UNIVERSITATEA DE ŞTIINŢELE VIEȚII
„REGELE MIHAI I” DIN TIMIŞOARA

FACULTATEA DE MEDICINĂ VETERINARĂ

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LASER THERAPY- AN ALTERNATIVE METHOD IN THE THERAPY OF MICROSPORUM CANIS INFECTIONS IN CARNIVORES

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Summary

Ring worm infections are caused by dermatophytes that affect the superficial layers of the skin and produce single or multiple lesions. The etiological agent responsible for ring worm infections in carnivores is *Microsporum canis* and the social importance of this dermatophytosis lies in its zoonotic nature. The importance of using laser therapy in the treatment of dermatophytosis lies in its ability to induce tissue biostimulation and to accelerate skin regeneration mechanisms compared to the conventional treatment based on ketoconazole, enilconazole and fluconazole. Thus, the aim of this study was to evaluate the efficacy of laser therapy in treating dermatophytosis in companion animals. In this context, five animals (three cats and two dogs) showing skin lesions characteristic for mycosis were examined. The presence of one or more circular, alopecic lesions with centrifugal pattern was observed following clinical observation. Skin scrapings and inoculation on DTM culture media was performed to establish the diagnosis. The skin scraping did not reveal the presence of mites. The result of the culture media revealed the presence of the *Microsporum canis* dermatophyte. Following the established diagnosis, treatment was performed using the *Foschi D5-810* laser for 10 consecutive days. During laser therapy, as well as after the treatment, the patients were not given any substance with antifungal action. After treatment, the clinical signs gradually improved until they dissapeared. Remission of clinical signs was observed within 10 days after the treatment. Laser therapy represents a safe and painless alternative protocol, a therapeutic challenge for future studies in carnivore dermatophytosis.

Keywords: *Microsporum spp.*, carnivores, laser therapy, *Foschi D5-810.*

The infection with *Microsporum canis* is a common, globally acknowledged dermatophytosis, which affects a wide range of animals. It has earned its importance due to its zoonotic character, causing multifocal alopecia, scaling, and circular lesions in both animals and humans (2). The disease is commonly referred to as ringworm.

In the aetiology of clinical cases in carnivores, the most common dermatophyte species are *Microsporum canis*, *Microsporum gypseum* and *Tricophyton mentagrophytes* (7). In dogs, about 70% of ringworm cases are caused by the fungus *Microsporum canis*, which is a geophilic and keratolytic dermatophyte that affects the superficial layers of the skin and the nails (20). The natural reservoir of *M. Canis* is the cat, which facilitates the transmission of the infection, despite it being asymptomatic in most cases. The favoring factors and clinical aspect of the disease depend rather on the immune system of the patient, than on factors related to the infective fungal strain (11). Direct microscopic examination is the most basic method of examination in case of cutaneous mycosis. The examined samples are: hairs, nail fragments, crusts, scales or exudate. Septated and branched hyphae and small arthrospores in clusters or chains can be observed microscopically (7). In veterinary practices, the most commonly used diagnostic methods are: inoculation on DTM (=Dermatophytes test medium), Wood lamp examination and trichogram examination. What distinguishes the DTM from other culture mediums is the addition of phenol red, a special dye that changes color when the pH increases. This change indicates the presence of a dermatophyte (14).

The fungal agent is highly contagious but responds well to the antifungal treatment, whether it is topical only or in combination with systemic medication. Systemic therapy is recommended for more extensive, chronic infections or when the application of a topical drug is not possible.

There is no reason to suspect clinical resistance to the antifungal treatment, unless we face clinical cases that show permanent infection or relapses within 4 weeks of an appropriate dosing regimen of an antifungal drug (3).

Even though the infection is widely spread throughout all continents, it has a well-established treatment protocol. A wide variety of topical treatments are available in different formulations such as: antifungal shampoo, gel and cream. Most of the agents belong to the "azole" and "allylamine" family (1). The most commonly used and tested antifungal substances are: enilconazole, clotrimazole, ketoconazole, miconazole and calcium sulphide (13).

Topical application of an antifungal cream is the preferred first step in the treatment of *Microsporum* infections and even though they are rare, side effects can be seen. These side effects consist of: irritation, burning sensation, stinging sensation, erythema and even contact dermatitis (10).

The applications of low-level laser treatments in veterinary medicine are many, the advantage of it being the facilitation of a faster healing process through the biostimulation of soft tissues. Mechanisms of action aimed at reducing pain and inflammation as well as tissue healing have been studied and identified. The side effects of the laser treatment are minimal to none (6, 19). ”Low-level laser therapy” is used in the treatment of certain types of cancer, in dermatology, ophthalmology in the treatment of skin lesions (congenital and acquired), scar management, tendon and cartilage healing. It has a very important role in the process of neovascularisation and angiogenesis, being able to stimulate a certain healing level of nerves and to accelerate wound healing and collagen synthesis (6). Thus, the aim of this study was to assess the efficacy of laser-therapy in the treatment of lesions caused by *Microsporum canis* in dogs and cats.

Materials and methods

The study was carried out on 5 animals, coming from different backgrounds and environments, from Timișoara, Timiș County, Romania. The animals taken into study were three cats and two dogs. All three cats were common breed stray cats. The felines were all females, between the ages of 2.5 and 5 years. The canine patients were a 9 year old male Pug and a 6 weeks old male American Staffordshire Terrier.

The patients included in the study were not prescribed nor given any other type of antifungal therapy throughout the study period.

All the patients came in presenting circular, alopecic lesions, that had a centrifugal spread, specific for the clinical picture of ringworm infection. Details on each patient as well as specific description of the lesions and their locations are described in Table 1.

