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Research Article

Antipruritic armamentarium with short term nutritional support solution involving silymarin and curcumin for atopic dermatitis in dogs

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ABSTRACT

The present researcher group hypothesized that commercially available oral/topical solution involving *Curcuma Longa* and *Silybium marianum* combination will significantly decrease the pruritus scores after short-term (1 week of twice-daily) topical treatment in 26 dogs with atopic dermatitis. Evaluations included, mean day 0 Owner Pruritus Visual Analogue Scale score were similar ($p>0.05$) among the Silifort paste treatment groups (6.90 - 6.93 cm for Silifort paste treated animals (range 2-10) and placebo control dogs (range 2-10), respectively, in which continued to gradually reduce over the remaining 6 days of research in the Silifort paste treatment group ($p<0.0001$) and at day 7, decreased to 0.93 cm (a 6 cm reduction, which corresponds to a decrease from "severe itching" to "normal"). The mean day 0 Veterinarian Dermatitis Visual Analogue Scale score were similar ($p>0.05$) between the Silifort paste treatment group (6.70 - 6.75 cm for the Silifort paste treated animals (range 2-10) and placebo control dogs (range 2-10), respectively, wheras at day 7 decreased to 1 cm (a 5.75 cm decrease from "moderately severe dermatitis" to "normal") and placebo control animals to 5.2 cm (a 1.5 cm decline from "moderately severe dermatitis" to "mildly moderate dermatitis") ($p<0.0001$). Veterinarian Visual Analogue Scale dermatitis scores in Silifort paste treated animals were notably reduce compared to placebo control dogs on day 7 ($p<0.0001$). The present study supports a potential benefit of topical Silifort paste for short term relieving itching sensation. This treatment modality may replace immunosuppressive applications, with its anti-inflammatory, anti-infectious and antioxidant formulation.

INTRODUCTION

Silymarin, a herbal derived flavonoid, obtanied from seeds and fruits from milk thistle (*Silybum marianum* L. Gaertn.), has been involved with the Asteraceae family Asteraceae (1). The latter mik thistle extract of milk thistle traditionaly used for therapeutic armamentarium for several disorders (2), which is now under consideration for treatment of several dermatological disorders (3).

Curcumin involved as diferuloylmethane, has long been spice, turmeric. Turmeric has gained medicinal properties (4-6). In the present study both herbal treasures were used and it was hypothesized that commercially available oral/topical nutritional support solution involving *Curcuma Longa* and *Silybium marianum*; Silifort paste (Silp), (Aurora, Italy, Turkish side distributor Pharmax, Turkey) combination will significantly decrease the pruritus scores after short term (1 week of twice-daily) topical treatment in dogs with atopic dermatitis (Ad).

MATERIAL and METHODS

Demographics

All necessary data of was shown on table 1. with detailed demographic values. A total of 26 dogs were included to the study (Table 1).

Interpretation of analytic methods

Diagnosis was based on necessary testing, laboratory evaluation and deemed necessary applications shown at Table 2. To those of slowly 26 cases were deemed eligible without pyoderma, mycotic secondary infection, demodicosis/scabies or endocrine disorders. This study was approved by HADYEK with number of 2019/022.

Topical usage of Silp nutritional support

Commercially available nutritional support solution invoving *C. longa* and *S. marianum* (*Silifort pasta 30 gr, Aurora biofarma, Italy, Turkish side distributor Pharmax, Turkey*); Silp was applied onto the lesional area(s) for a short term (1 week of twice-daily) topical treatment in dogs with Ad enrolled in this study. Control group dogs received setirizin (Zrytec oral drop) with 1 mg/kg dosage daily for a week.

CADESI-04 scores

Based on Hill's atopy index application by use of a iphone 8 plus device was recorded and obtained sum of data sent to e mail as a pdf file (automatically delivered by the application).

Statistical analysis

Treatment efficacy of the turmeric/Silymarin compared to placebo control dogs in the present study was assessed by the mean owner VAS pruritus via mean veterinarian VAS

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Table 1. Baseline characteristics of enrolled dogs

Variable	Control group	Treatment group
Breed distribution [n(%)]		
Mixed breed	6 (60)	9 (56.2)
Purebred	4 (40)	7 (43.8)
Sex distribution [n(%)]		
Male	5 (50)	7 (43.8)
Female	5 (50)	9 (56.2)
Mean age [years (range)]	6.7 (2-13)	5.8 (1-12)
Mean weight at study onset [kg (range)]	17.3 (4-34)	16.7 (4-42)
Owner Pruritus VAS score at study onset [arithmetic mean (cm)]	6.90	6.93
Veterinarian Dermatitis VAS score at study onset [arithmetic mean (cm)]	6.70	6.75

Table 2. Diagnosis criteria of atopic dogs.

Observational criteria/parameter	Prelaud criteria	Favrot criteria	Hill's atopy index handphone application
Pruritus evaluation	Owner Pruritus VAS score at study onset [mean]	Veterinarian Dermatitis VAS score at study onset [mean]	Clinical exam
Testing	Polycheck in vitro allergen determination	Non-invasive monitoring	Dermatological examination
	Acetate tape impression, deep skin scraping, Dermlite D4 dermatoscopic exam	Excluding other relevant disorders (i.e. microbiological,mycological lab work, endocrine profile: i.e. total T4, plasma cortisol values within reference ranges)	

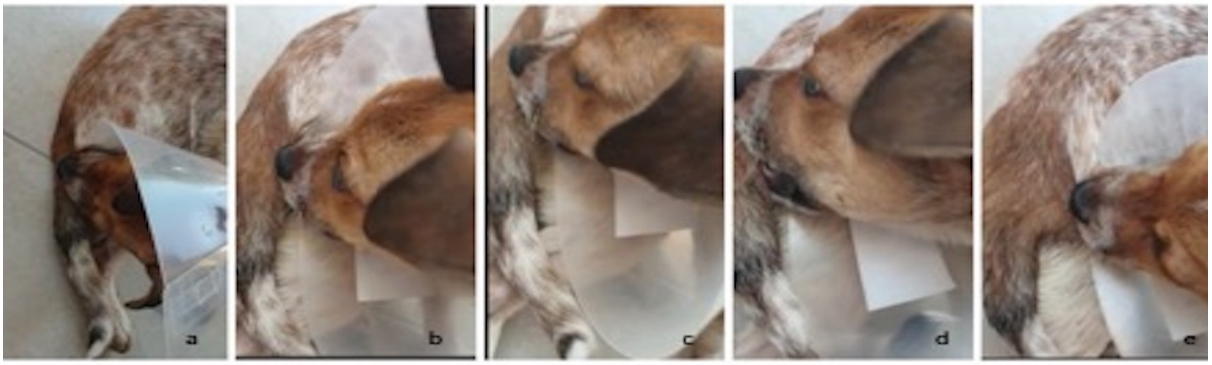

Figure 1. Slight view of pruritus behavior with in few seconds (a – e). Specifically dedicated to the gut microbiome which can modulate 'gut-skin axis', tryptophan existed out via gut microbiome could participate for the pruritus, as was the case herein showed. Several seconds thereafter showed itching sensetation, licking, chewing beahviour with a pruritus score of 9. After Silp treatment of 1 week, score declined to 1.

Figure 2. Two different cases diagnosed with Ad, with pododermatitis and pruritus scores of 6 and 7, respectively. After Silp treatment scores were evident as 0 and 1, respecticelyscore declined to 1.

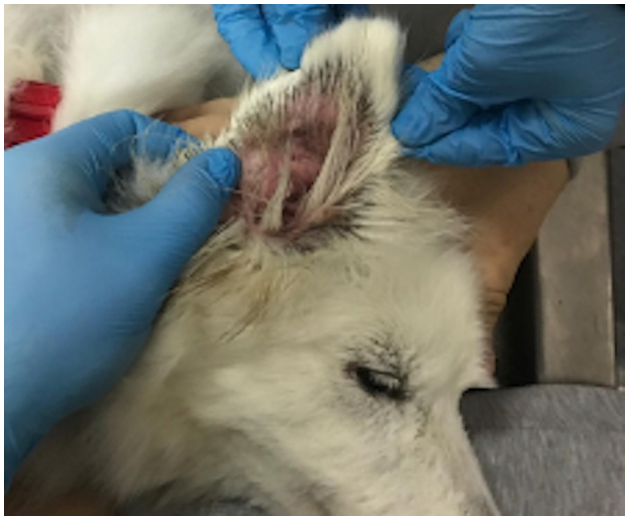

Figure 3. End stage inflammation resulting in otitis externa due to allergic reaction and histamin intolerance in Ad. This case was presented with a pruritus score of 7, which gradually decreased to 1, after Silp treatment.

Figure 4. Ad has been extensively determined in our clinical practice within the small breeds; as the pruritus scores were 6, 4, 5 and 7, respectively with notably observed itching sensetation..

dermatitis scores on daily basis for seven days and on day 0 and 7, respectively. Repeated measures ANOVA test were used to determine the time, group and group & time interactions. Friedman's two way-analysis of variance test were used to control the time interaction (days) for each group. To analyze the differences on each day between groups Mann-Whitney-U test were used and values <0.05 was recognized significant. All tests were done with package software (SPSS 22.0, SPSS Inc., USA)

RESULTS

Macroscopical view of selected cases

Owner VAS pruritus scores on each day of study

Mean 0. day owner VAS pruritus scores were similar (p>0.05) within the treatment groups (6.90 and 6.93 cm for the turmeric/silymarin treated dogs (range 2-10) and placebo control dogs (range 2-10), respectively. After 1 day of treatment, a 0.55 cm decrease of the mean owner VAS pruritus scores was observed in the treatment group, while the control dogs had a 0.27 cm reduction. The mean owner VAS pruritus scores continued to gradually reduction over the outstanding 6 days ($p < 0.0001$) of study in the treatment group. The mean owner VAS pruritus scores in the turmeric/silymarin treated dogs were notably lower compared to placebo treated animals on days 3 ($p < 0.01$), 4 ($p < 0.001$), 5, 6 and 7 ($p < 0.0001$). At day 7, the mean owner VAS pruritus score had reduced for the turmeric/black cumin treated dogs to 0.93 cm (a 6 cm reduction, which corresponds to a decrease from "severe itching" to "normal". The reduction in the owner VAS pruritus scores for placebo treated animals after 7 days was only 1.81 cm.

Veterinarian VAS dermatitis scores on each day of study

The mean day 0 veterinarian VAS dermatitis scores were similar (p>0.05) among the treatment groups (6.70 and 6.75 cm for turmeric/silymarin treated animals (range 2-10) and placebo control animals (range 2-10), separately. At day 7, mean veterinarian VAS dermatitis score for turmeric/black cumin treated animals declined to 1 cm (5.75 cm decrease from "moderately severe dermatitis" towards "normal"), the control animals to 5.2 cm (1.5 cm decrease from "moderately severe dermatitis" to "mildly moderate dermatitis") (p<0.0001). Veterinarian VAS dermatitis scores in the turmeric/silymarin treated animals were notably reduce compared to control dogs on day 7 (p<0.0001).

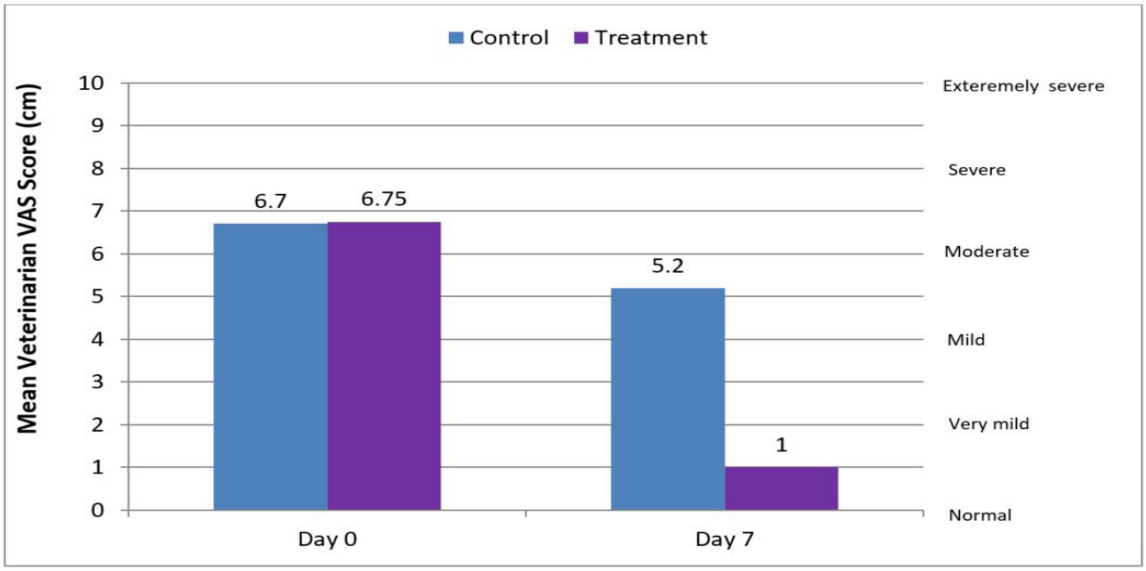

Figure 5. Mean veterinarian VAS scores throughout the study period.

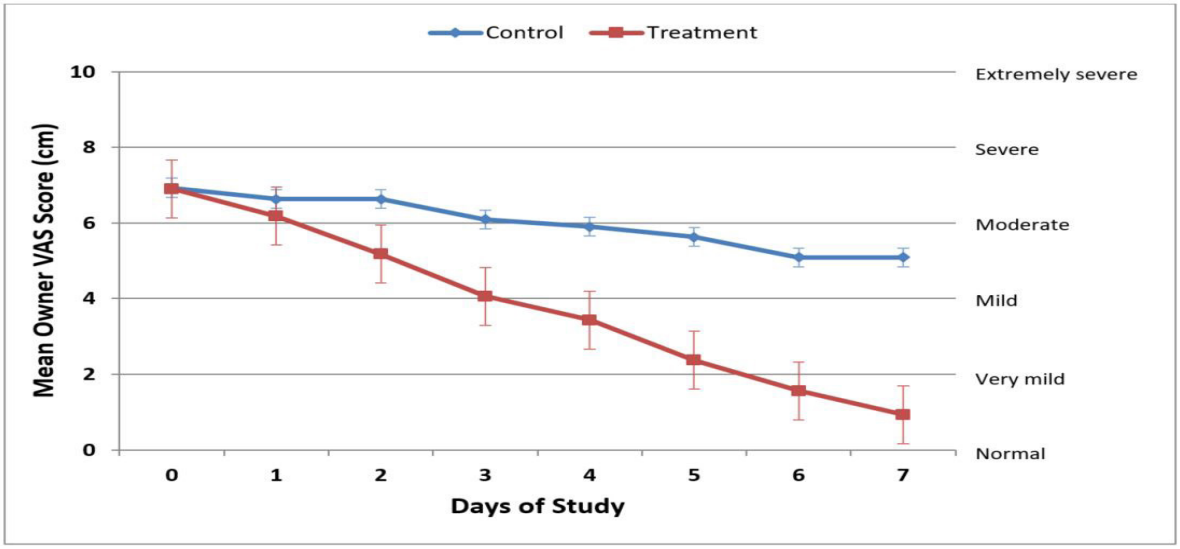

Figure 6. Mean owner VAS scores throughout the study period.

CADESI-04 analytes

Based on Hill's atopy index application by use of a iphone 8 plus device scores ranged from 11 (mild Ad) to 126 (severe Ad), which were switched to 10- 99 after Silp treatment.

DISCUSSION

The results of the present study might be comperable to a prior research investigating the efficacy of oclacitinib (ocl) against pruritus to those of dogs with allergic dermatitis. In that study after 1 week of ocl treatment, a 65% decrement among pruritus scores (changed from 'severe to very mild itching'). Within the first day of ocl therapy, pruritus scores declined by at least 2 cm (44%) compared to those of the control-treated animals (19%). On week 1, the vast majority of the ocl-treated dogs (86.4%) presented a 2 cm decline in Owner Pruritus VAS scores when compared to placebo-treated dogs (<42.5%). Furthermore, on week 1, 70.5% of ocl-treated animals represented a >50% decline in Owner Pruritus VAS scores in contrast to <23.2% of the placebo cases. In the latter study ocl therapy resulted in treatment success (66.5% in ocl-treated vs. 29.4% of the placebo), making ocl improving pruritus and dermatitis (7). In comparison in the present study mean 0. day owner VAS pruritus scores were similar ($p>0.05$) between the treatment groups (6.90 and 6.93 cm for the Silp treated dogs (range 2-10) and control dogs (range 2-10), respectively. After 1 day of Silp treatment, a 0.55 cm decrement of mean owner VAS pruritus scores was detected in treatment group, while the control dogs had a 0.27 cm reduction. The mean owner VAS pruritus scores persistent to gradually reduce over other 6 days of study in the treatment group ($p<0.0001$). The mean owner VAS pruritus scores in the Silp treated dogs were notably lower compared to control treated dogs on days 3 ($P<0.01$), 4 ($p<0.001$), 5, 6 and 7 ($p<0.0001$). At day 7, the mean owner VAS pruritus score had reduced for the Silp treated animals to 0.93 cm (a 6 cm decrement, which means a decrease from "severe itching" to "normal". The decrease in owner VAS pruritus scores for control treated animals after 7 days was only 1.81 cm.

Instant depression (probably) of the activity of pruritogenic cytokines [i.e. Interleukin-31], might briefly define the quick decline in pruritus thereafter ocl therapy (8). Hence by management of the pruritus in dogs, a direct anti-inflammatory action might also occur within the dermis. Similarly in the present study a better understanding for the Silp treatment option could be available with the expression of the compound ingredients. Turmeric has a well-known interaction with several molecular targets participating within inflammation (9). The latter spice, reduce the amplitude of the inflammatory respond by down-regulating the activities of cyclooxygenase-2, lipoxygenase and nitric oxide synthase (9). Anti-inflammatory effects of curcumin might be dedicated to downregulation of the expression of TNF-α, cyclin D and cell surface adhesion molecules (10). In addition inhibition the activity of protein serine/threonine kinases, c-Jun N-terminal kinase and protein tyrosine kinase, might participate (11). From another point of view curcumin selectively inhibits phosphorylase kinase (12), the enzyme participate for breaking down glycogen, for formation of ATP, with a significant role for phosphorylation (13). The latter enzyme may be released only 300 seconds after injury, activating inflammatory cells (13-15), wound healing and scar tissue formation (16). Given the efficacy of curcumin able to inhibit phosphorylase kinase activity might have the potential for modulation of the inflammatory respond for influencing above mentioned factors. In the present study the oral compund used involved curcumin, in which supported the decreased VAS scales and related scoring by probable antiinflammatoric action as reported previously and explorated from human studies (8-16).

