour and food preferences in horses. *Applied Animal Behaviour Science, 184*, 41-50.

van der Berg, M., Lee, C., Brown, W.Y., & Hinch, G.N. (2016c). Does energy intake influence diet selection of novel forages by horses? *Livestock Science, 186*, 6-15.

Vinassa, M., Cavallini, D., Galaverna, D., Baragli, P., Raspa, F., Nery, J., & Valle, E. (2020). Palatability assessment in horses in relation to lateralization and temperament. *Applied Animal Behaviour Science, 232*,105110.

Waters, A. J., Nicol, C. J., & French, N. P. (2002). Factors influencing the development of stereotypic and redirected behaviours in young horses: findings of a four year prospective epidemiological study. *Equine Veterinary Journal, 34*(6), 572–579.

Waters, A. J., Nicol, C. J., & French, N. P. (2010). Factors influencing the development of stereotypic and redirected behaviours in young horses: findings of a four year prospective epidemiological study. *Equine Veterinary Journal, 34*(6), 572-579.