Table 1

Description of patients taken into study

Species	Breed	Sex	Age	Type of lesions	Location
Feline	common	Female	5 years	3 circular, alopecic lesions with centrifugal spread of approximately the same size	- base of the tail, bilaterally - interscapular region
Feline	common	Female	4 years	1 round alopecic lesion	- base of the neck
Feline	common	Female	2,5 years	1 alopecic lesion (possibly as a result of the confluation of 2 round lesions)	- carpal region of the left thoracic limb
Canine	Mops	Male	9 years	1 round circular alopecic lesion	- dorsal cervical area
Canine	American Staffordshire Terrier	Male	6 weeks	2 alopecic lesions, varying from 0.5 mm to 3 cm in size	- above the scapulo-humeral joint of the right thoracic limb - above the C1-C2 joint

Hairs and scales from each patient were sampled and added to a DTM medium (Fig. 1). A hemostatic clamp was used for the collection of the biological materials, either by plucking the hair or by removing loose scales from the lesions.

The samples were taken from the outer margins of every lesion. The culture medium used for the diagnosis of the fungus was the *Kruuse Dermatophyte test*, a pre-poured agar plate that does not require an incubation period. The inoculated DTM plates were stored at room temperature, at 21-23 °C, for a maximum period of 10 days. The growth rate differed among the samples, ranging from 3 days post innoculation up to 9 days post innoculation.

Samples were collected from the colonies that grew on the inoculated culture medium and they were prepared for microscopic examination. The samples were collected using adhesive tape directly from the colony and stained using thiazine dye. Samples were then microscopically examined in order to establish the morphological characteristics (x100 magnification). The present macroconidia were spindle-shaped, with a thick outer wall and separated cells, which varied in numbers (from 3 to 7 cells). Septate hyphae and small, single-celled microconidia were also present (Fig. 2).

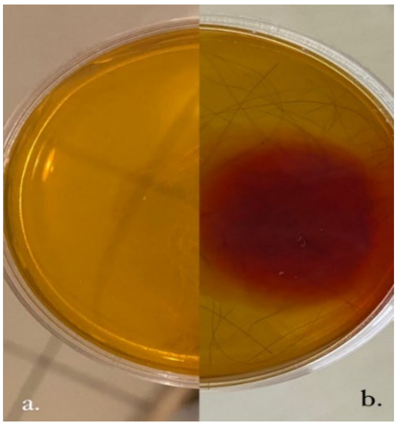

Fig. 1. a. Day 1 of the innoculation of the DTM medium; b. Day 5 after the innoculation of the DTM medium with visible specific color change of the medium (original)

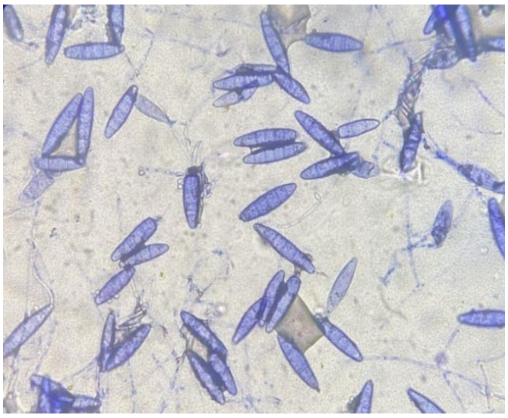

Fig. 2. *Microsporum canis* - microscopic aspect (original)

The laser used in this study was the Foschi D5-810 laser (Fig. 3). It was set to operate on biostimulation mode with a continuous wave emission, set at a 3W power (Fig. 4). The laser was used for 1 minute per lesion, for 10 consecutive days (Fig. 5).

Fig. 3. Laser Foschi D5-810

Fig. 4. Laser Foschi D5-810 settings

Fig. 5 Laser operating method

Results and discussions

All five animals taken into study had a satisfying clinical outcome following the elected laser therapy. The canine patients had a faster healing rate than the feline patients, with significant lesion improvement within 7 days following the end of the laser therapy period, in comparison to the feline patients, which showed an improvement 10 days after the end of the treatment (Fig. 6, 7, 8, 9, 10). The hair coat was fully regenerated at the 3 months check-up and there were no signs of persistent alopecic areas.

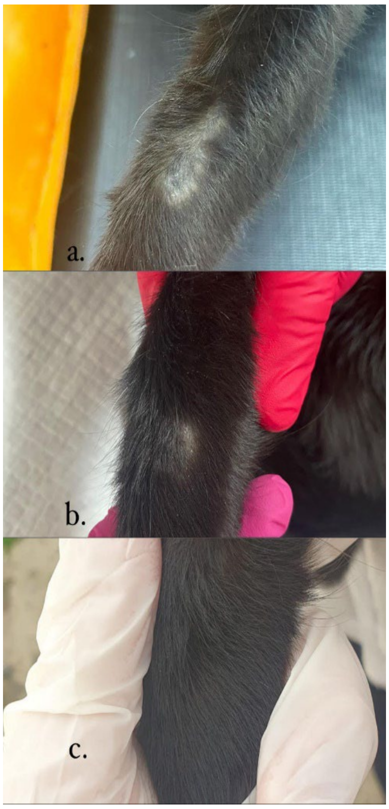

Fig. 6. Cat, 2.5 years old. a. Aspect of the lesion at the beginning of the treatment b. The aspect of the lesion 10 days after the laser treatment c. The hair coat 1 month after the treatment

No side effects were noticed during the treatment period in any of the patients. The animals were monitored for a possible recurrence of infection, over a 3-month period after the treatment protocol was finalized. None of the patients suffered from relapses.