In the present study interpretation of pruritus was based on VAS scales, as was also reported and determined previoulsy (7). In that research enhanced VAS scales were presented as an easy and quotable technique for practioners for assessing the severity of pruritus (20,22). In previous researches, a VAS scale was preferred for assessment of itching (23). Indeed, dogs may not truly present itching behaviour on referral. Thus scientists or practioners pruritus VAS score might have to lean on what the patient's owner report rather than observation

(7). On the other hand regarding Canine Atopic Dermatitis and Severity Index (CADESI), has long been known as an objective and validated assessment tools with limited usage confined now to atopic dermatitis, and might not be suitable for assessing the degree of the skin disorders (7), observed in this study. Enhanced dermatitis VAS used and adopted previously (7), which also adopted in the present study might have been useful for free veterinary surgeons participating at special practice with no special education nor certification in veterinary dermatology (7).

Berardesca *et al.* (17) denoted that silymarin alog with methylsulfonylmethane were found effective for relieving pruritus and relevant clinical signs at erythemato-telangiectatic phase of rosacea subtype-1. On the other hand topical silymarin diminished atopic dermatitis by suppression of mast cell infiltration in mice skin (18). Hence silymarin was able to reduce plasma IL-4 and IgE levels to those of mice. It has been postulated that silymarin be helpful for atopic dermatitis treatment (18). Mady *et al.(*19) used specifical silymarin formulation significantly diminshed redness, swelling, and inflammation in atopic dermatitis. Al those afromentioned mechanisms might be possibly interact with the anti-pruritic armamentarium obtained in this study.

CONCLUSION

In conclusion it may be suggested that, curcumin, a selective Janus kinase inhibitor, prescribed per twice daily, along with silymarin, were both safe and effective for releiving pruritus in association with allergy in dogs. This natural (herbal in origin) compounds offered itch relief within 24 hours as was observed throughout study period, with the vast majority of treated animals presented a 5.75 cm decrease on pruritus (from "moderately severe dermatitis" to "normal") by day 7. It should not be unwise to draw conclusion that this herbal compound might have helped pruritus relief and might be used with safety as an antipruritic agent.

DECLARATIONS

Ethics Approval

This study was approved by animal ethics committee of the Aydın Adnan Menderes University (No: 2019/022), Aydın.

Conflict of Interest

The authors declare that they have no competing interests.

Author Contribution

Idea, concept and design: K Ural

Data collection and analysis: K Ural, M Gültekin, S Erdoğan, H Erdoğan

Drafting of the manuscript: K Ural, M Gültekin, S Erdoğan, H Erdoğan

Critical review: K Ural, M Gültekin, S Erdoğan, H Erdoğan

Data Availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Research Article

Prevalence of dermatophytes isolated from domestic animals in Ankara within a three-year period (2014-2017)

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This study was presented as a poster at the '10th Balkan Congress of Microbiology / Microbiologia Balkanica '2017'. November 16th – 18th, 2017, Park Hotel Moskva, Sofia, Bulgaria

ABSTRACT

Dermatophytosis is an infectious and zoonotic disease caused by species belonging to the genera *Microsporum, Trichophyton, and Epidermophyton* that affects the hair follicles, nails, and keratin layer of the epidermis. The aim of this study was to determine the prevalence of dermatophytosis in different animal species with clinical lesions. To this aim, a total of 395 skin scraping and hair samples taken from cat, dog, horse, parrot, and calf with dermatophytosis suspicion, presented to the Department of Microbiology of Faculty of Veterinary Medicine Ankara University between 2014 and 2017 in different seasons were investigated. A mycological analysis of the samples was conducted involving direct microscopy and a fungal culture test. Of the 395 samples tested, 117 (29.62%) were positive for dermatophytosis with the following distribution: *Microsporum* spp., 34 of 195 (17.44%) cats, 24 of 181 (13.26%) dogs, two of 11 (18.18%) horses; *Trichophyton* spp., 26 of 181 (14.36%) dogs, 25 of 195 (12.82%) cats, one of 11 (9.09%) horses, one of three (33.33%) calves, and two of five (40%) parrots; *Epidermophyton* spp. two of 195 (1.02%) cats. The dermatophyte isolation rate was relatively higher in the summer (36.06%) and spring (29.51%) for cats, and in fall (30%) and spring (26%) for dogs. As a conclusion, the data contribute to the literature regarding the local epidemiology of dermatophytosis and define potential etiological agents in different animal species, especially cats and dogs.

INTRODUCTION

Dermatophytosis (ringworm) is the superficial infection of such keratinized tissue as the nails/claws, hair and stratum corneum caused by the *Microsporum, Trichophyton,* and *Epidermophyton* genera of fungi (1-4). Affecting several mammalian species, including humans and poultry, dermatophytes are classified as anthropophilic (mostly human-associated), zoophilic (animal-associated) and geophilic (soil-dwelling) based on their natural habitat and host preferences (2,3,5). Mostly zoophilic and geophilic species, and more rarely anthropophilic species, have been reported to cause infections in animals (1,5-7). Dermatophytes are known to be among the most common causes of dermatological problems in domesticated animals, and have also been reported to cause serious infections especially in the immunocompromised (AIDS, organ transplantation, diabetes mellitus, etc.) in many countries around the world (4,8-10). Dermatophytosis is considered a significant disease in veterinary medicine, due to its contagiousness and zoonotic potential, and in pet veterinary medicine in particular. Several mammals and poultry species are susceptible to infection, regardless of age, gender or breed, although it is inclined to occur more often in young, sick, and elderly patients. The incidence of dermatophytosis varies in terms of the natural host, climatic conditions, and geographical differences (1-3,6).

While there are more than 30 species that are known to cause dermatophytosis, it is believed that *Microsporum (M) canis*, *M. gypseum,* and *Trichophyton (T) mentagrophytes* are responsible for dermatophytosis in cats and dogs, *T. verrucosum* in cattle and other ruminants, *M. canis* and *T. equinum* in horses and *M. gallinae* in poultry (1,3,5,11). Transmission occurs primarily through direct contact with dermatophyte-infected animals or with contaminated fomites (via brushes, soil, etc). Germination occurs through the adherence of dermatophyte arthrospores to the cells of the stratum corneum, producing hyphae that then invade the stratum corneum through keratinases. Invasion induces an immune response, and the typical clinical presentation emerges within 1–3 weeks of exposure to the agent (5,10,12). Clinical presentations that may indicate dermatophytosis include such symptoms as

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alopecia, erythema, papules, scaling, and crusting, either individually or in combination (2,3,13). The clinical course and clinical signs of dermatophytosis contribute substantially to laboratory diagnosis, which is established through the direct microscopy of suspected samples and the subsequent isolation and identification of dermatophytes in cultures (9,13).

This study aimed to examine the samples of different animal species sent to the Department of Microbiology of Faculty of Veterinary Medicine Ankara University with suspected dermatophytosis between 2014 - 2017 and to determine the seasonal isolation rates.

MATERIAL and METHODS

A total of 395 skin scrapings and hair samples from 195 cats, 181 dogs, 11 horses, five parrots, and three calves that presented to the Department of Microbiology of Faculty of Veterinary Medicine Ankara University with suspected dermatophytosis in various seasons were investigated between 2014 and 2017.

Direct microscopic examination

The suspected skin scraping and hair samples were mixed with 10% potassium hydroxide (KOH) (Merck, Germany) in preparation for analysis. After being kept at room temperature for 15–20 minutes, the preparations were examined by lightmicroscope at 40x magnification to screen for hyphae and dermatophyte spores (9,14).

Mycological isolation

The suspected dermatophyte suspected samples were cultured in a Sabouraoud Dextrose Agar (SDA) medium (Oxoid, UK) supplemented with chloramphenicol (Oxoid, UK) (0.05 mg/ml), after being embedding using a sterile pen or lancet. The media was incubated under aerobic conditions and at 25°C for 1–4 weeks, and checked on a daily basis (9,14).

Macroscopic and microscopic examination of fungal colonies

The macroscopic examination was completed upon the evaluation of growing status and the time, shape, and pigmentation characteristics of the front and rear surfaces of the colonies, within and at the end of the incubation period. A microscopic examination was made to identify dermatophyte species in the hyphae, macroconidia, and microconidia on preparations made through the cellophane band method using a lactophenol cotton blue solution (Merck, Germany) (13,14).

Statistical analysis

The chi-square (x^2) test was used to examine the statistical significance of distribution of dermatophyte species and dermatophytosis prevalence in the population analyzed, distribution of dermatophyte species according to animal species, and dermatophytosis prevalence by seasons. $p < 0.001$ was considered statistically significant. The IBM SPSS Statistics V21.0 software package for Windows was used for the statistical analyses.

RESULTS

Of the 395 examined samples obtained from the various animal species, 117 (29.62%) were positive for dermatophytosis, and 60 (51.29%) isolates were identified as *Microsporum* spp., 55 (47%) as *Trichophyton* spp., and two (1.71%) as *Epidermophyton* spp., being the difference significant [x^2 (df=3, n=395)=454,752 ($p < 0.001$)] (Table 1). The distribution isolated dermatophytes and animal species was presented in Table 2. No statistically significant difference ($p < 0.001$) was detected among the animal species in terms of the identified dermatophyte species. The seasonal distribution of dermatophytosis indicated a proportionate increase in the summer months in cats (36.06%) and calves (100%), and in fall in dogs (30%) and horses (66.67%), while it was equally distributed across the fall and winter months for parrots (Table 3). No statistically significant association ($p < 0.001$) was found in the seasonal distribution of animal dermatophytoses.

Table 1. Distribution of results of samples examined according to animal specie.

Animal species	Materials (%)	Positive (%)
Cat	195 (49.37)	61 (31.28)
Dog	181 (45.82)	50 (27.62)
Horse	11 (2.79)	3 (27.27)
Parrot	5 (1.26)	2 (40)
Calf	3 (0.76)	1 (33.33)
Total	395 (100)	117 (29.62)

Table 2. Number and frequency of dermatophytes isolated from different animal species.

Dermatophytes	Cat	Dog	Horse	Parrot	Calf	Total
	n (%)					
Microsporum spp.	34 (55.74)	24 (48)	2 (66.67)	-	-	60 (51.29)
Trichophyton spp.	25 (40.98)	26 (52)	1 (33.33)	2 (100)	1 (100)	55 (47)
Epidermophyton spp.	2 (3.28)	-	-	-	-	2 (1.71)
Total	61 (52.14)	50 (42.74)	3 (2.56)	2 (1.71)	1 (0.85)	117 (100)

Table 3. Seasonal distribution of dermatophytes isolated from different animal species.

Seasons	Cat	Dog	Horse	Parrot	Calf	Total
	n (%)					
Winter	8 (13.11)	10 (20)	-	1 (50)	-	19 (16.24)
Spring	18 (29.51)	13 (26)	1 (33.33)	-	-	32 (27.35)
Summer	22 (36.06)	12 (24)	-	-	1 (100)	35 (29.91)
Fall	13 (21.31)	15 (30)	2 (66.67)	1 (50)	-	31 (26.49)

DISCUSSION

Dermatophytes are known to be among the most common causes of dermatological problems in domesticated animals and dermatophytosis are common in many countries around the world. Several studies are available on dermatophytosis in various animal species as varying. In a study in Finland, Aho (1980) reported a positivity rate of 10.9% for dermatophytes in 331 samples collected from various animals (3.9% of dogs, 21.3% of cats, 14.8% of cows, and no cage birds) with suspected dermatophytosis (15). Cabanes et al. (1997) reported a positivity rate of 33.7% for dermatophytes in 270 samples (38.9% of dogs, 33.9% of cats, 23.1% of horses, 25% of cows, and no parrots) in Spain (16). The several researchers indicated that 31.4% of 790 and 35.7% of 487 samples to be positive for dermatophytosis (55% of feline, 8% of canine, 38% of bovine, 19% of equine and 21.6% of canine, 43.5% of feline, 100% of bovine, 40% of equine samples, repectively) in Iran (17, 18). In Nigeria, Nweze (2011) reported that 39.8% of 538 samples were positive for dermatophytosis, with an animal origin-based rate of dermatophyte positivity of 22% in cats, 24.3% in dogs, 12.6% in cows, and 5.1% in horses (7).

The isolation of *Microsporum* spp. and *Trichophyton* spp. in various animal species (cat, dog, horse, and cow) ranges between 71-90 % and 52-74 %, respectively (7, 17-19). Cabanes et al. (1997) reported that *Microsporum* spp. and *Trichophyton* spp. isolation rates were 48.27% and 20.41% in same animal species (16). A comparison of the isolation rates established in the present study with those reported by the abovementioned studies reveals higher rates than those reported by Cabanes et al. (1997) and lower rates than those reported by Khosravi and Mahmoudi (2003), Yahyaraeyat et al. (2009), Nweze (2011), and İlhan (2015) (7, 17-19). In addition, the study findings were determined to be similar to the study findings (50% for *Microsporum* spp. and 41.67% for *Trichophyton* spp.) reported by Aho (1980) (15). It was considered that the differences in dermatophyte isolation rate might have resulted from the variability in animal breeds, and the quantities of samples collected.

It is reported that more than 95% of cases of dermatophytosis in cats are caused by *Microsporum* and *Trichophyton* species, and in particular, *M. canis*. *Epidermophyton* species among the anthropophilic dermatophytes have been reported to cause infections in animals on rare occasions (1,4,6). In the study, 55.74% of the 61 (31.28%) dermatophyte agents isolated from 195 suspected cat samples were identified as *Microsporum* spp. and 40.98% as *Trichophyton* spp. When compared with studies conducted in different regions of Turkey and the other countries, these rates are lower for *Microsporum* spp. and higher for *Trichophyton* spp. (19-29). Furthermore, 3.28% of the dermatophyte isolates were identified as *Epidermophyton* spp. in present study. The isolation of *Epidermophyton* spp. from animals reflected the human flora rather than the infection, while there are rare cases indicating infections in immunocompromised dogs (2). In our knowledge there was no reports of *Epidermophyton* spp. in animals in Turkey.

As with the case for cats, reports indicate that the causative agent of dermatophytosis in dogs are such *Microsporum and Trichophyton* species as *T. mentagrophytes* and *M. gypseum,* and in particular, *M. canis* (1,2,4). In the study, 48% of the 50 dermatophyte agents (27.62%) isolated from 181 dermatophytosis suspected dog samples were identified as *Microsporum* spp. and 52% as *Trichophyton* spp. Unlike cats, the rate of *Microsporum* spp. isolates in dogs was found to be lower than the rate of *Trichophyton* spp. isolates in the present study. *Trichophyton* spp. is known to be dominant in the back of dogs (27). It was considered that high *Trichophyton* spp. isolation in dogs may be related to the sampling area in this study. It has been ascertained from the studies carried out in several different parts of Turkey and around the world that species of the genus *Microsporum* account for the majority of dermatophytes isolated from dogs (21,24,26,28-32). In this regard, the findings of the present study differ from those of the aforementioned studies, while our study findings share similarities with those reported by Derincegöz and Parın (2016) in Turkey (*Trichophyton* spp. in 57.14% and *Microsporum* spp. in 42.86% of the isolates) and by Beraoldo et al. (2011) in Brazil (*Trichophyton* spp.in 57.89% and *Microsporum* spp. in 42.11%) (23,27).

It is reported that dermatophytosis in horses is caused by *Trichophyton* and *Microsporum* species, and in particular, such as *T. equinum, M. canis, T. mentagrophytes,* and *T. verrucosum* (3,4,11). It has been indicated that the isolation of *Trichophyton* spp. and *Microsporum* spp. in suspected horse samples ranges between 50-100% and 25-50%, respectively (7, 15, 16, 33-36). The isolation rate of *Microsporum* spp. in the present study was higher and the isolation rate of *Trichophyton* spp. was lower than those reported by the mentioned studies, but similar to those reported in the study by Khosravi and Mahmoudi (2003) conducted in Iran involving 79 horses (33.33% for *Trichophyton* spp., 66.67% for *Microsporum* spp.) (17). We believe that this difference might be a result of limited number of horse-origin

samples assessed.

It has been indicated that dermatophytosis in ruminants is caused by *Trichophyton* and *Microsporum* species, such as *T. mentagrophytes, T. equinum, M. canis,* and *M. gypseum,* and in particular *T. verrucosum* (1,2,4). In many studies, the researchers reported that that all of the dermatophytes isolated from calf- and cow-origin samples were species belonging to the genus *Trichophyton,* and in particular, *T. verrucosum* (15,17,19,37-41). In addition, Neweze (2011) reported that *Trichophyton* spp. and *Microsporum* spp. isolation rates were 59.26% and 40.74% in 55 samples collected from cows in a study in Nigeria (7). It was used a small number of samples calf in the study. However, it can be considered that the study findings (100% for *Trichophyton* spp.) similar to the mentioned study findings in terms of the *Trichophyton* spp. isolation.

Literature indicates that dermatophytosis is rare in poultry, and is usually reported only as sporadic cases. It is thought that *M. gallinae* is responsible for dermatophytosis in poultry, in additional, *T. simii, T. mentagrophytes* and *T. terrestre* have also been reported to cause infection (1,2). *Trichophyon* spp. from two (40%) of the five parrot-origin samples were isolated in present study. The dermatophytosis studies in domesticated or wild birds are limited. The study by Cabanes et al. (1997), conducted with different animal species in Spain, reported that no dermatophyte agent was isolated from the samples collected from a parrot, while Gungnani et al. (2012) from Saint Kitts and Nevis reported isolating *M. gypseum* from samples collected from brown doves, pigeons, and ducks, but no isolation of dermatophyte agents from parrot-origin samples (16,42). Furthermore, the study by Mandeel et al. (2009), conducted with clinically healthy birds in Bahrain, reported the collection of two *T. terrestre* isolates from five Alexandrian parrot samples (43). Alteraş and Cojoca (1970) reported the isolation and identification of *M. canis* from a parakeet and the owner of the bird in Romania (44). The fact that dermatophytes were isolated from parrot samples in this study and that these isolates belong to the *Trichophyton* species are similar to be the findings of Mandeel et al. (2009) (43). In addition, it is considered to the findings provide a significant contribution to literature given the limited number of studies in this field to date.

The prevalence of dermatophytosis in cats and dogs is known to vary by location and season due to differences in climatic conditions (26,29). There have been several reports in this regard in many countries around the world and different parts of Turkey (16,17,21,22,24,26,29,31). Although the seasonal distribution did not indicate a significant association with dermatophytosis, the present study established an increased rate of feline dermatophytosis in summer (36.06%) and spring (29.51%), and canine dermatophytosis in fall (30%) and spring (26%), differing from the study by Çiftçi et al. (2005) that was carried out in the same region, but in a different year (22).

CONCLUSION

In the present study, it was determinated that *Microsporum* spp. was the most common species isolated from the samples belong to different animal species and the isolation rates of dermatophytes may differ according to the season and animal species. It was considered that *Epidermophyton* spp. isolation from cats is valuable finding in terms of transmission of dermatophytes to animal from human. There have been a number of studies of feline and canine dermatophytoses in Turkey, but few reports about the other animal species as poultry etc. Based on the results, it is suggested that the identification of other animal species that are prone to dermatophytosis infection, and that are in close contact with humans, is important for both animal and human health. Furthermore, laboratory diagnoses should be obtained in cases of suspected dermatophytosis, and it would be beneficial to raise awareness among animal owners and animal keepers of this issue due to the potential for zoonotichumans, is important for both animal and human health. Furthermore, laboratory diagnoses should be obtained in cases of suspected dermatophytosis, and it would be beneficial to raise awareness among animal owners and animal keepers of this issue due to the potential for zoonotic transmission.

DECLARATIONS

Ethics Approval

Not applicable.

Conflict of Interest

The authors declare that they have no competing interests.

Author Contribution

Idea, concept and design: N Karacan-Sever,

Data collection and analysis: N Karacan-Sever, T Üstün, M Omerovic, M Önol, AK Zahiri, B Doğan

Drafting of the manuscript: N Karacan-Sever

Critical review: N Karacan-Sever, T Üstün, M Omerovic, M Önol, AK Zahiri, B Doğan

Data Availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Research Article

Sagittal anatomic investigation of the rabbit liver

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ABSTRACT

The aim of the research was to study the topography of the liver and to image on computed tomography of the white New Zealand rabbit. We used ten rabbit cadavers. We obtained sagittal frozen cuts. At the level of the plane 10 mm to the left, the left medial lobe was cranial to the left lateral lobe. Caudally were the spleen, the left kidney and parts of the small and large intestines. At the level of the plane 20 mm to the left, the left lateral lobe touched caudally the stomach fundus and body, the papillary process was dorsal to the stomach fundus. At the level of the plane 10 mm to the right, the right lobe was cranially situated to the other lobes. Between the right lobe and caudate lobe were fundus and body of the stomach. Caudate process was caudal to the fundus of the stomach and dorsal to the cranial part of duodenum and ascending colon. It had anatomical contact with the right kidney. Papillary process covered the dorsal part of the stomach. At the level of the plane 20 mm to the right, the right lobe was cranial to the other lobes of the liver. The left medial lobe was covered partially by quadrate lobe. Gall bladder did not reach the ventral border of the liver. The left medial lobe was cranial to the body of the stomach. Caudate lobe touched the muscles of the spine.

INTRODUCTION

New Zealand rabbits are used to determine topographical features of the rabbit liver and other organs (1-5). Computed tomography (CT) methods are widely used in non-clinical basic research to study anatomical objects that are individually specific (6). Some authors introduce CT as innovative method for describing the abdominal anatomy in small animals. The used algorithm for determine the topography of the abdominal organs is for transverse CT (7) The sagittal CT is appropriate for visualization of the anatomical peculiarties of the human abdominal organs. Even more it gives detailed information via graphic displaying (8).

Precontrast computed tomography of the abdominal organs is an appropriate method for obtaining detailed anatomical information about the organs and vessels of small domestic mammals (9).

The anatomical representation of the liver in the domestic rabbit by means of CT corresponds to the data concerning the topography of the examined organ. CT is a comprehensive method for interpreting the anatomical features of the liver (lobes, topography and boundaries) in the rabbit in modern aspect (10).

In previous studies, Stamatova-Yovcheva et al. (11) and Stamatova-Yovcheva et al. (12) compared the anatomical features of the liver in the domestic rabbit on frozen transverse sections with the transverse CT visualization of the examined organ. The authors prove that the data obtained in the anatomical examination (topography, number of sections, location of *vesica fellea* and adjacencies with other abdominal organs) complement the CT anatomical features of the liver in this animal species Dimitrov et al. (13) conducted a comparative CT anatomical investigation in sagittal aspect of the prostate and bubourethral glands in the domestic rabbit. The obtained imaging anatomical results present in detail the morphological features of the studied glands - location, bone markers, marking their topography and as well the presence of a prostate complex specific for this species.

CT imaging material is preferred for visualization of the normal topography of the rabbit liver compared to black and white photographic images of native frozen transverse sections, due to their lower quality. Image anatomical soft tissue findings are interpreted according to variations in CT density and gray-white scale (10).

Schapiro and Chiu (14) investigated the possibilities of axial CT for the anatomical visualization of human epigastric organs. The differentiation of anatomical structures from pathologically altered ones is based on the difference in densities that are specific to each tissue. CT and anatomical features of the organs in the *epigastrium* are used as a morphological basis in the differentiation of normal tissues in this abdominal area.

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The disadvantage of the axial CT method is that it does not take into account the changes that occur in the resulting images during respiration. The difference between the axial and spiral CT method is that in the axial each tomographic slice is parallel to the other, while in the spiral, the images are spring-like (spiral) and each slice is at an angle to the other (15, 16).

It is evident that the scientific literature provides data mainly on the anatomical features of the liver in the rabbit in a transversal aspect. The information on the sagittal CT presentation of this organ is insufficient. These facts were motive to undertake this study. The aim of the research was to study the topography of the liver and to image on computed tomography of the white New Zealand rabbit.

MATERIAL and METHODS

Sagittal postmortem anatomical examination

We examined the cadavers of three male mature New Zealand rabbits which weights to 2.8 to 3 kilogram. Using an electric saw with a replaceable blade MAX RTR RTM908 (Turkey) we obtained sagittal frozen anatomical sections with a thickness of 10 mm. The first postmortem frozen anatomical sections were obtained at the level of the longitudinal plane, passed 10 mm parallel and laterally to the median plane in the left and right directions, and the last - at 40 mm. The findings observed in one native section were compared with those of three consecutive computed tomography scans.

Axial CT anatomical study

We studied 12 mature (6 male, 6 female) clinically healthy New Zealand rabbits which weight to 2.8 to 3.2 kilogram. An axial computed tomography Picker Marconi-USA, 1995, has been used. The table height was 395 mm, the field of view (FOV) - 180, filter - 1, anode force 125 mA, anode voltage 100 kV and time of scanning - 1.2 sec. The resolution was high - 512 and gentry (GT) -0 °. The window (W) was 399 and the center - 53. The experimental animals were positioned in a ventrodorsal position. Computed tomography sagittal investigation of the abdominal cavity was from 8 intercostal space to L7 at a slice thickness of 5 mm. The scan slices were consecutively chosen. The results were interpreted following the terms of NAV 2017 (17). CT parameters measured on sagittal sections were dorsoventral size (DV - the distance between the dorsal and ventral border of the liver) and craniocaudal size (CC - the distance between the most prominent part of *facies diaphragmatica* and the caudal contour of the liver border) (10, 18).

Statistical methods

The obtained values were measured in mm to the second decimal place. Data were statistically processed by Statistica 8 (19). Descriptive statistical analysis was applied at P = 95%.

RESULTS

The results from the postmortem anatomical study on sagittal sections obtained at the level of the sagittal plane, passed 10 mm parallel to the median and to the left, showed the intrathoracic anatomical location of the liver in the domestic rabbit. *Lobus hepatis sinister medialis* was cranially to *lobus hepatis sinister lateralis* and covered it on *facies diaphragmatica*. *Proc. papillaris* was observed as an oval structure, caudodorsal to the left lobes. The ventral margin of the organ touched the soft abdominal wall. The spleen, left kidney, segments of the small and large intestines were found caudally from the organ, and at this level they did not have direct anatomical contact with the liver (Figure 1).

On the sagittal *postmortem* anatomical study on frozen sections obtained at the level of the longitudinal plane passed 20 mm laterally from the median plane and on the left, the anatomical

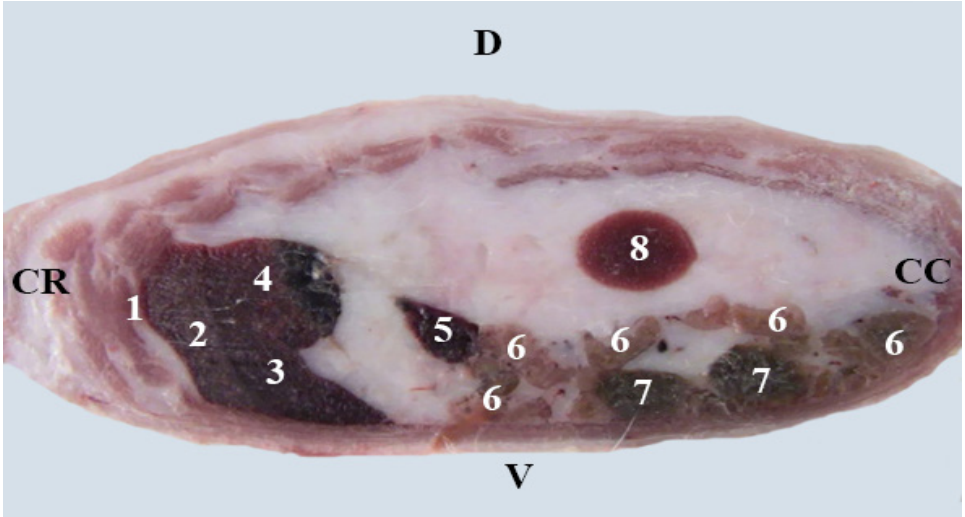

Figure 1. Sagittal postmortem anatomical section of cavum abdominis in a rabbit, at the level of the longitudinal plane passed 10 mm parallel to the median plane and to the left (D-dorsal; V-ventral). (1) diaphragm, (2) lobus hepatis sinister medialis; (3) lobus hepatis sinister lateralis; (4) proc. papillaris; (5) lien; (6) jejunum; (7) caecum, (8) ren sinister.

borders of the liver in the domestic rabbit with the adjacent abdominal organs from the left half of the abdominal cavity were outlined. *Lobus hepatis sinister lateralis* was located caudally from the diaphragm and it covered *lobus hepatis sinister lateralis* at *facies diaphragmatica*. The lateral left lobe touched caudally to *fundus et corpus ventriculi*, and *proc. papillaris* was located caudally to the most dorsal parts of the stomach (Figure 2).

The postmortem anatomical study on sagittal sections from *regio abdominis* obtained at the level of the longitudinal plane passed 10 mm parallel and to the right of the median plane showed that *lobus hepatis dexter* was cranially located relative to the remaining parts of the liver. The bottom and body of the stomach remained located between *lobus hepatis dexter* and *lobus caudatus*. *Lobus hepatis dexter* touched the parietal surface of the stomach. *Proc. caudatus* was found caudally relative to *fundus ventric*uli and dorsally from *pars cranialis duodeni* and segments of *colon ascendens*. Caudally, it touched the right kidney. *Proc. papillaris* covered the dorsal parts of the stomach and touched dorsally the muscles of the spine (Figure 3).

On the sagittal *postmortem* anatomical study on frozen sections obtained at the level of the longitudinal plane passed 20 mm parallel and to the right of the median plane, it was found that *lobus hepatis dexter* was observed as a well-defined anatomical structure cranially located relative to the other sections. *Lobus hepatis sinister medialis* was partially covered by *lobus quadratus*. The latter was found medially to the gallbladder as a poorly defined anatomical structure. *Vesica fellea* was round in shape and did not reach the ventral edge of the liver. *Corpus ventriculi* was topographed caudally to *lobus hepatis sinister medialis*. *Lobus caudatus* was in direct contact with the muscles of the spine, as the longitudinal section of *v. cava caudalis* was found just below the muscles of the spine and on the visceral surface of the dorsal right segments of the liver (Figure 4).

The results of the sagittal anatomical CT examination of *regio abdominis*, at the level of the longitudinal plane, passing 10 mm parallel and to the right of the median plane, demonstrated the peculiarities of the localization of the liver in *regio abdominis cranialis* and its adjacent soft tissue structures defining their shade according to the gray-white scale. *Lobus hepatis dexter*

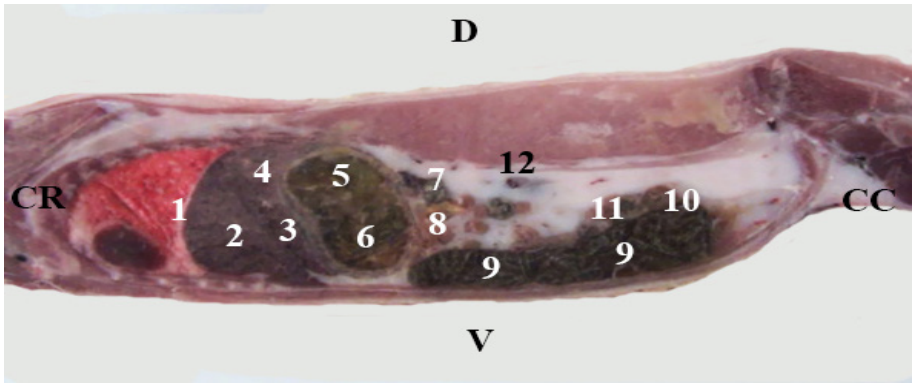

Figure 2. Sagittal postmortem anatomical section of the cavum abdominis at the level of the longitudinal plane passed 20 mm parallel and to the left of the median plane (D-dorsal; V-ventral). (1) diaphragm; (2) lobus hepatis sinister medialis; (3) lobus hepatis sinister lateralis; (4) proc. papillaris; (5) fundus ventriculi; (6) corpus ventriculi; (7) lien; (8) jejunum; (9) caecum; (10) duodenum; (11) ascending colon; (12) ren sinister.

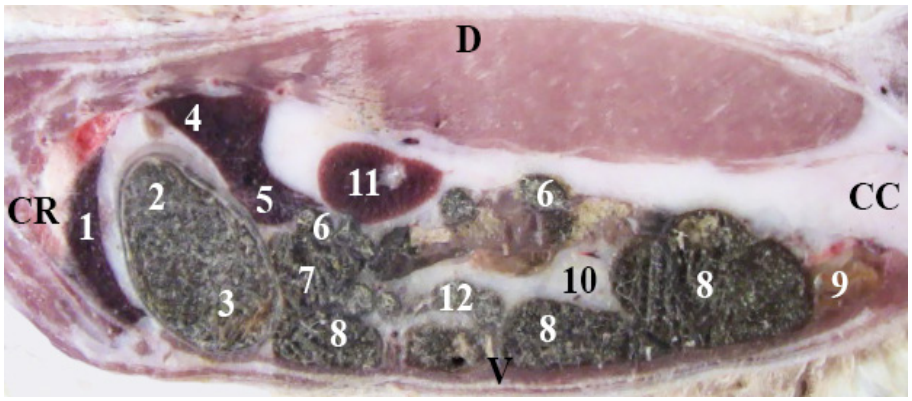

Figure 3. Sagittal postmortem anatomical section of cavum abdominis at the level of the longitudinal plane passed 10 mm parallel and to the right of the median plane (D-dorsal; V-ventral). (1) lobus hepatis dexter; (2) fundus ventriculi; (3) corpus ventriculi; (4) proc. papillaris; (5) proc. caudatus; (6) pars cranialis duodeni; (7) haustral part of colon ascendens; (8) caecum; (9) urinary bladder; (10) adipose tissue; (11) ren dexter; (12) ileum.