In recent decades, interest in pets has grown significantly. As people share the same living environment with animals, the risk of contamination with many zoonoses increases. According to a 2020 study review that focuses on the prevalence and clinical signs of dermatophytosis, the incidence of dermatophyte infections in cats and dogs, ranges from 20% to 30%. The species *Microsporum canis* was identified as the infectant agent in 81.8%-97% of the cases (15). The epidemiological importance lies both in the existence of carnivores with apparent clinical infections and in the asymptomatic carriers. One of the causes of the disease multiplication is the subclinical evolution. Patients that show mild to no clinical signs, have a very important role in spreading the pathogenic agent. The treatment of choice in these mycotic infections consists of topical application of antifungal creams, ointments or gels, systemic administration of antifungals or administration of an inactivated vaccine.

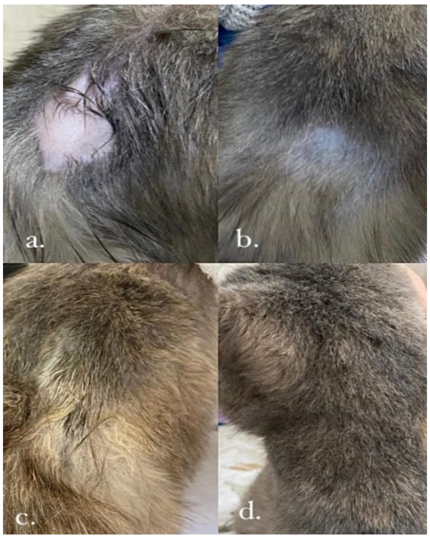

Fig. 7. Cat, 5 years old. a. Aspect of the lesion at the beginning of laser therapy; b. Aspect of the lesion 10 days after laser therapy; c. The alopecic lesion 3 weeks post treatment; d. Aspect of the hair coat 4 weeks post treatment (original)

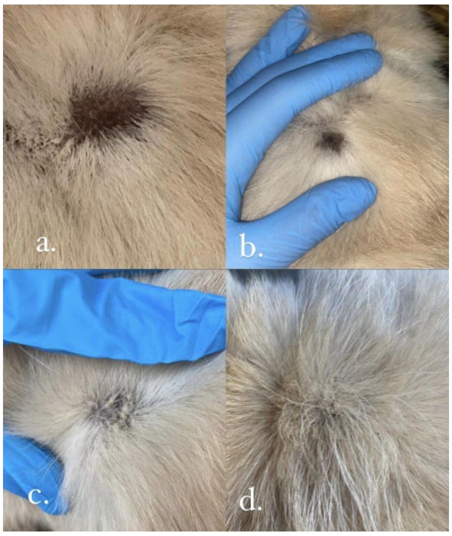

Fig. 8. Dog, 9 years old. a. Aspect of the lesion at the beginning of laser therapy; b. The lesion aspect on day 10 of treatment; c. Aspect of the lesion 10 days after laser therapy; d. Aspect of the hair coat 6 weeks post treatment (original)

Currently, eight classes of antifungal drugs are being used in veterinary practices, of which only four are elected in the treatment of dermatomycoses (8). The use of lasers in clinical dermatophytosis cases in companion animals is yet to be in-depth researched. In human medicine, some cases of onycomycosis were treated with a 1.064 nm wavelength energy laser. At the 8 month follow up, a significant improvement of the lesion aspect could be observed (5). Another study comparing the laser treatment to an antimycotic agent showed a higher efficacy of the Nd:YAG laser (1064 nm) compared to intraconazole use (9).

In Veterinary Medicine, the low-level laser is one of the elected treatment options in: wound healing, inflammation sites, post-surgical incisions and arthritis. There are multiple techniques regarding the laser operating mode; the most used technique is: moving the laser “in a scanning fashion” over the area that needs to be treated (4, 17). When treating an acute condition, it is ideal that the patient undergoes daily laser treatments, compared to more chronic conditions, where the time span between treatments becomes bigger as time passes.

Fig. 9. Dog, 6 weeks old. a. The aspect of the lesion before the treatment; b. The aspect of the lesion 4 weeks after the treatment

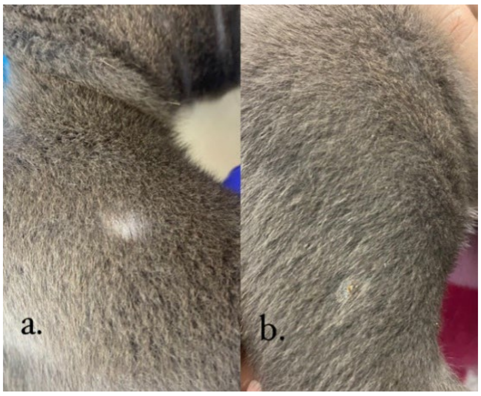

Fig. 10. Dog, 6 weeks old. a. The aspect of the lesion before the treatment; b. The aspect of the lesion 4 weeks after the treatment

Comparing the use of the Foschi D5-810 in dermatophytosis infections to the regular laser treatments conducted in veterinary medicine, the technique was more similar to the one used in acute diseases. The area of affected skin was treated in an “off-contact” method, the operator moving the laser beam slowly over the entire lesion.

Laser therapy applied on post hemilaminectomy incisions showed a significant improvement rate in the healing process and in the scar scale, the laser being used once a day, for 7 consecutive days (17, 19). Comparing the evolution rate of incision healing with the improvement rate of dermatophytosis lesions, the latter shows slower progress, healing signs being observed after a minimum of 10 days of treatment.

With increasing prevalence of dermatophytosis among companion animals and humans, the risk of antifungal resistance increases as well. Inadequate and irregular use of antifungal drugs will lead to an increase in the number of resistant strains, that will present a challenge for human and veterinary medicine (12). Laser treatment should be taken into consideration as a future treatment possibility.