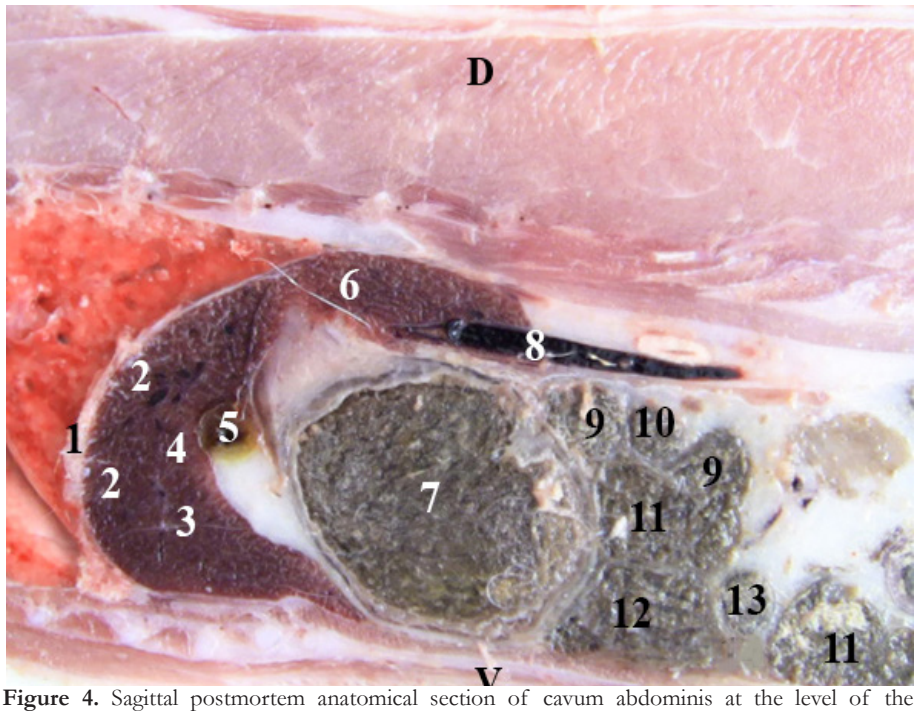

Figure 4. Sagittal postmortem anatomical section of cavum abdominis at the level of the longitudinal plane passed 20 mm parallel and to the right of the median plane (D-dorsal; V-ventral). (1) diaphragm; (2) lobus hepatis dexter; (3) lobus hepatis sinister medialis; (4) lobus quadratus; (5) vesica fellea; (6) lobus caudatus; (7) corpus ventriculi; (8) v. cava caudalis; (9) duodenum; (10) ileum; (11) caecum; (12) ascending colon; (13) jejunum.

was located cranially relative to the rest of the liver and was visualized as a single clearly defined norm attenuated structure, sharply distinguishable from *lobus hepatis sinister medialis* and *lobus caudatus*. There was no clear line between the *lobus hepatis sinister medialis* and *lobus hepatis dexter*. The hypo attenuated intraperitoneally distinct soft tissue image of *fundus ventriculi* was found between *lobus hepatis dexter* and *lobus caudatus*, with the relatively hyper dense liver borders defining its topography. *Proc caudatus* showed intermediate density relative to the surrounding soft tissue findings and was found caudodorsally to *fundus ventriculi* and dorsally from *pars cranialis duodeni* and the segments of *caecum*. At this sagittal scan level, CT anatomical contact between *proc. caudatus* and right kidney were visualized. The norm dense anatomical image of *proc. papillaris* covered the dorsal hypo dense parts of the stomach and was opposite to *proc. caudatus* (Figure 5).

On the sagittal anatomical CT study of *regio abdominis* at the level of the longitudinal plane passed 20 mm parallel and to the right of the median plane, *lobus hepatis dexter* was observed as a clearly defined CT norm attenuated anatomical structure caudally situated to the relative hypo dense diaphragm. *Lobus hepatis dexter* covered *lobus hepatis sinister medialis* at *facies diaphragmatica*. A soft tissue anatomical marker for their

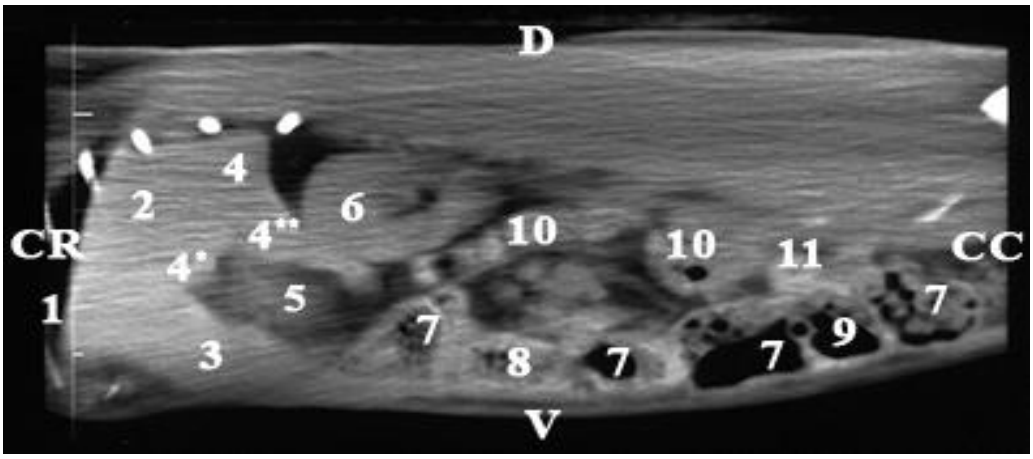

Figure 5. Sagittal anatomical CT section of cavum abdominis at the level of the longitudinal plane passed 10 mm parallel and to the right of the median plane. CR - cranial, CC - caudal, D - dorsal, V - ventral. (1) diaphragm; (2) lobus hepatis dexter; (3) lobus hepatis sinister medialis; (4) lobus caudatus; (4 *) proc. papillaris; (4 **) proc. caudatus; (5) fundus ventriculi with peritoneum; (6) ren dexter; (7) caecum; (8) ileum; (9) ascending colon; (10) duodenum; (11) jejunum.

distinction was a longitudinal hypo dense linear band. The norm attenuated CT image of *lobus caudatus* partially overlapped with that of *lobus hepatis dexter* and was found caudally as a single structure, without the presence of separation. *Lobus caudatus* was located dorsally relative to the rest of the liver and showed close density to that of the muscles around the spine. The cranial pole of the relatively hypo dense right kidney partially touched and overlapped the norm attenuated *lobus caudatus*, and pars *cranialis duodeni* and segments of the caecum were observed caudally from the visceral surface of *lobus hepatis sinister medialis*. A soft tissue marker for defining their CT anatomical images was the relatively hyper dense outlines of the liver margins (Figure 6).

On sagittal CT anatomical study of *regio abdominis*, at the level of the longitudinal plane passed 30 mm parallel and to the right of the median plane, it was found that *lobus hepatis dexter* was located cranially to *lobus hepatis sinister medialis*. Dorsally to the left and right lobes of the liver was the norm dense anatomical image of *lobus caudatus*. The soft-tissue peripheral outlines of *proc. caudatus* were hyper attenuated compared to the norm dense right kidney. Ventrocaudally to the ventral border of *lobus hepatis dexter* were visualized the left parts of the stomach (*fundus et corpus ventriculi*), which in accordance to the gray-white scale showed intermediate density close to that of the liver (Figure 7).

On sagittal CT anatomical study of *regio abdominis* at the level of the longitudinal plane passed 10 mm parallel and to the left of the median plane, *lobus hepatis sinister medialis* was visualized as a clearly defined CT norm attenuated soft tissue anatomical finding. *Lobus hepatis sinister medialis* covered *lobus hepatis sinister lateralis* on *facies diaphragmatica*. The norm attenuated CT image of *proc. papillaris* and *lobus caudatus* were found dorsally from the left lobes of the liver. *Proc papillaris* showed intermediate density to the surrounding soft tissue findings and was found caudorsally to *fundus ventriculi*. The relatively hyper dense outlines of the liver borders and the hypo dense mesentery were used as a soft tissue marker to define the CT anatomical image of the liver. (Figure 8). In the sagittal anatomical CT

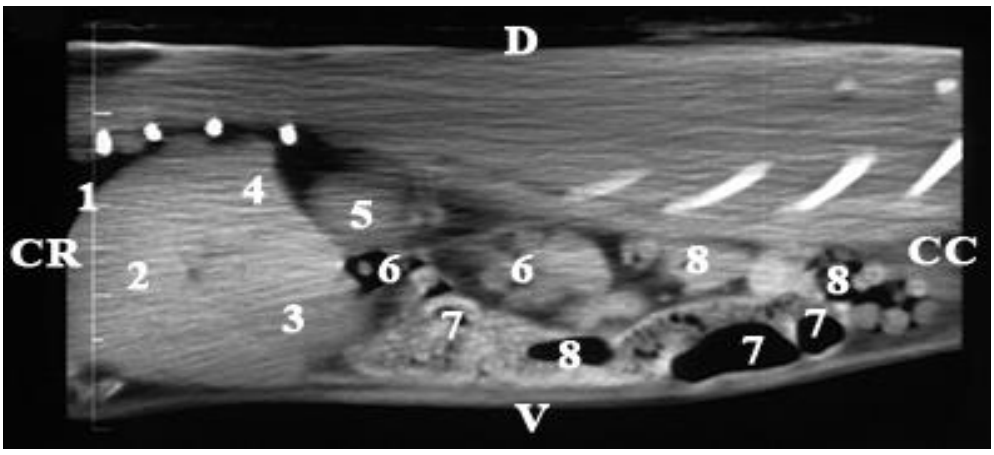

Figure 6. Sagittal anatomical CT section of the cavum abdominis at the level of the longitudinal plane passed 20 mm parallel and to the right of the median plane. CR - cranial, CC - caudal, D - dorsal, V - ventral. (1) diaphragm; (2) lobus hepatis dexter; (3) lobus hepatis sinister medialis; (4) lobus caudatus; (5) ren dexter; (6) duodenum; (7) caecum; (8) jejunum.

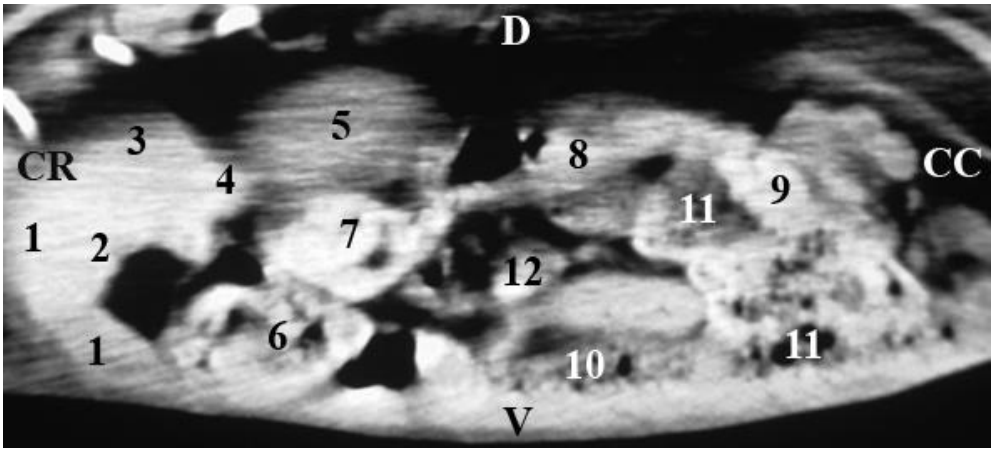

Figure 7. Sagittal anatomical CT section of cavum abdominis at the level of the longitudinal plane passed 30 mm parallel and to the right of the median plane. CR - cranial, CC - caudal, D - dorsal, V - ventral. (1) lobus hepatis dexter; (2) lobus hepatis sinister medialis; (3) lobus caudatus; (4); proc. caudatus; (5) ren dexter; (6) corpus ventriculi and pars pylorica; (7) pars cranialis duodeni; (8) pars descendens duodeni; (9) pars transversa duodeni; (10) ascending colon; (11) caecum; (12) jejunum

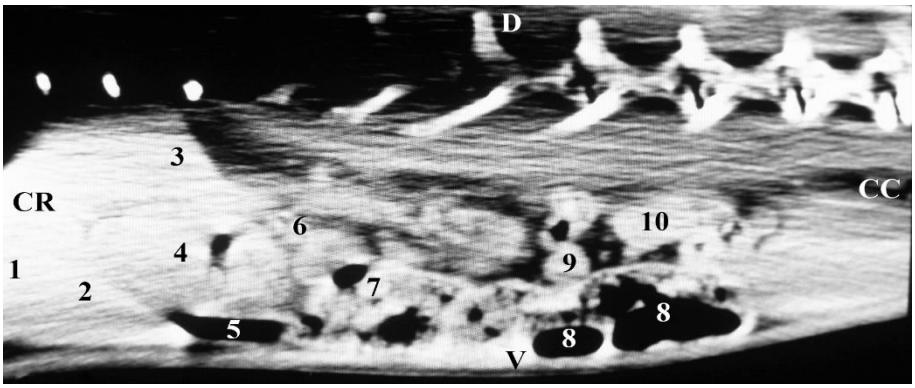

Figure 8. Sagittal anatomical CT section of cavum abdominis at the level of the longitudinal plane passed 10 mm parallel and to the left of the median plane. CR - cranial, CC - caudal, D - dorsal, V - ventral. (1) lobus hepatis sinister medialis; (2) lobus hepatis sinister lateralis; (3) lobus caudatus (proc. papillaris); (4) fundus et corpus ventriculi; (5) mesentery; (6) lien; (7) ansae jejunales; (8) caecum; (9) colon ascendens (non-haustral part); (10) duodenum

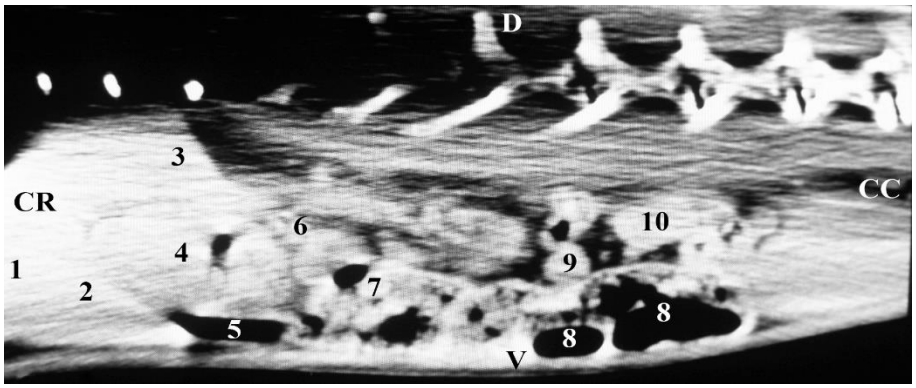

Figure 9. Sagittal anatomical CT section of cavum abdominis at the level of the longitudinal plane passed 20 mm parallel and to the left of the median plane. CR - cranial, CC - caudal, D - dorsal, V - ventral. (1) lobus hepatis sinister medialis; (2) lobus hepatis sinister lateralis; (3) lobus caudatus (proc. papillaris); (4) fundus et corpus ventriculi; (5) lobus caudatus; (6) ren sinister; (7) colon ascendens (haustral part); (8) caecum; (9) jejunum; (10) colon ascendens (non-haustral part); (11) duodenum.

study of *regio abdominis*, at the level of the longitudinal plane passed 20 mm parallel and to the left of the median plane, the CT anatomical boundaries of the liver toward the adjacent abdominal organs of the left abdominal half were defined. The norm dense anatomical image of *lobus hepatis sinister medialis* covered *lobus hepatis sinister lateralis* on *facies diaphragmatica*. *Lobus caudatus* was found dorsally to the left lobes of the liver, as *proc. papillaris* touched *fundus et corpus ventriculi* and was visualized as a relatively hyper dense structure relative to the hypo dense borders of the stomach. The relatively hyper dense outlines of the liver's borders and the hypo dense mesentery were used as a soft tissue marker to define the computed tomographic anatomical image of the liver at this level (Figure 9).

On sagittal anatomical CT study of *regio abdominis*, at the level of the longitudinal plane passed 30 mm in parallel and to the left of the median plane, the whole anatomical image of *lobus hepatis sinister medialis* was visualized. The left medial part of the liver was cranially to *lobus hepatis sinister lateralis*, partially covering it. *Lobus hepatis sinister lateralis* and *lobus caudatus* touched the left parts of the stomach (*fundus et corpus ventriculi*) (Figure 10).

The average computed tomography metric values of the rabbit liver, measured in the field of visualization of the organ on sagittal sections, were presented graphically. The results presented the average values of the test metrics. The diagram was confirmative (Figure 11).

DISCUSSION

The imaging anatomical data present in detail the anatomical position of the examined organ in different sagittal planes. In addition, we interpret the lobes of the organ according to the variations on the gray-white scale, but at the same time we find the topography and the adjacency of the organ with other abdominal organs (diaphragm, *fundus et corpus ventriculi*, *pars pylorica*, *duodenum*, *ren dexter* and segments of the caecum). These facts give us reason to propose axial CT as an appropriate anatomical method for the visualization of the liver in domestic rabbits, similar to the findings of some authors for the applicability of CT (6).

We apply a sagittal CT scan of the liver in the domestic rabbit. This approach is suitable to obtain objective anatomical data concerning the topography of the organ. Our algorithm differs to that of the transverse CT study to investigate the topography of the abdominal organs in small mammals (7)

The sagittal CT study is a sufficiently comprehensive

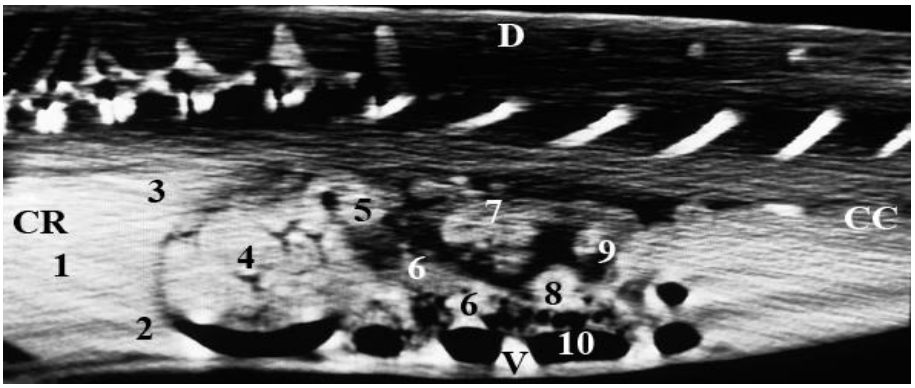

Figure 10. Sagittal anatomical CT scan of cavum abdominis at the level of the longitudinal plane passed 30 mm parallel and to the left of the median plane. CR - cranial, CC - caudal, D - dorsal, V - ventral. (1) lobus hepatis sinister medialis; (2) lobus hepatis sinister lateralis; (3) lobus caudatus (proc. papillaris); (4) fundus et corpus ventriculi; (5) lien; (6) jejunum; (7) ren sinister; (8) ascending colon; (9) duodenum; (10) caecum.