Conclusions

The results obtained after the laser treatments seemed to have a favorable outcome. The regeneration of the skin and coat took place at a different pace, depending on the patient.

The overall observation throughout the case evolutions, was that the hair growth of canine patients seemed to occur at a faster pace compared to the feline patients. The time difference between the rate of the hair growth was, however, only 3 days. A slight improvement in the aspect of the lesion was seen 7 days after the end of the treatment in dogs, whereas in the feline patients, such results were seen 10 days post treatment.

During the treatment, there was no visible improvement of the lesions. In this time, the alopecia did not spread. Improvement was observed only after the laser therapy was finished.

All patients had full recovery between 4 - 6 weeks post laser treatment. At the 3 months post-therapeutic consult, the hair coat was healthy and intact and there were no signs of infection reoccurrence.

Laser therapy represents a safe and painless alternative protocol, a therapeutic challenge for future studies in carnivore dermatophytosis.

References

1. **Aneke, C., Otranto, D., Cafarchia, C.,** Therapy and antifungal susceptibility profile of *Microsporum canis*, Journal of Fungi, 2018, 4, 3, 107.
2. **Bishnoi, A., Vinay, K., Dogra, S.,** Emergence of recalcitrant dermatophytosis in India, The Lancet Infectious diseases, 2018, 18, 3, 250-251.
3. **Dărăbuş, Gh., Oprescu, I., Morariu, S., Mederle, N.,** Parazitologie și boli parazitare, Editura Mirton ,Timișoara, 2016.
4. **Espírito Santo, R.B., Deps, P.D.,** Case study of onychomycosis patients treated with 1,064-nm Nd:YAG Laser. Case Reports, Dermatology, 2018, 10, 2, 216-225.
5. **Fesseha, H.,** Laser therapy and its potential application in veterinary practice- A Review, Journal of Light and Laser Current Trends, 2020, 3, 007.
6. **Gnat, S., Łagowski, D., Nowakiewicz, A.,** Major challenges and perspectives in the diagnostics and treatment of dermatophyte infections, Journal of Applied Microbiology, 2020, 129, 2, 212-232.
7. **Kandpal, R., Arora, S., Arora, D.,** A study of Q-switched Nd:YAG Laser versus itraconazole in management of onychomycosis, Journal of Cutaneous and Aesthetic Surgery, 2021, 14, 1, 93-100.
8. **Kaur, I.P., Kakkar, S.,** Topical delivery of antifungal agents, Expert Opinion on Drug Delivery, 2010, 7, 11, 1303-1327.
9. **Mahajan, S., Tilak, R., Kaushal, S.K., Mishra, R.N., Pandey, S.S.,** Clinico-mycological study of dermatophytic infections and their sensitivity to antifungal

drugs in a tertiary care center, Indian Journal of Dermatology, Venereology and Leprology, 2017, 83, 4, 436-440.

10. **Mathy, A., Baldo, A., Schoofs, L., Cambier, L., Defaweux, V., Tabart, J., Mignon, B.,** Fungalysin and dipeptidyl-peptidase gene transcription in *Microsporum canis* strains isolated from symptomatic and asymptomatic cats, Veterinary Microbiology, 2010, 146, 1-2, 179-182.
11. **Moriello, K.,** Dermatophytosis in cats and dogs: a practical guide to diagnosis and treatment, Practice, 2019, 41, 4, 138-147.
12. **Moskaluk, A.E., VandeWoude, S.,** Current topics in dermatophyte classification and clinical diagnosis, Pathogens, 2022, 11, 9, 957.
13. **Paryuni, A.D., Indarjulianto, S., Widyarini, S.,** Dermatophytosis in companion animals: A review, Veterinary World, 2020, 13, 6, 1174-1181.
14. **Pryor, B., Millis, D.L.,** Therapeutic laser in veterinary medicine, Veterinary Clinics of North America: Small Animal Practice, 2015, 45, 1, 45-56.
15. **Riegel, R.J., Godbold Jr, J.C.,** Laser therapy in veterinary medicine: photobiomodulation, Wiley Blackwell, Hoboken, 2017.
16. **Wardlaw, J.L., Gazzola, K.M., Wagoner, A., Brinkman, E., Burt, J., Butler, R., Senter, L.H.,** Laser therapy for incision healing in 9 dogs, Frontiers in Veterinary Science, 2019, 5, 349.
17. **Zorab, H.K., Amin, S.Q., Mahmood, H.J., Mustafa, H.H., Abdulrahman, N.M.A.,** Dermatophytosis, In: Aguilar-Marcelino L, Younus M, Khan A, Saeed NM and Abbas RZ (eds), One Health Triad, 2023, 3, 99-106.
18. ***https://www.advancedanimalcare.com/services/dogs/laser-therapy-for-dogs
19. ***https://www.frontiersin.org/articles/10.3389/fvets.2018.00349
20. ***https://www.msdvetmanual.com/dog-owners/skin-disorders-of-dogs/ringworm-dermatophytosis-in-dogs

THE PREVALENCE OF VARROOSIS IN BEES FROM VÂLCEA COUNTY

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Summary

Varroosis, an acariosis that affects honey bees and causes severe disorders, colony weakening, and high mortality, is considered the most damaging bee disease. *Varroa destructor* is an obligate parasite of bees with a social lifestyle and which forms perennial colonies, belonging to the genus Apis. The presence of *V. destructor* on other insects is only accidental, as none of its developmental stages can develop on them. This study was performed in Vâlcea County and presents data on the evolution of varroosis over a period of 10 years (2014-2023), in accordance with the data recorded at the Directorate for Sanitary, Veterinary and Food Safety (DSVFS). The laboratory diagnosis was made by examining the bees in order to identify the *Varroa* mite. The *Varroa* mite was identified annually in bees from Vâlcea County. The lowest prevalence was reported in 2018 (3.72%) and the highest prevalence was in 2023 (13.52%). Due to the decrease in production associated with the loss of bee colonies, varroosis is and remains a threat to the health of bees in Vâlcea County. The implementation of a new and complex strategy of parasitological control is a recommendation in accordance with the results obtained in this study.