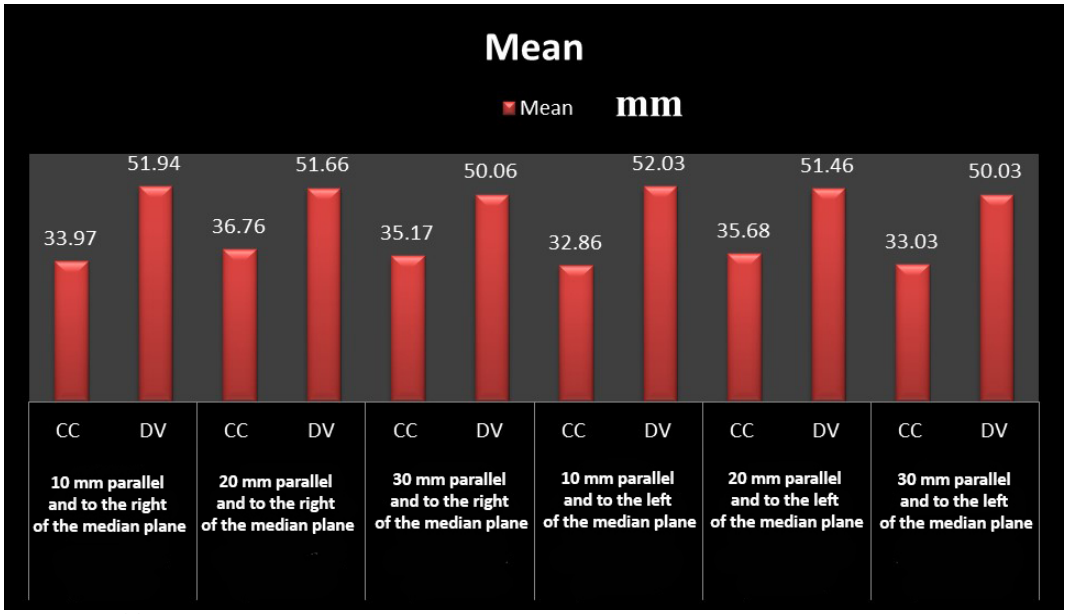

Figure 11. CT metric values (mm) of the liver in a rabbit, measured in the field of visualization of the organ on sagittal sections. (SS-craniocaudal size; DV-dorsoventral size)

method for investigation either the anatomical features of the liver (presence of five separate sections - *lobus hepatis dexter*, *lobus hepatis sinister medialis*, *lobus hepatis sinister lateralis*, *lobus caudatus*), adjacent to other abdominal organs, either to achieve reliable metric results (the cranial caudal and dorsal ventral size of the liver). Our thesis on the precision of the sagittal CT as an anatomical method complements the findings of some authors in humans (8).

Our imaging results provide information on the anatomical location of the liver in the rabbit in four sagittal planes (10 mm, 20 mm, 30 mm and 40 mm to the left and right to the median plane). In addition, these CT planes correspond to the applied anatomical planes in the *postmortem* examination. This thesis complements the theory of some authors about the accuracy of axial CT as an anatomical method (9, 10, 13).

Our data correspond to the opinion of Schapiro and Chiu (14) regarding the application and capabilities of axial CT for the anatomical visualization of soft tissue structures of *regio abdominis cranialis* in humans. The interpretation of CT anatomical images of the liver in the rabbit, according to the gray-white scale, is related to the degree of X-ray absorption. We find the anatomical CT imaging of the liver.

CONCLUSION

We resume that the axial CT of the liver in the rabbit gives detailed data on the topography and anatomical features of the organ. We present information for *lobus hepatis dexter* (a single clearly defined norm attenuated find, sharply distinguishable from *lobus hepatis sinister medialis* and *lobus caudatus*). Our data demonstrated the anatomical contact between the liver and other organs (diaphragm, *fundus et corpus ventriculi*, *pars pylorica*, *duodenum*, *ren dexter* and segments of the caecum).

DECLARATIONS

Ethics Approval

This study was approved by animal ethics committee of the Trakai University (No: 51/2012, No:59/2013), Bulgaria.

Conflict of Interest

The authors declare that they have no competing interests.

Author Contribution

Idea, concept and design: K Stamatova-Yovcheva, R Dimitrov

Data collection and analysis: K Stamatova-Yovcheva, R Dimitrov, ÖG Dilek, D Yovchev

Drafting of the manuscript: K Stamatova-Yovcheva

Critical review: R Dimitrov, ÖG Dilek, D Yovchev

Data Availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Araştırma Makalesi

Burdur yöresinde bir sağmal süt sığırı (simental) işletmesinde gözlenen ayak hastalıklarının incelenmesi

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ÖZ

Çalışmada Burdur ilinin Kemer ilçesinde sağmal bir işletmede bulunan Simental ırkı sığırların ayak hastalıklarının belirlenmesi ve lezyonlu ayakların tedavi edilmesi, koruyucu önlemlerin alınması ve efektif sürü sağlığının sağlanması amaçlanmıştır. Araştırma materyali olarak rutin tırnak kesimi yapılan 281 adet, farklı yaş ve ağırlıktaki Simental ırkı sığırlar kullanıldı. İncelenen olgularda 7 hayvanda sadece tırnak deformitesi, 104 vakada ayak hastalığı görülürken 165 olguda da tırnak deformitesi ile birlikte ayak hastalığı saptandı. 221 adet deformasyon tespit edildi; olguların 78'i yayvan ve geniş tırnak, 65'i düzenli uzamış tırnak, 49'u tirbuşon tırnak, 16'sı makasvari tırnak, 12'si küt tırnak, 1'i gaga tırnak olarak saptandı. Ayak hastalığı gözlenen 269 sığırda toplam 578 adet lezyon tespit edildi. Belirlenen lezyonların 195'i subklinik laminitis, 198'i beyaz çizgi hastalığı, 125'i çift taban oluşumu, 31'i tırnak çatlağı, 12'si yabancı cisim, 5'i ince taban oluşumu, 3'ü taban ucu ülseri, 2'si interdigital dermatitis, 2'si taban ülseri, 1'i digital dermatitis, 1'i ökçe erozyonu, 1'i ökçe ülseri, 1'i ökçeden tırnak ayrılması ve 1'i podoartritis purulenta idi. Elde edilen veriler doğrultusunda hasta sahipleri ayak hastalıkları ve tırnak deformasyonları hakkında bilgilendirildi ve işletmenin yönetimsel bazda bazı değişiklere gitmesi gerektiği belirtildi.

Investigation of foot diseases observed in dairy farm (simmental) in Burdur province

ABSTRACT

In the study, it was aimed to determine the foot diseases of the Simmental breed cattle in a dairy farm, which in Kemer town of Burdur city and to take preventive measures together with the treatment of the lesioned hoofs and to ensure effective herd health. As the research material, 281 Simmental breeds, cattle of different ages and weights, which were routinely trimming toenails, were used. There were only digital deformities in 7 claws and only foot diseases in 104 claws. Foot diseases with digital deformities were seen in 165 cattle. Classification of the deformities and diseases were as follows; splay claw in 78, regularly elongated claw in 65, corkscrew claw in 49, scissor claw in 16, blunt claw in 12, beak claw in 1 cases. . In this study, a total of 578 lesions were identified in 269 cattle with foot disease. 195 of the identified lesions were subclinical laminitis, 198 white line disease, 125 occurrence of twin solear, 31 fissure ungulae, 12 foreign body invasion, 5 thin base formation, 3 base tip ulcers, 2 interdigital dermatitis, 2 solea ulcers 1 digital dermatitis, 1 heel erosion, 1 heel ulcer, 1 nail detachment from the heel and 1 podoarthritis purulenta. In line with the data obtained, the owners were informed about foot diseases and nail deformities, and it was stated that the business had to make some changes on a managerial basis.

GİRİŞ

Dünyanın farklı ülkelerinde gerçekleştirilen epidemiyolojik çalışmaların sonuçlarına göre topallık, süt sığırlarında mastitis ve infertiliteden sonra ciddi ekonomik kayıplara sebep olan en önemli sağlık problemlerinden biridir (1-3). Ülkemizde modern süt sığırcılığı işletmelerinin yaygınlaşması ile birlikte ayak hastalıklarının sığırlarda görülme oranı artmıştır. Örneğin, İngiltere'de, ayak hastalıkları gözlenen ineklerin oranının (insidans) 1957/58 yıllarında %4, 1980'li yılların başında %25 ve 1990'lı yıllarda ise % 54.6 civarında olduğu belirtilmektedir (4). Yapılan araştırmalarda süt sığırlarında görülen ayak hastalıkları insidansının %1-25 oranları arasında değiştiği bazı kaynaklarda ise %30 ve üzerinde olduğu bildirilmiştir (1). Yapılan bir araştırma, ayak ve tırnak lezyonlarının modern sayılabilecek ve çok bakımlı işletmelerde bile %18.3 oranında görüldüğünü belirtmektedir (5). Ayak hastalıkları insidansı; Güney Kore'de %0.3–3, Avustralya'da %3.7, ABD'de %6.6, Pakistan'da %7.6, İrlanda'da %9.5, Fransa'da %10.9, İsviçre'de %16.4 ve İngiltere'de %17.4 şeklindedir (6).

Ayak hastalıklarının sebep olduğu öncelikli ekonomik kayıplar canlı ağırlık kaybı, ağırlık artışında azalma, üretimden erken çıkma, tedavi giderleri, laktasyon süresi ile süt veriminde azalma ve infertilite şeklindedir (3,7,8,9). Ayak hastalığı saptanan sığırlarda sağlıklı olan sığırlara oranla günlük süt verimi 1,12 kg ile 3,1 kg arasında azalmakta, gebe kalma süreleri 12 gün daha

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uzamakta ve %1,17'si sürüden çıkarılmaktadır (10). Birçok araştırmacı sığır ayak hastalıklarının büyük oranda kültür ırkı sığırlarda görüldüğünü vurgulamışlardır (1,7,11,12).

Sığırlarda görülen ayak hastalıklarının bildirilen birçok sebebi bulunmaktadır. Uygun olmayan koşullarda barındırma, iyi beslememe, mevsim, yaş, ırk ve cinsiyet, gebelik ve laktasyon ayak hastalıklarının şekillenmesinde önemli rol oynamaktadırlar (3,12).

Bu çalışmada Burdur ilinin Kemer ilçesinde sağmal bir işletmede bulunan Simental ırkı sığırların ayak hastalıklarının belirlenmesi ve lezyonlu ayakların tedavisi, koruyucu önlemlerin alınması ve efektif sürü sağlığı amaçlanmıştır.

GEREÇ ve YÖNTEM

Araştırma materyali olarak Burdur ilinin Kemer ilçesinde 1100 başlık sağmal bir işletmede bulunan rutin tırnak kesimi yapılan 281 adet, Simental ırk, farklı yaş ve ağırlıktaki sığırlar kullanıldı. Araştırma Kasım ayında gerçekleştirildi. İşletmenin fiziki yapısı, ahırların durumu, temizliği, kapasitesi ve hayvanların beslenme programı incelendi. İşletme sahibine ayak hastalıkları ve tırnak deformasyonlarının nedenleri ve alınabilecek önlemler, tırnak kesim programı ve tekniği ile ilgili bilgiler verildi.

Bütün hayvanların ayakları mekanik olarak temizlendi ve ortopedik tırnak kesimi yapıldı. Ortopedik tırnak kesimi yapılan ayaklarda canlı ve cansız tırnak lezyonları ortaya çıkarıldı. Lezyonun durumuna göre uygun sağaltım yöntemleri uygulandı. Hayvanların anamnez ve klinik muayeneleri ile elde edilen veriler "Hasta Muayene Formlarına" işlendi (Şekil 1) ve istatistiksel olarak değerlendirilmek üzere kayıt altına alındı.

BULGULAR

İşletmenin her yerinde zeminin kilitli parke taşı olduğu, hayvanların yataklığı olmadığı, padok içinde serbest dolaştıkları ve ek gezinti alanları olmadığı görüldü ancak bulunan alanın hayvan yoğunluğu için yeterli olduğu belirlendi. Bazı padoklarda dışkı ve idrar için kanalların bulunduğu bir kısmında bu kanalların bulunmadığı, haftada bir veya iki haftada bir kepçe ile temizlendiği öğrenildi. İşletmede önceden ayak banyolarının bulunmadığı yapılan düzenleme ile tüm sığırların her gün sağım öncesi ve sonrası ayak banyolarından geçişinin sağlandığı öğrenildi. Sığırların ortalama süt veriminin 27 litre olduğu ve en son tırnak kesiminin beş ay önce yapıldığı, işletmenin geçmişte digital dermatit ile mücadele ettiği ve alınan önlemler ile bu problemlerin azaldığı bilgisi alındı.

Araştırmada ayak hastalıkları olarak; taban ülseri (Şekil 2), ökçe ülseri (Şekil 3), tırnak çatlağı (Şekil 4) ve beyaz çizgi hastalığı (Şekil 5) gibi hastalıklar ile tırnak deformasyonları olarak; yayvan ve geniş tırnak (Şekil 6), küt tırnak (Şekil 7), makasvari tırnak, tirbuşon tırnak olguları gözlemlendi.

Hayvanların muayenesinde 281 baş sığırın 7'sinde (%2,49) sadece tırnak deformasyonu, 165'inde (%58,72) tırnak deformasyonu ile birlikte ayak hastalığı, 104'ünde (%37,01) ise sadece lezyonlu ayak hastalığı saptandı. 281 baş sığırdan 5'inde (%1,78) ne tırnak deformasyonu ne de ayak hastalığı bulunduğu görüldü. Tırnak kesimi yapılan 281 sığırdan 35'inde (%12,46) topallık tespit edildi.

İşletme Adı: | İşletme Tipi:
İşletme Sahibi: | İşletme Numarası:
Küpe No: | Cinsiyet: | Kilo:
Irk: Holstein ☐ Simental ☐ Montafon ☐ Melez ☐ Yerli ☐
Yaş: 0-3 Ay ☐ 3-6 Ay ☐ 6-12 Ay ☐ 1 - 2 - 3 - 4 - 5 - 6 - 7 - 8 - 9
VKS: 1 - 2 - 3 - 4 - 5 | Gebelik/Laktasyon | 0-3 Ay / 3-6 Ay / 6-9 Ay / Kuru
Barınak Tipi: Kapalı ☐ Yarı Açık ☐ Açık ☐
Zemin: Beton ☐ Çakıl ☐ Kum ☐ Kauçuk ☐ Toprak ☐
İdrar/Dışkı: Az ☐ Orta ☐ Yoğun ☐ Zemin: Nemli ☐ Kuru ☐
Barınak Yoğunluğu? E / H | Tırnak kesimi düzenli yapılıyor mu? E / H
Yataklık sayısı yeterli mi? E / H | Yataklık hayvan boyutuna uygun mu? E / H
Tırnak banyosu var mı? E / H | Doğru uygulanıyor mu? E / H
Beslenme: Kuru/Konsantre | Yem Oranı:
Protein: % | Vit % | Min % | Yem Katkı
KH: %
Yağ : %

Bacak Duruşu

Ön - Önden	Ön - Yandan	Arka - Yandan	Arka - Arkadan
Düzgün ☐	Düzgün ☐	Düzgün ☐	Düzgün ☐
Önleri Açık/Kapalı ☐	Önleri İleri/Geri ☐	Arkaları İleri/Geri ☐	Arkaları Açık/Kapalı ☐
Fıçı ☐	Çukur/Büküк dizl ☐		X Bacaklı ☐
İt Elli ☐	Yatık/Dik Bilekli ☐		O Bacaklı ☐
Paytak ☐			Fıçı ☐
Öküz Dizli ☐			Paytak ☐
			İtelli ☐

Lezyon

	R / L	A / P	M / L		R / L	A / P	M / L
Akut Laminitis ☐	R / L	A / P	M / L	Distal Falanks Kırığı ☐	R / L	A / P	M / L
Subakut Laminitis ☐	R / L	A / P	M / L	İnce Taban Sendromu ☐	R / L	A / P	M / L
Subklinik Laminitis ☐	R / L	A / P	M / L	İnterdigital Dermatitis ☐	R / L	A / P	M / L
Kronik Laminitis ☐	R / L	A / P	M / L	İnterdigital Flegmon ☐	R / L	A / P	M / L
Beyaz Çizgi ☐	R / L	A / P	M / L	İnterdigital Hiperplazi(Limax) ☐	R / L	A / P	M / L
Çamur Humması ☐	R / L	A / P	M / L	Ölçeden Tırnak Ayrılması ☐	R / L	A / P	M / L
Çift Taban ☐	R / L	A / P	M / L	Padodermatitis Purulenta ☐	R / L	A / P	M / L
Digital Dermatitis ☐	R / L	A / P	M / L	Tabana Yabancı Cisim Batması ☐	R / L	A / P	M / L
Felç ☐	R / L	A / P	M / L	Tırnak Ucu Nekrozu ☐	R / L	A / P	M / L
Ökçe Apsesi ☐	R / L	A / P	M / L	Topuk Ekleminin Yangısı ☐	R / L	A / P	M / L
Ökçe Erozyonu ☐	R / L	A / P	M / L	Tırnak Deformasyonu			
Ökçe Ülseri(Apse) ☐	R / L	A / P	M / L	Düzenli Uzamış ☐	R / L	A / P	M / L
Pedal Osteitis ☐	R / L	A / P	M / L	Ayrık ☐	R / L	A / P	M / L
Taban Eziği ☐	R / L	A / P	M / L	Gaga ☐	R / L	A / P	M / L
Taban Ucu Absesi ☐	R / L	A / P	M / L	Geniş ve yayvan ☐	R / L	A / P	M / L
Taban Ucu Ülseri ☐	R / L	A / P	M / L	Küt ☐	R / L	A / P	M / L
Taban Ülseri ☐	R / L	A / P	M / L	Makas ☐	R / L	A / P	M / L
Tenosinovitis ☐	R / L	A / P	M / L	Tirbüşon ☐	R / L	A / P	M / L
Tırnak Çatlağı ☐	R / L	A / P	M / L	Vücut Kondüsyon Skoru			
Tırnak Düşmesi ☐	R / L	A / P	M / L	1 - 2 - 3 - 4 - 5			

NOT:

Şekil 1. Hasta muayene formu

Şekil 2. Taban ülseri

Şekil 3. Ökçe ülseri

Şekil 4. Beyaz çizgi hastalığı

Şekil 5. Tırnak çatlağı

Şekil 6. Geniş ve yayvan tırnak

Şekil 7. Küt tırnak

Ayak hastalığı gözlenen 269 sığırda toplam 578 adet lezyon tespit edildi. Belirlenen lezyonların 195'ini (%33,74) subklinik laminitis, 198'ini (%34,26) beyaz çizgi hastalığı, 125'ini (%21,63) çift taban oluşumu, 31'ini (%5,36) tırnak çatlağı, 12'sini (%2,08) yabancı cisim, 5'ini (%0,87) ince taban oluşumu, 3'ünü (%0,52) taban ucu ülseri, 2'sini (%0,35) interdigital dermatitis, 2'sini (%0,35) taban ülseri, 1'ini (%0,17) digital dermatitis, 1'ini (%0,17) ökçe erozyonu, 1'ini (%0,17) ökçe ülseri, 1'ini (%0,17) ökçeden tırnak ayrılması ve 1'ini (%0,17) podoartritis purulenta oluşturdu.

Ayak hastalığı tespit edilen 269 baş hayvanda lezyonların ön ve arka ayaklarda görülme oranları ön ayaklarda %63,23 (ön sağ %29,66, ön sol %33,57), arka ayaklarda %36,77 (arka sağ %18,38, arka sol %18,38) olarak belirlendi. Lezyonlar ön ayak medial tırnakta 379 (%53,23), lateral tırnakta 333 (%46,44), arka ayak medial tırnakta 158 (%38,16), lateral tırnakta 256 (%61,84) olarak görüldü.

Tırnak deformasyonları tespit edilen 172 baş hayvanda 221 adet deformasyon tespit edildi; deformasyonların %35,29'u yayvan ve geniş tırnak, %29,41'i düzenli uzamış tırnak, %22,17'si tirbuşon tırnak, %7,24 makasvari tırnak, %5,43'ü küt tırnak, %0,45'i gaga tırnak olarak saptandı, görülme oranları ise; ön ayaklarda 130 (%42,07) (ön sağ %16,83, ön sol %25,24), arka ayaklarda 179 (%57,93) (arka sağ %28,80, arka sol %29,13) olarak tespit edildi.

TARTIŞMA

Sığır yetiştiriciliğinde ayak hastalıklarının önlenebilmesi için ahır ısısının uygun olması, ahır zeminin kuru ve yumuşak olması, ayrıca zeminin düşmelere sebep olacak kadar kaygan olmaması gerekmektedir (13). Serbest dolaşımlı ahırlarda hayvanların başıboş gezinmeleri nedeniyle ahır zemininin dışkı ve idrarla daha fazla kirleneceği, dışkı ve idrar içinde kalan ayaklarda hastalık riskinin artacağı vurgulanmıştır (14). İncelenen işletmede sığırların serbest dolaştığı, yataklık ve altlığın kullanılmadığı, idrar ve dışkı için yeterli kanalın bulunmadığı tespit edilmiştir ancak dışkı ve idrarın sık aralıklarla uzaklaştırıldığı bilinmektedir.

Bir tırnağın küçük, diğerinin büyük olması veya tırnak uzunluklarının eşit olmaması, vücut ağırlığının eşit dağılmamasına neden olur. Önlerde medial arkalarda ise lateral tırnaklar daha büyüktür (15). Çoğu kez ayak hastalıkları önlerde medial arkalarda ise lateral tırnaklarda oluşur (15,16,17). Bu araştırmada lezyonlar ön ayak medial tırnakta 379 (%53,23), lateral tırnakta 333 (%46,44), arka ayak medial tırnakta 158 (%38,16), lateral tırnakta 256(%61,84) olarak görüldü. Araştırmanın sonuçları bu konuda daha önce yapılmış araştırmaların sonuçlarıyla paralellik göstermektedir (1,10,18).

Tırnağın uzaması, bakımının yapılamaması, tırnakların kesilip düzeltilmemesi, ahır hijyeni ve zeminin durumu tırnakların değişimine ve bozulmasına sebep olur. Bunun sonucunda deforme tırnaklar oluşur. Deforme tırnaklar genetik faktörlerle de oluşabilir (19). Anteplioğlu ve Akın (1978)(20) özellikle kış aylarında uzun süre ahır bakımında hareketsiz kalmış inek-

lerde aşırı tırnak uzamalarına bağlı yürüyüş bozukluklarının gözlendiğini belirtmişlerdir. Deforme tırnak yapılarının, tırnak hastalıklarının oluşması üzerine ciddi etkilerinin olduğu ifade edilmiştir. Yapılan bir çalışmada ülkemizdeki tırnak deformasyonlarının % 25'in üzerinde olduğu bildirilmiştir (21). Tırnak deformitelerinin ayak hastalıkları içerisinde %58,72 gibi yüksek oranda görülmesi ayak hastalıklarının oluşmasında deforme tırnak yapısının önemli rol oynadığı ve bu durum bundan önceki araştırmacıların bulguları ile benzerlik göstermektedir (10,13,18,22).

Laminitis çok faktörlü bir olaydır ve büyüme hızı, yaş, doğum, laktasyon, beslenme, davranış, barınma, mevsim, tırnak kesimi uygulamaları ve üreme bozuklukları ile ortaya çıkabilir (3). Karbonhidratlarca zengin yemlerin fazla miktarda verilmesi ile rumen asidozunun oluşması, pH düşmesi ve aşırı miktarda laktik asidin artması sonucunda laminitis meydana gelmektedir. Buna bağlı olarak da kornu üretimi etkilenerek deforme tırnak yapıları ve solea ülseri gibi ayak hastalıkları oluşmaktadır (23,24,25). Subklinik laminitin, tek başına ülserler ve beyaz çizgi hastalığı gibi topallığa neden olabilen lezyonların önemli bir predispozan nedeni olduğu düşünülmektedir (26,27). İngiltere ve Galler'in 4 bölgesinde 37 süt çiftliği üzerinde yapılan bir araştırmada, topallığa neden olan 8645 lezyondan % 40'ını taban ülserinin ve % 29'unu beyaz çizgi lezyonlarının oluşturduğu gözlenmiştir (27).

Araştırma yapılan işletmedeki sığırlarda görülen lezyonların büyük çoğunluğunu, subklinik laminitisin (%33,74), laminitisle alakalı beyaz çizgi hastalığının (%34,26), çift taban oluşumunun (%21,63) oluşturduğu gözlenmiştir. İşletmenin bir süredir rasyon ile ilgili sıkıntılar yaşadığı bilinmektedir dolayısıyla doğum sonrası sığırların süt veriminin arttırılması amacıyla karbonhidrat ve enerji bakımından zengin rasyonla beslenmesi, rasyonların dengeli hazırlanmaması, ayrıca ahır zemininin sert olması ve sürekli idrar ve gübre ile kirli halde bulunması gibi sebeplerin laminitis, dolaylı ve doğrudan beyaz çizgi hastalığı ve çift taban oluşumunda artışa sebep olabileceği düşünülmektedir.

Ayak hastalıklarının ortak belirtisi hafiften şiddetliye değişen topallıklardır (28) Topallığın erken tespiti son derece önemlidir. Erken müdahaleye izin verir ve hafif ayak hastalıklarına göre neredeyse 3 kat daha fazla maliyete neden olan daha şiddetli ayak hastalıklarının önlenmesine katkıda bulunur (29). Belge ve ark., (2005)(26) çalışma sonuçlarına göre süt sığırlarında topallık insidansının % 13 ile % 58 arasında değişkenlik gösterdiğini saptamışlardır. Birçok yazar ayak hastalıklarının diagnoz öncesi ve sonrası, haftalar hatta aylar boyunca süt verimini düşürdüğünü rapor etmişlerdir (30,31,32). Huxley (2013) (30) topal bir ineğin laktasyon başına 270 ila 574 kg daha az süt üretebileceğini özetlemiş ve topallığı tanımlamanın farklı yolları ve analiz için kullanılan metodolojiler nedeniyle literatürde verilen çok çeşitli değerleri karşılaştırmanın zorluğunun altını çizmiştir (29). Amory ve ark. (2008)(33), İngiltere ve Galler'deki 30 süt ineği çiftliğinde spesifik lezyonlar ve süt üretimi arasındaki ilişkiyi analiz ettikleri çalışmada taban ülseri ve beyaz çizgi hastalığına bağlı süt kaybını sırasıyla 1,5 ve 0,8 kg / gün olarak tahmin etmişlerdir ve bu laktasyon boyunca sırasıyla 574 ve 369 kg toplam kayba neden olmaktadır.

Tırnaklardaki lezyonları önlemek ve ayak simetrisinin ve şeklinin düzeltilmesi ve korunmasıyla yürüyüşü iyileştirmek için tırnak kesimi yapılır, bu da ağırlığın doğru dağılımını sağlar. Tırnak bozukluklarının neden olduğu topallık, doğru tırnak kesimi ile tedavi edilebilir. Sağlıklı tırnaklarda tırnak kesimi, süt yağı ve süt proteini bileşimleri üzerinde önemli ölçüde etkilidir (34). İncelenen işletmede, tırnak kesimi yapılan 281 sığırda topallık oranının %12,46 bulunması ile düzenli tırnak kesiminin topallık görülme oranındaki azalmaya katkı sağladığı bilgisi desteklemektedir. Yetiştiriciler, işletmelerinde ayak hastalıklarının hiç olmamasını temenni etseler de, hastalıkların görülmesi kaçınılmazdır. Bu sebepten dolayı sürüde bulunan sığırların topallık puanlarına (1, 2, 3, 4 ve 5) göre ideal oranlarının sırasıyla %75, %15, %9, %0.5 ve %0.5 seviyelerinde olması önerilmektedir (4).

SONUÇ

Elde edilen veriler doğrultusunda hasta sahipleri ayak hastalıkları ve tırnak deformasyonları hakkında bilgilendirildi ve işletmenin yönetimsel bazda bazı değişiklere gitmesi gerektiği belirtildi.

BEYANNAMELER

Etik Onayı

Uygulanamaz.

Çıkar Çatışması

Yazarlar, herhangi bir çıkar çatışması beyan etmemektedir.

Yazar Katkıları

Fikir, Kavram ve Tasarım: Ö Şengöz-Şirin, A Önür, F Şavklıyıldız

Veri Toplama ve Analiz: Ö Şengöz-Şirin, A Önür, F Şavklıyıldız

Makalenin Yazımı: Ö Şengöz-Şirin, A Önür, F Şavklıyıldız

Eleştirel İnceleme: Ö Şengöz-Şirin, A Önür, F Şavklıyıldız

Veri kullanılabilirliği

Bu çalışmanın bulgularını destekleyen veriler makul talep üzerine sorumlu yazardan temin edilebilir.

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Review

The use of effect size in veterinary medicine

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ABSTRACT

Effect size is a statistical index that measures the magnitude of the effect generated by the variable of interest in a study, in a sense, reflecting the practical or clinical value of the study in addition to the statistical results. In recent years, it has become preferable to report the effect size expressing practical significance in addition to the statistical significance expressed by the p-value in hypothesis tests in scientific research, and even it has been required by some scientific journals. By reporting the effect size, it is possible to use it in statistical power analysis, to compare the results of the studies, and to determine the amount of the effect in the study. In this study, by mentioning the concept of effect size, the main effect size indices used according to research types are introduced. In addition, the calculation methods of the effect size indices commonly used for continuous and categorical outcome variables were given and interpreted with scenarios from the field of veterinary medicine. In conclusion, in order to be able to interpret the results of a study in clinical or practical terms, to present the analyzed data in more detail than the p-value, and to ensure its use in power analysis, it was suggested that researchers report effect size in their studies.

Etki büyüklüğünün veteriner hekimliği alanında kullanımı

ÖZ

Etki büyüklüğü, bir çalışmada ilgilenilen değişkenin meydana getirdiği etkinin büyüklüğünü ölçen, bir anlamda çalışmanın istatistiksel sonuçlarına ek olarak, pratik veya klinik anlamdaki değerini de yansıtan istatistiksel bir indekstir. Son yıllarda bilimsel araştırmalarda hipotez testlerinde p değeri ile ifade edilen istatistiksel anlamlılığa ek olarak pratik anlamlılığı ifade eden etki büyüklüğünün de raporlanması tercih edilir hale gelmiş, hatta bazı bilimsel dergiler tarafından zorunlu kılınmıştır. Etki büyüklüğünün raporlanması ile istatistiksel güç analizinde yararlanılması, çalışmaların sonuçlarının karşılaştırılması ve çalışmada belirlenen etkinin miktarının belirlenmesi mümkün olmaktadır. Bu çalışmada etki büyüklüğü kavramından bahsedilerek, araştırma türlerine göre kullanılan başlıca etki büyüklüğü indeksleri tanıtılmıştır. Ayrıca, sürekli ve kategorik sonuç değişkenler için yaygın olarak kullanılan etki büyüklüğü indekslerinin hesaplama yöntemleri verilerek, veteriner hekimliği alanından senaryolar ile örneklendirilmiş ve yorumlanmıştır. Sonuç olarak, klinik veya pratik anlamda çalışma sonuçlarını yorumlayabilmek, incelenen veriyi p değerinden daha ayrıntılı şekilde sunabilmek, güç analizlerinde kullanımını sağlamak amacıyla, araştırmacıların çalışmalarında etki büyüklüğü raporlaması önerilmiştir.

INTRODUCTION

Statistical significance tests have a history dating back to the 1700s. It was first used by the Scottish physician John Arbuthnot in evaluating the birth rate in London according to the sex of the newborn babies, but its use was not widespread until the 1900s (1). The use of hypothesis tests has become widespread with the development of Karl Pearson's Chi-Square Goodness of Fit Test in 1900, William S. Gossett's Student t-Test in 1908, and Ronald Fisher's Analysis of Variance (ANOVA) in 1918 (2,3,4). It became popular after Fisher published his first book in 1925, Statistical Methods for Research Workers, and then The Design of Experiments in 1935 (1,5,6).

Although the decision-making process based on observation data in scientific research is actually a complex process, the fact that the deterministic algorithm of hypothesis testing approach has reduced this process to a dichotomous form of accepting and rejecting the hypothesis. For this reason, the hypothesis testing approach has spread rapidly as an easy-to-use process for researchers (7). However, over time, as researchers perceived this process only as a tool used to reach statistical significance, it has become difficult to reach scientific generalizations, and consequently, it received serious criticism (8,9,10). Criticisms of the hypothesis testing approach argue that the effect size should be used to express practical or clinical significance, in addition to the statistical significance expressed by hypothesis testing. Indeed, organizations such as the American Educational Research Association (AERA) and the American

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Psychological Association (APA) have made it mandatory to report effect sizes and confidence intervals in their publishing guidance (11). In summary, hypothesis tests provide information only about the probability of confirming the null hypothesis of observed data, as mentioned above. This information, used as the p-value, forces the researcher to make a dichotomous decision in the form of rejecting or not being able to reject the null hypothesis (12). In order to go beyond this process, additional values such as statistical power, effect size, and confidence interval should be evaluated (13).

1. What is effect size?

The effect size is the statistical value showing the deviation level between the results obtained from the sample and the expectations defined in the null hypothesis (14). Effect size is also defined as a statistical index that measures the magnitude of the effect created by the variable of interest in a study and, in a sense, reflects the practical or clinical value of the study in addition to the statistical results.

Including the effect size while reporting the research results generally serves three main purposes;

- The first of these is the use of the effect size in the statistical power analysis to calculate the sample size at the beginning of the study. Effect size is an important part of statistical power analysis. Although not applied consciously, the effect size contributes to a good experiment design (12). In other words, during the power analysis, the required sample size is chosen on purpose, taking into account the importance of the effect between the phenomena of interest, the sensitivity of the tools used to detect this effect, and the research design (13).

- The second purpose of using effect size is to allow comparison between studies answering the same hypothesis. These studies may have been done using different test statistics, different sample sizes, and designs. Therefore, effect size, which is a standardized index that eliminates different features between studies, is needed in order to compare study results (13).

- Reporting the effect size also makes it possible to interpret the magnitude of effect determined in the studies. In addition to making comparative interpretations of different studies, it is also possible to classify a single effect size as small, medium, large effect size as determined by Cohen. Cohen states that the cut-off values he gives for the interpretation of the effect level will be useful in new areas where there are not many studies. That is, when an effect is observed in a study, it is functional if there are no studies that can be compared to understand its magnitude (14). The classification of some effect size indices of most common used statistical tests are given in Table 1 (14, 15)

2. The calculation and interpretation of effect size

Just as there are different hypothesis tests used for different research designs in inferential statistics, effect size calculations also vary according to the structure of the variables. It is possible to evaluate the frequently used effect size indices under two main titles: those used for continuous outcomes and dichotomous outcomes.

Table 1. Effect size classifications for common used test

		Classification		
	Test	**Small**	**Medium**	**Large**
Cohen's d	t-Test	0.20	0.50	0.80
Cohen's f	Variance analysis	0.10	0.25	0.40
f^2	Regression analysis	0.02	0.15	0.35
Odds ratio (OR)	Contingency tables (2x2)	1.5	2	3
Risk ratio (RR)	Contingency tables (2x2)	2	3	4
W (Φ)	Contingency tables	0.10	0.30	0.50
Cohen's h	Contingency tables	0.20	0.50	0.80
r	Correlation	±0.20	±0.50	±0.80
r^2	Variance analysis/ Regression analysis	0,04	0,25	0,64

2.1. Effect size indices for continuous outcomes

In the research design where the means of the two independent groups are compared, the effect size can be calculated by the mean difference or standardized mean difference.

2.1.1. Mean difference

Let us assume that one compares the monthly live weight gains of Angus and Simental cattle in a breeding farm. The mean and standard deviation values of the live weight gain of two breeds are given in Table 2. It is seen that the difference between the means of the two groups, ie the effect size, is d = 9.03-7.46 = 1.57. However, it is difficult to comment on the difference between groups based on the pure mean difference. Because this difference is also related to the variation in the dependent variable. If the dependent variable is distributed with a wide variation, the difference of 1.57 units represents a very small effect, while the dependent variable is distributed in a narrow range may infer that the difference of 1.57 units is a significant effect.