Keywords: honey bees, varroosis, Vâlcea County.

Bee parasites, and their worldwide spread, are one of the most serious problems affecting modern beekeeping today. Parasitic diseases of bees in Romania are: nosemosis, varroosis, braulosis and ascospherosis (4, 19, 20, 21, 22).

The most damaging bee disease is varroosis, an acariosis that affects honey bees and causes severe disorders, colony weakening, and high mortality (9, 11). *V. destructor* is an obligate parasite of bees with a social lifestyle and which forms perennial colonies, belonging to the genus *Apis*. The presence of *V. destructor* on other insects is only accidental, as none of its developmental stages can develop on them (7, 8).

For the purpose of reproduction, *Apis mellifera* gentler and more productive has been introduced into the area where *Apis cerana* (Asian honeybee) has its natural distribution. This new introduction has led to contact between allopatric species, i.e. species that come from separate areas. *A. mellifera* has become a new host for several genotypes of *Varroa*, and is widespread almost worldwide (12).

In Romania, there are studies describing that the prevalence of varroosis reaches high values and adult bees and brood are affected (4, 13).

The aim of the present study is to provide information on the evolution, over ten years (2014-2023), of the most important parasitic disease affecting bees, varroosis, in accordance with the data registered at the DSVFS from Valcea County.

Materials and methods

Stages for performing the study

The study was performed at the apiaries in Vâlcea County. The main activities to reach the purpose of the work are:

- collection of samples of live/dead bees and brood combs;
- collection of epidemiological data recorded in the period 2014-2023 (10 years) from the database of the DSVFS from Valcea County;
- examination of samples in the Parasitology Laboratory of DSVFS Vâlcea (Fig. 1-3);
- interpretation of results.

Fig. 1. Examination of samples in the Parasitology Laboratory of DSVFS Valcea

The laboratory examinations within DSVFS Vâlcea were carried out in compliance with Order No. March 35/30, 2016 regarding the National Program for Surveillance, Prevention, Control, and Eradication of Diseases in Animals, those transmissible from animals to humans, animal protection and environmental protection, as well as the methodological norms for the application of the Surveillance and Control Program in the field of food safety. Issuer - National Sanitary Veterinary and Food Safety Authority. Reference documents: Standard SR EN ISO/CEI 17025/2018; Manual of Diagnostics Tests and Vaccines for Terrestrial Animals OIE.

Fig. 2. Examination of bees

Fig. 3. Examination of the brood comb

Procedure (28).

25 - 50 live or dying bees per sample, combs, and residues collected from the bottom of the hive are examined.

Materials subject to examination: portions of honeycomb with overgrown brood; live, dying, or recently dead bees, queens, and drones; residues collected from the bottom of the hives.

Preparation of materials for examination: the portions of the honeycomb with covered brood are placed in the freezer at -20°C for 30 minutes; live bees are put in the freezer at - 20°C for 30 minutes or put in Ethanol or petroleum ether to kill them (to avoid stings); dead bees are washed in Ethanol or petroleum ether or in water with detergent to separate the parasites present (if positive) from their bodies; the residues collected from the bottom of the hives are dried for 24 hours, after which they are introduced into a vessel with Ethanol, mixed for 10-20 minutes. The residue is deposited on the bottom of the vessel, and the parasites float on its surface.

Examination: parts of the honeycomb are detached and the ventral side of the lids and cell walls are examined directly, macroscopically, or with a stereomicroscope; the pupae are extracted from the cells and examined macroscopically or washed repeatedly with distilled or tap water over a sieve with 1 mm mesh that retains the parasites, the interior of the cell is examined with a stereomicroscope and all developmental stages of the parasite can be detected: egg, larva, protonymph, deutonymph, and adult; bees, queens and drones are examined macroscopically or stereo microscopically and the inserts must be taken into account: head-thorax, thorax-abdomen, the base of the wings and the membranes between the abdominal tergites, the main sites of the election of the parasite on the body of adult bees;

In the case of bees killed with Ethanol, petroleum ether, or after washing with water and other detergents, the mixture is filtered through a sieve, and parasites (if positive) are detected with the naked eye or with a stereomicroscope in the deposit at the bottom of the vessel; in residues subjected to drying-washing operations, adult parasites will be able to be observed on the surface of the dish in the washing liquid.

Evaluation and interpretation of the result: normal values in the case of this parasite mean its non-existence in bee families. One parasite seen means another nine parasites unseen. The female is recognized by its flat, transversely oval body, 1.1 mm long and 1.6 mm wide, reddish-brown chitinous sleeping envelope, covered with bristles, four pairs of legs, and sucking and stinging mouthparts. *Varroa destructor* infestation goes unnoticed in the first two years after infestation, but untreated in the 3^{rd}-4^{th} year the whole family dies.

Results and discussion

The 10-years epidemiological situation for *Varroa* spp. is shown in Table 1 and Fig. 4.