Table 2. The measurements of live weight gain of Angus ve Simental cattle

	N	Mean	Standard Deviation	Variance
Angus	150	9.03	1.96	3.84
Simental	150	7.46	2.13	4.53

2.1.2. Standardized mean difference

If there is a predetermined standard of measurement for the variable of interest, it may be possible to comment on the effect of the difference between the two groups. However, as it is seen in the above example and most studies, generally there is no standard scale for the variable of interest. Therefore, in order to comment on the amount of difference between means, it is necessary to evaluate the means together with the variations of the distributions (16). Accordingly, the effect size of two independent group designs is calculated as in Equation 1.

$$d = \frac{\bar{x}_1 - \bar{x}_2}{S_{pooled}} \quad (1)$$

$$S_{pooled} = \sqrt{\frac{(n_1-1)S_1^2 + (n_2-1)S_2^2}{n_1+n_2-2}} \quad (2)$$

In the formula for the effect size expressed as "Cohen's d" $\bar{x}_i$ refers to the mean, S_i^2 refers to the variance and n_i refers to the sample size of each group.

In the example in Table 2, Angus and Simental beef cattle in a farm were intended to be compared in terms of monthly live weight gains.The variance (V) and standard error of d are calculated as in Equations 3 and 4, respectively (17).

$$V_d = \frac{n_1+n_2}{n_1 n_2} + \frac{d^2}{2(n_1+n_2)} \quad (3)$$

$$SE_d = \sqrt{V_d} \quad (4)$$

Herefrom;

$$S_{pooled} = \sqrt{\frac{(150-1)3{,}84 + (150-1)4{,}53}{150+150-2}} = 2{,}04$$

$$d = \frac{9{,}03-7{,}46}{2{,}04} = 0{,}77$$

When the above formulas are examined, it does not seem difficult to estimate d, if the population parameters are known or it is possible to obtain the data of interest. However, as is frequently encountered today, there may be a new variable that has not been subjected to any experiment, and has no available data. Under these conditions, it is not possible to obtain the required mean difference and standard deviation information for calculating the effect size. For similar cases, Cohen developed the categories of "small", "medium" and "large" effect size and enabled an approximate interpretation (14). For example, a new nutrition program is aimed to compare, which is thought to affect the milk yield of Holstein breed cows, with the standard nutrition program in terms of milk yield. The effect size was around d = 0.2-0.3 in expectation of small and the effect size could be around d = 0.8-1.00 in expectation of large. When the effect of the nutrition program on milk yield is expected to be moderate, the effect size may be about d = 0.5. If this interpretation is to be generalized, it can be expressed as (14);

d ≅0.20 small effect size

d≅0.50 medium effect size

d≅0.80 large effect size

Hedges suggested a degree of freedom correction as in Equation 5 because Cohen's d overestimates the effect size when the sample size is small (18).

$$J(df) = 1 - \frac{3}{4df-1} \quad (5)$$

$$g = j(df)d \quad (6)$$

$$V_g = [J(df)]^2 V_d \quad (7)$$

$$SE_g = \sqrt{V_g} \quad (8)$$

2.2. Effect size indices for dichotomous outcomes

If both dependent and independent variables are dichotomized, the most frequently used effect sizes are; risk difference, risk ratio, or odds ratio.

2.2.1. Risk difference

The effect size which expresses the difference between the two proportions P_1 and P_2 is shown as

$$j = P_1 - P_2 \quad (9)$$

But for example, let be $P_1 = 0.65$ and $P_2 = 0.45$, the effect size is calculated as $j = 0.20$; and let be $P_1 = 0.25$ and $P_2 = 0.05$, the effect size is still calculated as $j = 0.20$. This situation shows that the index j is insufficient to scale equal units. Therefore Cohen developed the index h in Equation 11, which he obtained with a non-parametric transformation on P values (14).

$$\Phi = 2 \arcsin \sqrt{P} \quad (10)$$

$$h = \Phi_1 - \Phi_2 \quad (11)$$

A generalization can be made about the interpretation of the index h as follows (14):

h≅0.20 small effect size

h≅0.50 medium effect size

h≅0.80 large effect size

2.2.2. Risk ratio

Risk ratio or relative risk (RR) is another effect size index frequently used in cross-sectional or prospective studies (17). It expresses the ratio of the probability of observing the event of interest in two independent samples.

$$RR = {}^{P_1}/_{P_2} \quad (12)$$

$$SE_{\ln(RR)} = \left(\frac{1-P_1}{n_1 P_1} + \frac{1-P_2}{n_2 P_2}\right)^{1/2} \quad (13)$$

As an illustrative example, let the data of a research design investigating the efficacy of the drug A developed for the treatment of Feline Infectious Peritonitis (FIP) disease seen in cats, compared to placebo, given in Table 3.

According to Table 3, the risk of disease occurrence in cats treated with placebo is calculated as $P_1 = 40 / 50 = 0.80$, while the risk of disease occurrence in cats treated with drug A is calculated as $P_2 = 5 / 50 = 0.10$. Accordingly, the risk ratio is found as RR= $P_1 / P_2 = 0.80 / 0.10 = 8$. This result is interpreted as the risk of disease in cats treated with placebo is 8 times higher than in cats treated with drug A. The point to be considered in relative risk is that one of the ratios of interest should belong to the unpreferable situation and the other to the preferred situation (17).

Table 3. The distribution of FIP positive and FIP negative cases in Drug A and Placebo

	Y		
X	**FIP positive**	**FIP Negative**	**Total**
Placebo	40	10	50
Drug A	5	45	50
Total	45	55	100

2.2.3. Odds ratio

Odds is defined as the ratio of the probability of occurrence of an event to the probability of non-occurrence. And the odds ratio (OR) is defined as the ratio of the odds of two groups (eg, treatment and placebo groups) whose effects were examined (19). While the risk ratio is an effect size measure used in cross-sectional and prospective studies, the odds ratio can also be used in retrospective research design (17). The observed positive and negative values of the X and Y variables are given in Table 4, and the calculation of the odds ratio according to these values in Equation 14 and the calculation of its standard error (SE) in Equation 15 are shown.

Table 4. Odds Ratio

	Y		
X	**Positive**	**Negative**	**Total**
Positive	n_{11}	n_{12}	$n_{1.}$
Negative	n_{21}	n_{22}	$n_{2.}$
Total	$n_{.1}$	$n_{.2}$	$n_{..}$

$$OR = \frac{n_{11}n_{22}}{n_{12}n_{21}} \quad (14)$$

$$SE_{OR} = \left(\frac{1}{n_{11}} + \frac{1}{n_{12}} + \frac{1}{n_{21}} + \frac{1}{n_{22}}\right)^{1/2} \quad (15)$$

Odds ratio based on the example in Table 4 is calculated as;

$$OR = 40x45/5x10 = 36$$

According to this result, it is interpreted that the likelihood of being positive for FIP disease in cats treated with placebo is 36 times more than cats treated with drug A. In other words, cats treated with drug A had 36 times more likelihood of recovery than cats treated with placebo.

CONCLUSION

In this study, we evaluated not only the effect size of calculations that can be used in different research designs but also evaluated how these calculated indexes should be interpreted. Furthermore, Cohen's effect size classification as "small", "medium" and "large" is also mentioned. Some researchers attribute Cohen's popularity about effect size to this classification system that he brought to the interpretation of effect size (10, 20). However, Vacha-Haase and Thompson argued that the use of this classification system is unreasonable, as it resembles the rigidity in the $p < 0.05$ system used in the hypothesis testing approach (10). Therefore, it was stated that a specific evaluation should be made for each study without sticking to a classification in the interpretation of the effect size. For example, in a study investigating the effect of smoking on lifetime, even if the effect size is found to be low, this is considered a valuable result. Because first of all, the outcome we are interested in is, the lifetime, clinically very important and it would also be seen that it is approximately similar to the effect size found in previous studies conducted on the same subject. Accordingly, while interpreting the effect size, interpretation should be made by considering both the characteristics of the outcome evaluated in the study and the effect sizes found in previous studies on the same subject.

The recomendations using effect size in addition to p-value aim to overcome the deficiencies of p-value. The most important limitation of the p-value is that it is affected by the sample size. Even though the effect size is zero or very small, p-value would indicate a statistically significant difference, if the sample size is adequately big. Statistically significance depends upon both effect size and sample size, while effect size is independent of sample size. The other limitation of p-value is that it is provided information only about the existance of the effect, not its effect. Thus, reporting only the p-value is not sufficient to fully understand the results (15).

Finally, it should be noted that, even though Cohen's small-medium-large effect size classification seems like it prevents to avoid the inflexibility of the p-value, it can be used as a rough guide in the absence of any preliminary information during the design phase of the research. In addition, researchers should prefer to report effect size to give information about the amount of the effect revealed in the intervention.

DECLARATIONS

Ethics Approval

Not applicable.

Conflict of Interest

The authors declare that they have no competing interests.

Author Contribution

Idea, concept and design: P Ambarcıoğlu

Data collection and analysis: P Ambarcıoğlu

Drafting of the manuscript: P Ambarcıoğlu

Critical review: P Ambarcıoğlu

Data Availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Review

Embryonic diapause

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ABSTRACT

Embryonic diapause in other words temporary cessation of embryonic development is a common some plant and animal species. Embryonic diapause is a temporary cessation of the development of embryogenesis in the blastocyst stage and is a reproductive strategy characterized by delayed implantation in the uterus. It is defined in over 130 species of mammals. It occurs obligate or facultative in cases where the development of the embryo from the blastocyst stage to later stages is not appropriate (eg, during environmental conditions or lactation). The embryonic diapause begins with the blastocyst entering the metabolic and proliferative state of silence so reduction or interruption of mitosis in the embryo. When exit from the diapause, reactivation, blastocyst returns to active metabolism, mitotic activity restarts and with cell proliferation, the implantation process begins in the uterus. Embryonic diapause is a protective phenomenon, it represents an important developmental advantage for species survival and should be evolutionarily protected.

Embriyonik diyapoz

ÖZ

Embriyonik diyapoz veya embriyo gelişiminin geçici olarak durdurulması, bitki ve hayvan türlerinde yaygın bir fenomendir. Embriyonik diyapoz, embriyogenezisin blastosist aşamasında gelişiminin geçici olarak durdurulmasıdır ve uterusta gecikmeli implantasyon ile karakterize bir üreme stratejisidir. 130'dan fazla memeli türünde tanımlanmıştır. Embriyonun blastosist asmamasından daha ileriki aşamalara gelişiminin uygun olmadığı durumlarda (örneğin çevresel koşullar veya laktasyon döneminde) zorunlu olarak ya da fakültatif olarak meydana gelmektedir. Embriyonik diyapoz, blastosistin metabolik ve proliferatif sessizlik durumuna girmesiyle başlamaktadır yani embriyodaki mitozun azaltılması veya kesilmesidir. Diyapozdan çıkış , reaktivasyon, şekillendiğinde blastosist aktif metabolizmaya geri dönmektedir, mitotik aktivite yeniden başlamaktadır ve hücre proliferasyonu ile beraber uterusa implantasyon süreci başlamaktadır. Embriyonik diyapoz koruyucu bir olgudur, türlerin hayatta kalması için önemli bir gelişimsel avantajı temsil etmektedir ve evrimsel olarak sürdürülmelidir.

INTRODUCTION

The embryo develops on its own before implanting into the uterus. However, when it reaches the blastocyst stage, its metabolism inherently slows down and cannot develop further in the absence of appropriate stimuli in the uterus (16). Pregnancy can be blocked in the presence of an unfavorable environment such as uterine causes, metabolic, climatic conditions. Carnivore, Rodentia or Diprotodontia embryos who event hard climates, malnutrition, metabolic stress or breastfeeding, enter diapause (29). Embryonic diapause, also known as delayed implantation, is the arrest of blastocysts in the early stage of the embryo. So cell division, development and blastocyst expansion cease or are very considerably reduced and there is down-regulation of metabolism. It includes the uncoupling of mating and fertilization from birth. So it is an evolutionary strategy by ensuring that postnatal development occurs under the most favourable environmental conditions for the survival of the offspring. It is known to occur in more than 130 species (for example roe deer, polar bear, giant panda) in blastocyst stage (7). It can take from a few days to 11 months depending on the species. Although the property of diapause differ between species according to the endocrine status the purpose is; extending the gestational period, ensuring that the offspring are born at the propitious moment of the year according to the feeding, environmental conditions, and if the mother has given birth and breastfeeding before conception, she gives all her metabolic resources to her newborn babies (19). The striking thing about diapause is that early embryonic cells stop dividing, but this is reversible. To understand how this is controlled at the molecular level; it has the potential to aid in many situations, such as improving the viability of blastocysts in assisted reproductive technologies, deriving embryonic stem cells or slowing cell division and identifying new cancer therapies by halting cancer cells. In

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essence embryonic diapause is the study of how to stop and restart cell growth (8).

1. The embryo in diapause

The success of reproduction is depends on the external (photoperiod, season as) and internal (hormones as) the conditions. In the absence of conditions for successful reproduction, different adaptations may occur depending on the species. An example of these adaptation situations is delayed fertilization (31), delayed implantation or post-implantation delay (seen some in bats). All this adaptation status has to aim: the gestational period is extended so the birth of offspring when environmental conditions are better (2). Embryonic diapause is a period in early development during which an embryo remains temporarily suspended at the blastocyst stage (30). It was the first species in which embryonic diapause exists in the roe deer (8). One of the main properties of diapause is regardless of the mating time or the normal birth time and it is the cessation of development in a period of pregnancy and controlling the birth time according to the environmental or physiological conditions. Although embryonic diapause varies between species, it can vary from a few days to 11 months. For example, rodents embryo can last from 1 day to several weeks in diapause or the tammar embryo can remain in diapause for 11 months. According to studies, it has been determined that diapause occurs in more than 130 mammal species. During diapause, there is minimal or no cell division, reduced cell metabolism, cell development is put on hold and despite all these situations, when the conditions become suitable, the pregnancy continues without any problems. (30). Diapause has effects on cell division apart from its effects on cell metabolism. It causes the mitosis in the embryo to decrease or stop during cell division. Although it depends on the species, cell cycle arrest may occur in the G0, G1 or G2 phase. The resumption of mitotic activity also means the end of diapause (19). Diapause occurs when the early embryo is in the blastocyst stage, blastocyst development either stops completely or develops very slowly, and after blastocyst stage continuity of pregnancy requires contact with the uterus and uterine secretory activity, which are dependent on ovarian steroids, blastocyst development either stops completely or develops very slowly, and after blastocyst stage continuity of pregnancy requires contact with the uterus and uterine secretory activity, which are dependent on ovarian steroids (30). In carnivores during diapause, blastocysts are not complete metabolically inactive since their oxygen consumption is continuous, and they continue to synthesize RNA, DNA and protein, although they are at lower rates compared to active blastocysts (22). Many factors can induce diapause; season, food, temperature, photoperiod and breastfeeding like (30). So, embryonic diapause in animals is a result of physiological stress factors (photoperiod, lactation), and all physiological and psychological stress factors directly change uterine receptors through the hypothalamic-pituitary-gonadal (HPG) axis and affect the hypothalamic-pituitary-adrenal axis (15). Diapause is divided into 2 types according to physiological conditions (facultative) and reproductive season (obligate) in animal species. (22).

1.1 Facultative embryonic diapause

Facultative embryonic diapause is best known in rodents and marsupials. Rodents are the most used species in facultative diapause studies. In this study after fertilization, ovariectomy operation and hormone application (progesterone) is performed (27), and it can also be finished with estradiol application (36). If mating occurs with oestrus in mice, zygotes are transported to the uterus by reaching the blastocyst stage on the 4th day (10). In the uterus, optimal progesterone levels must be maintained for embryonic development to continue (37). However, if mating occurs while the mouse is breastfeeding its offspring, progesterone will remain below optimal concentrations and embryonic diapause takes shape. Embryonic diapause in rodents can last from 1 day to several weeks, depending on the number of pups in the suckling litter (25). The amount of progesterone released from the corpus luteum under the effect of the prolactin hormone is not sufficient since the mother is still breastfeeding her offspring in postnatal oestrus. That is, a low amount of released progesterone induces embryonic diapause. The prolactin secretion can also be enhanced by melatonin in Marsupialia (2). (Figure 1).

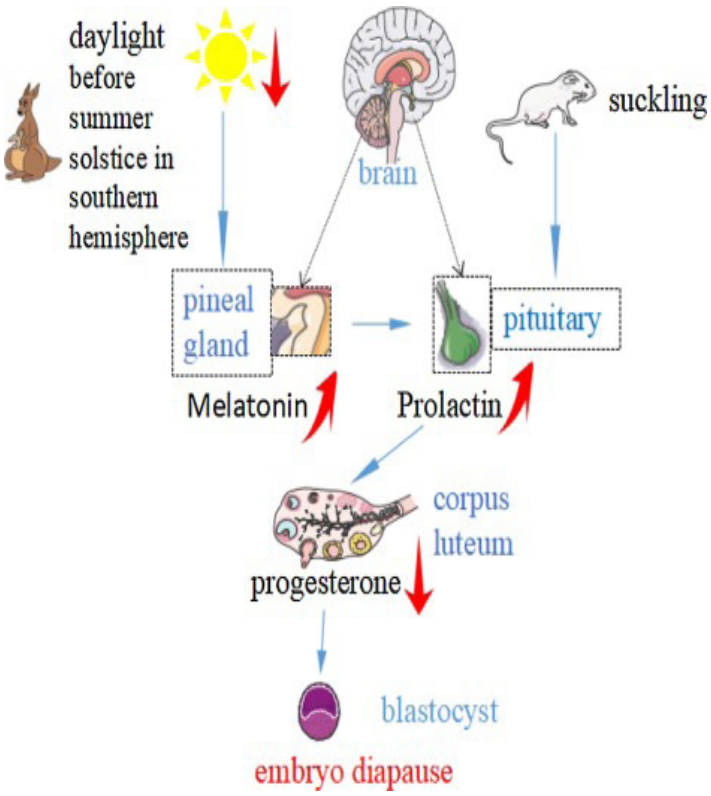

Figure 1. Regulation of facultative diapause (2).