Table 1

Epidemiological data for *Varroa* spp. collected from DSVFS Vâlcea

No. Crt.	**The year**	**Total bees examined**	**Infested with Varroa spp.**	**Percent (%)**
1.	2014	1835	79	4.31%
2.	2015	1858	163	8.77%
3.	2016	1717	182	10.83%
4.	2017	1684	103	6.12%
5.	2018	2122	76	3.72%
6.	2019	2183	169	7.74%
7.	2020	1887	86	4.56%
8.	2021	1851	106	5.73%
9.	2022	2804	295	10.52%
10.	2023	2974	402	13.52%

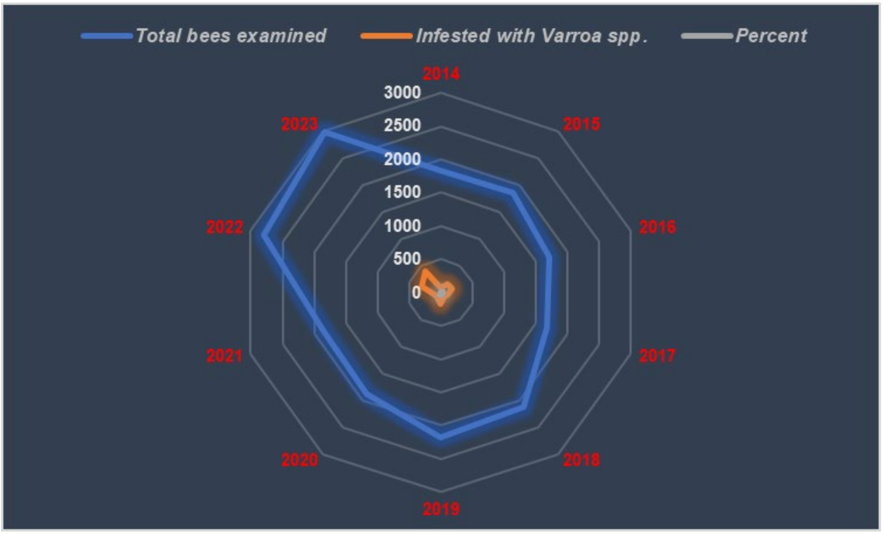

Fig. 4. Epidemiological data for *Varroa* spp. collected from DSVFS Vâlcea

The epidemiological situation over a 10-year period for *Varroa* mites in brood combs is shown in Table 2 and Figure 5 respectively.

Table 2

Epidemiological data for *Varroa* mite present in honeycombs with brood collected at DSVFS Vâlcea

No. Crt.	**The year**	**Total honeycomb with brood examined**	**Infested with *Varroa* spp.**	**Percent (%)**
1.	2014	1805	60	3.32%
2.	2015	1803	49	2.72%
3.	2016	1703	102	6.22%
4.	2017	1671	62	3.71%
5.	2018	2103	8	0.62%
6.	2019	2155	75	3.48%
7.	2020	1848	5	0.27%
8.	2021	1788	42	2.35%
9.	2022	2740	100	3.65%
10.	2023	2942	35	1.19%

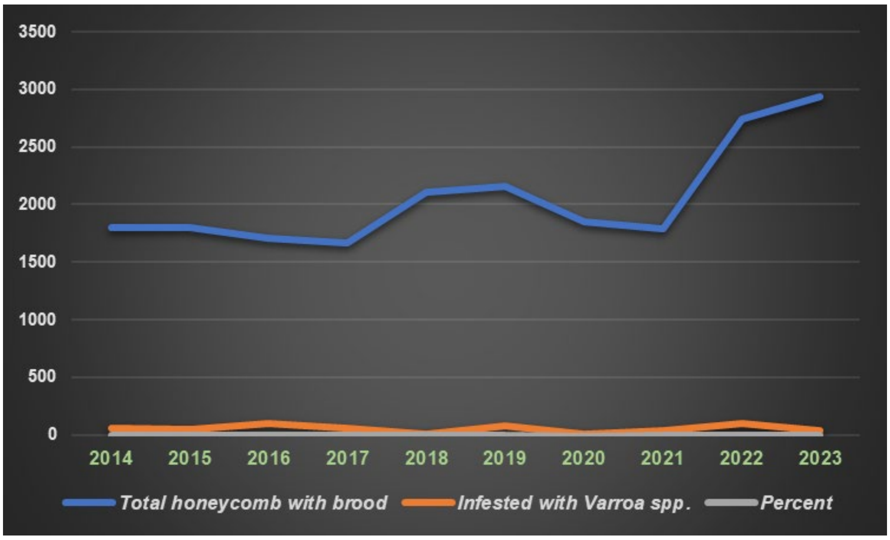

Fig. 5. Epidemiological data for *Varroa* mite present in honeycombs with brood collected at DSVFS Valcea

The results of the examination of the samples in the Parasitology Laboratory of DSVFS Valcea are shown in Figure 6 a, b.

Fig. 6. a, b *Varroa* mites

The present study evokes a ten-year overview of the prevalence of varroosis, the acariosis so important for the life of bees. The year 2023 was the representative year for the highest varroosis prevalence (13.52%), as opposed to the lowest prevalence recorded in 2018 (3.72%).

Human intervention through incorrectly executed maneuvers, rainfall regime, and transport stress in the pastoral forage system can influence the development of varroosis in a certain area over a certain period of time (3, 23).

Varroosis remains an acariosis affecting honey bees, causing severe disturbance, colony weakening, and high mortality (2, 15, 18).

In Romania, in the counties of Arad, Timis, Caras-Severin, and Bihor, the mite *V. destructor* was present in all hives examined, with a prevalence of 72% in adult bees and 96% in brood (4).

Dobrotă et al. (13) conducted a study over a period of 10 years to identify the prevalence of varroosis in apiaries from Mehedinti County (Romania). The limits of varroosis prevalence were 4.76% and 0.15%, respectively (13).

In neighboring countries (Hungary), but also France and Austria, the prevalence of varroosis reached values similar to those identified in Romania, as hives located in different regions are affected by the presence of *V. destructor* mite (16).

In Italy, Bava et al. (5) determined the level of *V. destructor* infestation by examining 840 samples of adult bees and reported a 54.7% prevalence of the mite.

In Ethiopia, Shegaw et al. (25) reported a mite prevalence ranging from 48.44% to 83.33% in 384 bee colonies, with maximum values during wet seasons. One year later, Robi et al. (24) reported a *V. destructor* mite prevalence of 39.3% in adult bees and 43.2% in brood, respectively.

Keshlaf et al. (17) conducted a study of 66 beehives in different regions of Libya. The research showed with certainty the distribution of *Varroa* mites in all regions examined, with a different degree of mite density in hives located in the south of the country compared to colonies located in the north.

In northern Iran, Bokaie et al. (6) identified 80 infested bee centers and founded the prevalence of *V. destructor* mite to be 92%.

In Thailand, Thongsawang et al. (26) sampled 1152 bees from 144 hives. The results revealed a prevalence of *V. destructor* mites of 50.69% at the colony level and 22.74% at the hive level, respectively, with the most affected area being the northern region of the country.

In 2020, Fanelli and Tizzani (14) turned to the OIE database on the global distribution of *Varroa* spp. over a 13-year period (2005-2018) and identified that in the period studied, 53.4% of countries reported the presence of the mite at least once. At the opposite pole of the statistic, 9% of countries were reported free (14).

In the USA, Abban et al. (1) examined 4039 samples of adult bees from 2015-2022 and reported a prevalence of *V. destructor* ranging from 8.21% to 85.14%. The steady increase in prevalence from 2015 to 2018 by up to 4.7% was followed by a stagnation between 2018 and 2021, followed by a significant decrease in 2022 (1).

In Mexico, in 2023, Correa-Benítez et al. (10) assessed the mite situation in 369 apiaries and reported a prevalence of 83.5% in the apiaries examined, although the Pacific Coast region is recognized as an endemic area for *V. destructor* whose prevalence reached values of up to 95% (10).

In Alabama, Ward et al. (27) monitored *V. destructor* mite infestations in different seasons and reported that one mite per 100 bees was present in late winter and 5-10 mites per 100 bees in late summer, respectively.

It should be noted that over a period of 10 years, the presence of *Varroa* mites is constant, with cases of varroosis being diagnosed every year in bees in Valcea County. At the beginning of the study, in 2014, the prevalence of varroosis reached a value of 4.31% and a maximum of 13.52% in 2023.

Conclusions

In Vâlcea County, varroosis is and remains a threat to bee health, through decreased production of bee products, loss of bee colonies, and, implicitly, the aplication of new and more complex control strategies.

Over a period of 10 years, the presence of *Varroa* mite is constant, with cases of varroosis being diagnosed every year in bees in Vâlcea County. The prevalence of *Varroa* mite was recorded with a minimum value in 2018 and the higher value in 2023.

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References

1. **Abban, S., Smith, B., Corona, M., Cook, S.C., Evans, J.D., Chen, Y., Alburaki, M.,** Prevalence and distribution of *Varroa destructor* and *Nosema spp.* in symptomatic honey bee colonies across the USA from 2015 to 2022, Scientific Reports, 2024, 14, 1726.
2. **Amdam, G.V., Hartfelder, K., Norberg, K., Hagen, A., Omholt, S.W.,** Altered physiology in worker honey bees (*Hymenoptera: Apidae*) infested with the mite *Varroa destructor* (*Acari: Varroidae*): a factor in colony loss during overwintering?, Journal of Economic Entomology, 2004, 97, 741-747.
3. **Bakonyi, T., Farkas, R., Szendroi, A., Dobos-Kovacs, M., Rusvai, M.,** Detection of acute bee paralysis virus by RT-PCR in honey bee and *Varroa destructor* field samples: rapid screening of representative Hungarian apiaries, Apidologie, 2002, 33, 63-74.