1.2 Obligate (Seasonal) embryonic diapause

In species in which obligate diapause occurs, the embryo undergoes diapause in during every reproductive season. When the environmental conditions are suitable for birth, the diapause ends and the pregnancy continues (33). Studies have shown that this process in mammals with obligate diapause is regulated by seasonal changes in the photoperiod (28). Mink, weasel, skunk has seasonal diapause, which also affects day length and melatonin levels in regulating diapause through prolactin. In skunks (Spilogale Gracilis) mating occurs in autumn and embryos remain in diapause for 200 days (21), likewise in minkes (Neovision Vison) they undergo a long or short diapause phase that can last about 2 weeks after mating in March (23). In carnivores, blastocysts continue to cluster in the uterus in diapause. The number and diversity of cells in embryos undergoing diapause is higher than in rodents. Embryos in obligate diapause are encapsulated in the zona pellucida of the oocyte (4). Minkes mate before the spring equinox when daylight is less than 12 hours. While this situation suppresses prolactin secretion, it causes an increase in melatonin secretion. At the same time, diapause begins, which will continue until after the spring equinox. The new studies show that polyamines as the factors that are required for reactivation of the embryo in diapause. Ornithine decarboxylase (ODC) is the rate-limiting enzyme for transformation of ornithine to putrescine. It can reactivate the development of mink embryos that are in the state of diapause (24) (Figure 2).

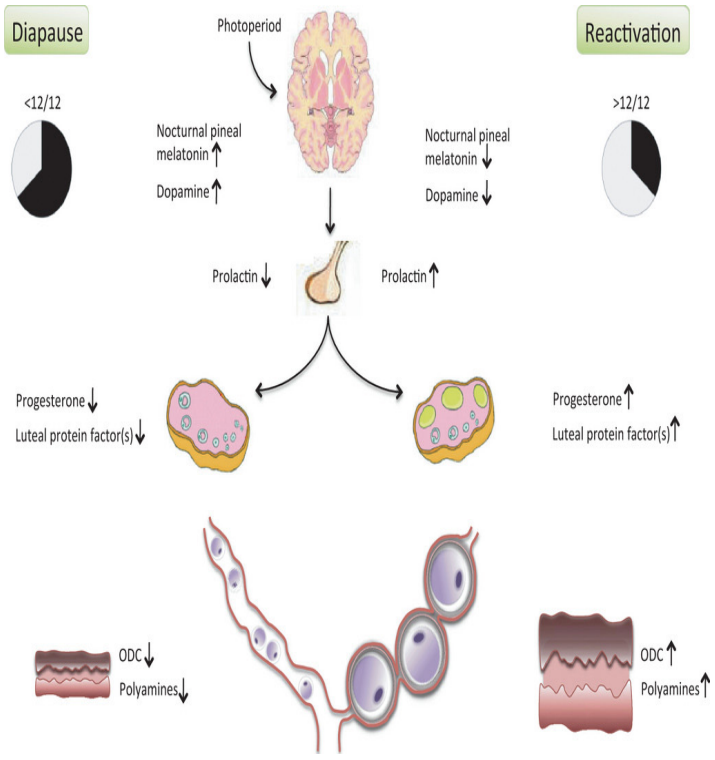

Figure 2. Endocrine and uterine regulation during diapause and reactivation in the mink (24).

2. Hormones involved in diapause

Prolactin, melatonin, progesterone, estrogen are important hormones in the initiation of diapause and reactivation. Prolactin hormone is secreted by the pituitary gland. Prolactin is an important hormone that has an effect on the initiation and continuation of breastfeeding in mammary gland development and the implantation of the embryo. It is also effective in the initiation of both obligate and facultative diapause. In obligate diapause photoperiod influences embryonic diapause and following reinitiating of embryo development by regulating the secretion of pituitary prolactin (5). In facultative diapause, fluctuations in prolactin concentration cause suppression of secretion from the corpus luteum and development. In facultative diapause, fluctuations in prolactin concentration cause suppression of secretion from the corpus luteum and development. Melatonin hormone is produced in the pineal gland and its biosynthesis is controlled by the photoperiod. Melatonin hormone is also effective in regulating embryonic development. Estrogen hormone optimizes embryonic implantation time, affecting both uterine and blastocyst activation, especially in mice. Estradiol-17B is used to start implantation in laboratory mice. Progesterone is a necessary hormone for both the regulation of diapause and implantation. The hormonal mechanism required for initiation, maintenance, and termination of diapause is complex and many molecules are required (2). To sum up the hormonal control of diapause depends on prolactin, estrogen and progesterone levels (29) (Figure 3).

3. Molecular control of diapause

Mouse, mink and tammar wallaby are the species in which the molecular part of diapause is best understood according to studies. and mink blastocysts are surrounded by multiple acellular layers and do not implant until a few days before reactivation, suggesting that the factors controlling diapause must reach the embryo via uterine secretions (28). It is thought that the mechanism that provides communication between the endometrium with blastocyst may be uterine secretions. Recent studies have revealed that a large number of proteins, hormones, cytokines, transcription factors are important in regulating embryonic development and are also effective in entering into diapause and reactivation. It has been shown that amino acids found in uterine fluids affect embryo development, but it has not been determined what the amino acids specifically affect (35). The uterine endometrium secretes cytokines and growth factors that affect the development of the preimplantation embryo. Some of these are known to control the arrested growth that occurs in diapause. Among these factors, Leukaemia Inhibitory Factor (LIF), Insulin-Like Growth Factor (IGF), Epidermal Growth Factor (EGF), Fibroblast Growth Factor (FGF), Transforming Growth Factor-b (TGF-b) (11;14), PAF (formerly platelet activating factor), Vascular Endothelial Growth Factor (VEGF). However, molecular control of diapause is still not fully resolved (30).

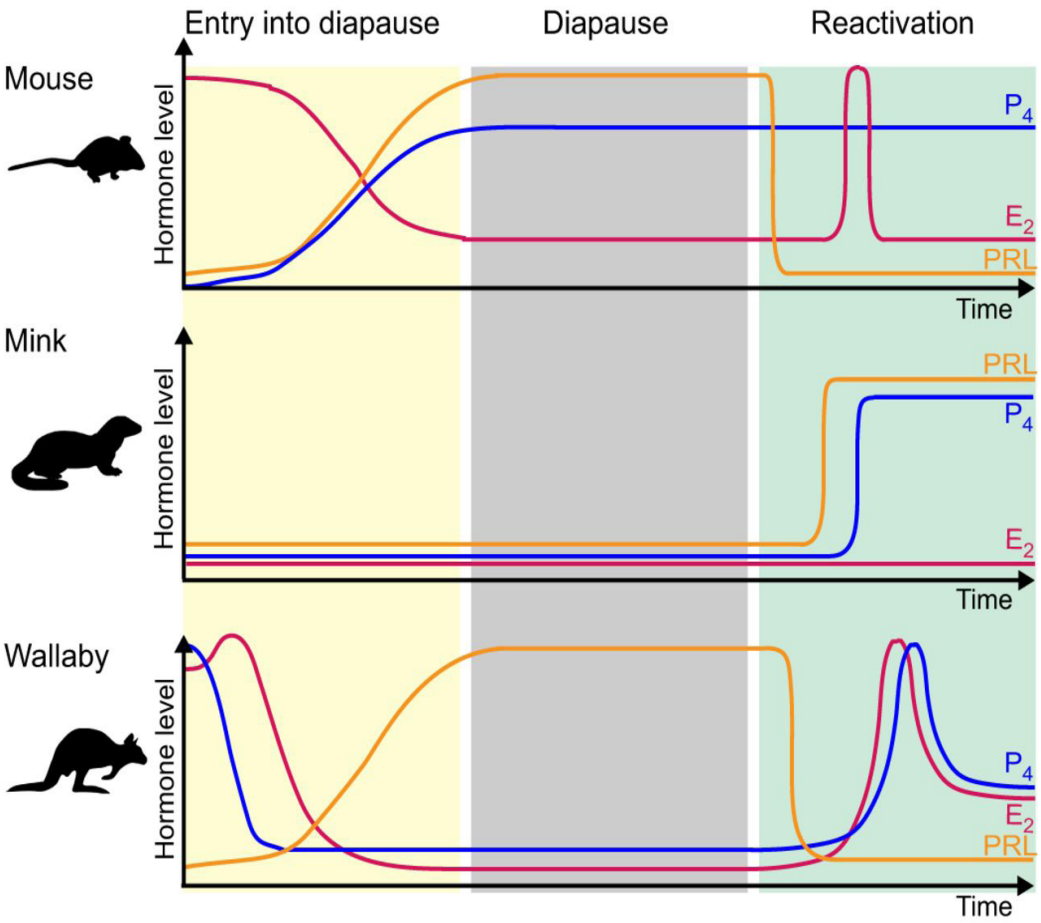

Figure 3. Hormonal changes during embryonic diapause in some species. Progesterone (P4), estradiol (E2), prolactin (PRL) (29).

4. Cell cycle effect

Mammalian embryo transforms from zygote to embryo by going through the stages of cell division and differentiation. The main cause of events occurring in diapause is the inhibition of the mitotic cell cycle in embryonic cells, stopping or decreasing cell proliferation. Cells enter a dormant state and apoptosis is prevented by maintaining basal metabolism with protein, RNA synthesis and oxygen consumption (31). Quantification of DNA in mammalian embryos shows that diapause occurs before the S phase of the cell cycle (34), but the absence of 5-bromo-2-deoxyuridine uptake in mink shows that diapause occurs the G0/G1 phase of the cell cycle (3) Immobilized embryonic cells maintain the ability to continue the cell cycle when the diapause ends (31).

5. Changes in gene expression

Many known and unknown factors play a role in inducing, maintaining, and ending the diapause. It is still not known how the factors act on the blastocyst at the molecular level, so proteomics and transcriptomic studies continue to eliminate these uncertainties (30). A study in mouse looked at the characterization of blastocysts using microarray technology to identify genes between blastocysts at the diapause stage and blastocysts activated following diapause. In this study, mRNAs were isolated from 100 diapause and activated embryos. 229 different genes have been identified between activated and diapause blastocysts. 229 different genes have been identified between activated and diapause blastocysts. Blastocysts in diapause have 149 genes upregulated compared to active blastocysts and 80 genes that increase in expression level. Genes ranging from diapause and activated blastocysts: genes specifically involved in cell cycle or cell proliferation control; genes involved in energy pathways and carbohydrate mechanisms; genes involved in signalling; genes involved in nuclear transport; genes involved in chromatin remodelling involved in adhesion divided into six functional groups as genes. In accordance with the physiological state of the embryos, an increase was observed in the expression levels of genes that stop cell proliferation at the G0 and G1 stages in the cell (9). In general, the expression of many genes that differ between diapause and activated blastocysts is consistent with the cellular and physiological events that are expected to change with activation. However, none of these 229 genes have been identified as the 'key' to regulate diapause (12). Likewise, 91 genes are upregulated in the mink blastocyst at reactivation from diapause (5). In mink, there was also a clear increase in the amount of polyamines (spermine, spermidine, and putrescine) among the genes upregulated in the activated uterus (17). In recent studies it was found that rapamycin (mTOR) can induce diapause in the mouse blastocyst (1). In a recent study, they identified pathways in gene ontology that are up and down regulated during diapause. What stood out in the study were the genes in lipolysis, glycolysis and pyruvate pathways, and those

involved in cholesterol metabolism. The results showed that an important metabolic profile characterizes diapause. They also emphasized that mTOR inhibition or failure of mouse embryonic stem cells (mESC) results in a transciptome that summarizes the diapause cellular phenotype (13).There are many factors that are interlinked in this increasingly complex network. Also, there are many factors that have been identified, including microRNAs. Proteomic studies have shown that there are a lot of new effective candidates for diapause (18).

6. Future directions

A thorough understanding of how diapause is controlled by molecular aspects has the potential to assist in many situations, enhancing viability of blastocysts in assisted reproductive technologies (ART), generating embryonic stem cells, or identifying new cancer therapies by halting and slowing cell division. It will provide information about the requirements to keep the embryo alive for a long time, thus bringing new approaches to assisted reproductive technologies, helping to improve the embryo culture environment, and be able to introduce approaches that can offer an alternative to cryopreservation. It will also be effective before transfer in determining the selection criteria for the best embryo. Same time ıt will also be effective before transfer in determining the selection criteria for the best embryo (8). It has a lot of similarities to the invasive nature of cancer cells including embryo reactivation, rapid resumption of cell proliferation and the implantation process (20). Preventing the reactivation of a cancer cell or encouraging it to enter diapause could provide a new treatment method for cancer in the future or prevent metastasis (8). Another important point to be wondered and studied is whether embryonic diapause can occur in other mammalian species, including humans. If embryonic diapause is an evolutionarily conserved phenomenon, it must also be inducible in blastocysts of non-diapause mammals. In the study conducted to prove this hypothesis, blastocysts from domestic sheep were transplanted into mice in which diapause conditions were induced. Sheep blastocysts have been found to stop growing and ıt has been found to express a gene specific to diapause and to be in diapause. This is shows that embryos from a domestic mammal, the sheep, can enter into diapause when adequate conditions are created (27) (Figure 4). They demonstrated that a non-diapausing species, the sheep, is capable of embryonic diapause, put forward a new hypothesis; could all mammals, including humans, have a common ancestral trait? (26). Understanding the mechanism of embryonic diapause in humans might be a tool for prolonging or enhancement of embryo culture durations in some patients of infertility (32). The study on mTOR has opened the door to new studies on assisted reproductive technologies that can help in many areas such as regenerative medicine, preservation of cell viability after trauma and aging, and stopping mTOR inhibition in cancer cells (1).

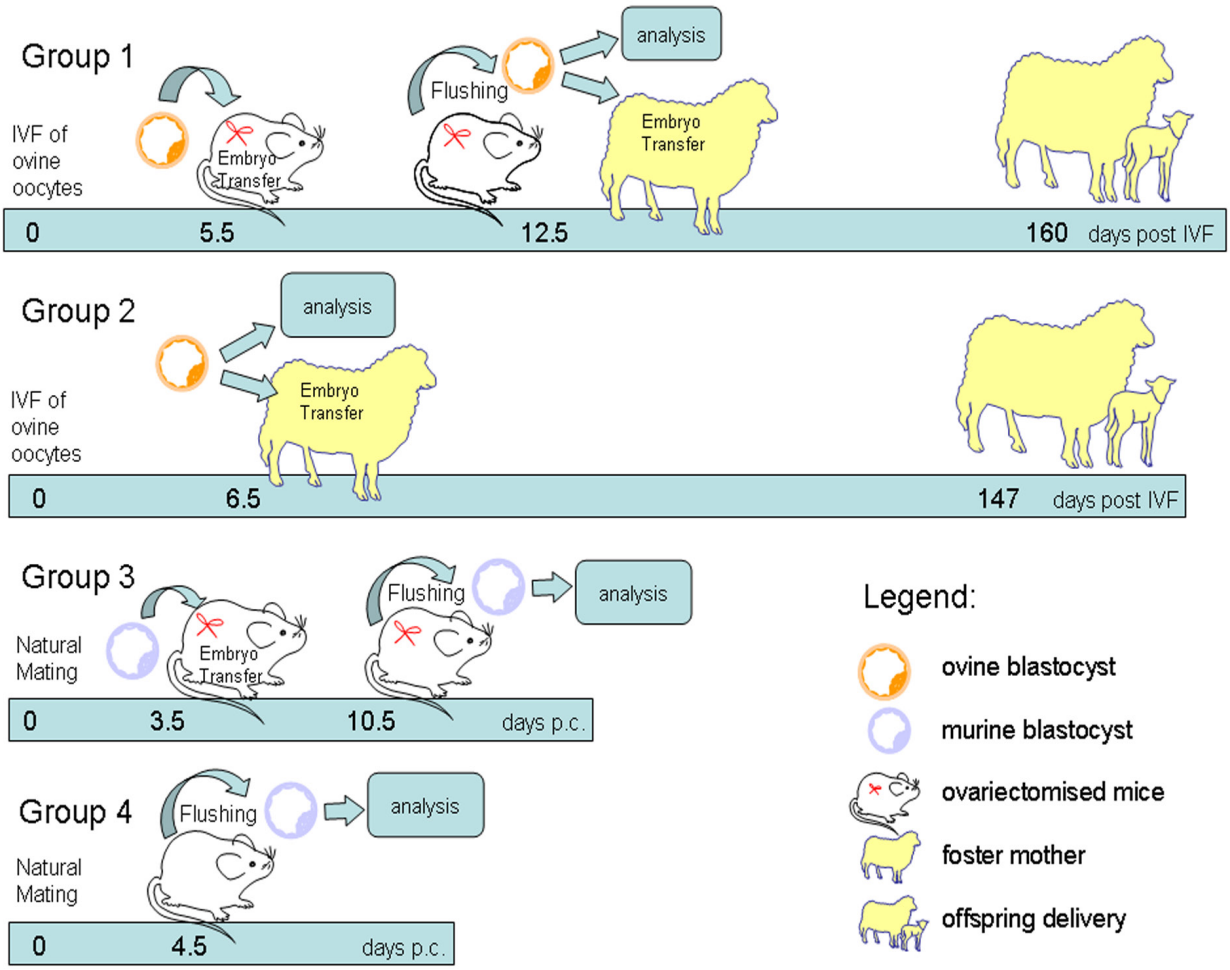

Figure 4. Experimental design of a study of sheep blastocysts in embryonic diapause by transferring ovariectomized pseudo-pregnant mice. Times in the diagram refers to embryos (25).

CONCLUSION

Embryonic diapause studies also hold promise for reproductive research. There is a molecular communication between the uterus and blastocyst in the entry and continuation or even the ending of diapause. Understanding all the molecular stages of diapause will not only shed light on evolution, but will also have important contributions in many areas such as assisted reproductive technologies (ART) By determining the molecular level of the mechanism of diapause formation, maintenance and reactivation, it will be understood to what extent it is protected among species and whether all mammalian embryos can enter diapause. New proteomic studies have shown the wealth of new candidates. There are still many unanswered questions on embryonic diapause and many species to work on. Cell and molecular biology techniques, which continues to evolve today, can provide tools to answer all other questions about diapause. Diapause will continue to be a powerful research topic with all its mechanisms.

DECLARATIONS

Ethics Approval

Not applicable.

Conflict of Interest

The authors declare that they have no competing interests.

Author Contribution

Idea, concept and design: M Çevik, MD Genç

Data collection and analysis: M Çevik, MD Genç

Drafting of the manuscript: M Çevik, MD Genç

Critical review: M Çevik, MD Genç

Data Availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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