4. **Balint, A.,** Parazitozele albinelor în vestul României. Strategii de control, Teză de doctorat, USAMVB Timișoara, 2002.
5. **Bava, R., Castagna, F., Palma, E., Carlotta, Ceniti, C., Maurizio Millea, M., Carmine Lupia, C., Domenico, Britti D., Vincenzo Musella, V.,** Prevalence of *Varroa destructor* in Honeybee (*Apis mellifera*) Farms and *Varroosis* Control Practices in Southern Italy, Microorganisms, 2023, 11, 5, 1228.
6. **Bokaie, S., Sharifi, L., Mehrabadi, M.,** Prevalence and epizootical aspects of *Varroasis* in Golestan province, northern Iran, Journal of Arthropod-Borne Diseases, 2013, 8, 1, 102-107.
7. **Boot, W., Calis, J., Beetsma, J.,** Differential periods of *Varroa* mite invasion into worker and drone cells of honey bees, Experimental and Applied Acarology, 1992, 16, 295-301.
8. **Boot, W., Calis, J., Beetsma, J.,** Invasion of *Varroa* mites into honeybee brood cells. When do brood cells attract *Varroa* mites?, Proceedings of the Section Experimental and Applied Entomology of the Netherlands Entomological Society, 1991, 2, 154-156.
9. **Calis, J., Fries, I., Ryrie, S.C.,** Population modelling of *Varroa jacobsoni* Oud, Apidologie, 1999, 30, 111-124.
10. **Correa-Benítez, A., Anguiano-Baez, R. Heneidi-Zeckua, A. Dávalos-Flores, J.L., Peña-Haaz, N.T., Pérez-Martínez, E.E., Carbajal-Rodríguez, M., Vasquez-Valencia, I., Almazán-Maldonado, N., Petukhova, T., Guzman-Novoa, E.,** Prevalence of Adult Honey Bee (*Apis mellifera L.)* Pests and Pathogens in the Five Beekeeping Regions of Mexico, Animals, 2023, 13, 11, 1734.
11. **Dărăbuş, G., Oprescu, I., Morariu, S., Mederle N., Ilie M., Imre M.,** Parazitologie şi boli parazitare, Ed. Mirton, Timişoara, 2022.
12. **De Guzman, L.I., Rinderer, T.E.,** Identification and comparasion of *Varroa* species infesting honey bees, Apidologie, 1999, 30, 85-95.
13. **Dobrotă, R.S., Lungu, G., Ghilean, B.M., Marin, A.M., Savici, J., Mederle, N.,** Epidemiological study on the evolution over a 10-year period of varroosis in bees from Mehedinti county, Lucrări Științifice Medicină Veterinară Timișoara, 2023, 56, 3, 38-46.
14. **Fanelli, A., Tizzani, P.,** Spatial and temporal analysis of varroosis from 2005 to 2018, Research in Veterinary Science, 2020, 131, 215-221.
15. **Fernandez, N., Coineau, Y.,** *Varroa*: The serial bee killer mite, Atlantica, Biarritz, Franţa, 2006.
16. **Forgách P., Bakonyi T., Tapaszti Z., Nowotny N., Rusvai M.,** Prevalence of pathogenic bee viruses in Hungarian apiaries: situation before joining the European Union, Journal of Invertebrate Pathology, 2008,98, 2, 235-238.
17. **Keshlaf, M.M., Mirwan, H.B., Ghana, S., Mubrok, S., Shaibi, T.,** Prevalence of *Varroa* mites (*Varroa destructor* Anderson & Trueman) and bee lice (*Bruala coeca* Nitzsch) in honey bee (*Apis mellifera* L.) colonies in Libya, Open Veterinary Journal, 2023, 13, 7, 834-838.

18. **Mederle, N., Balint, A., Morariu, S., Dărăbuș, G.,** Colony Collapse Disorder (CCD) - a review, Romanian Journal of Veterinary Medicine & Pharmacology, 2017, 2, 142-149.
19. **Mederle, N., Balint, A., Morariu, S., Hora, F., Darabus, G.,** Morphometric study of *Nosema* spores isolated from honey bee from Western Romania, Lucrări Științifice Medicină Veterinară, Timisoara, 2015, 48, 103-108.
20. **Mederle, N., Balint, A., Morariu, S., Hora, F.Ş., Mederle, O., Marincu, I., Darabus, Gh.,** Research on the prevalence of honey bees nosemosis in Arad County, Journal of Biotechnology, 2015, 208, S5-S120.
21. **Mederle, N., Kaya, A., Balint, A., Morariu, S., Oprescu, I., Ilie, M., Imre, M., Ciobanu, Gh., Dărăbuș, Gh.,** Epidemiological investigations on honey bees nosemosis in Timis County, Lucrări Științifice Medicină Veterinară, Timisoara, 2017, 2, 142-147.
22. **Mederle, N., Kaya, A., Morariu, S., Crăciun, C., Balint, A., Radulov, I., Dărăbuș, Gh.,** Innovative therapeutic approaches for the control and prevention of honeybee nosemosis: implications in the bee's life and the quality of their product, Geografica Timisensis, 2016.
23. **Mendoza, Y., Diaz-Cetti, S., Ramallo, G., Santos, E., Porrini, M., Invernizzi, C.,** *Nosema ceranae:* Winter Control: Study of the Effectiveness of Different Fumagillin Treatments and Consequences on the Strength of Honey Bee (Hymenoptera: *Apidae*) Colonies, Journal of Economic Entomology, 2017, 110, 1-5.
24. **Robi, D.T., Temteme, S., Aleme, M., Bogale, A., Getachew, A., Mendesil, E.,** Epidemiology, factors influencing prevalence and level of varroosis infestation (*Varroa destructor*) in honeybee (*Apis mellifera*) colonies in different agroecologies of Southwest Ethiopia, Parasite Epidemiology Control, 2023, 23, e00325.
25. **Shegaw, T.,** Diagnostic survey on varroa mite (*Varroa destructor*) prevalence in South-Western Ethiopia, Bonga Agricultural Research Article, 2022, 2143610.
26. **Thongsawang, T., Rueangsonn, P., Boonyo, K., Wongphruksasoong, V., Suphanchaimat, R.**, Situation Analysis of Varroosis and Tropilaelaps Infestation of Honeybees in Thailand, 2017-2018, Veterinary Medicine: Research and Reports, 2021, 12, 169-176.
27. **Ward, K., Danka, R., Ward, R.,** Comparative performance of two mite-resistant stocks of honey bees (*Hymenoptera: Apidae*) in Alabama beekeeping operations, Journal of Economic Entomology, 2008, 101, 3, 654-659.
28. ***https://www.aca.org.ro/wp-content/uploads/2020/05/Raspuns-ANSVSA-1719-din-15.05.2020-la-adresa-ACA-124-din-09.04.2020.pdf.

TICK INFESTATION IN MOUFLON (*OVIS ARIES MUSIMON*) FROM TIMIȘ COUNTY – CASE REPORT

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Summary

The mouflon (*Ovis aries musimon*) is a wild mammal, a subspecies of primitive sheep, which originates from Corsica and Sardinia, but whose population has also expanded to other regions of Europe. This animal can also be a host for ticks. Ticks are obligatory ectoparasitic arthropod vectors of terrestrial vertebrates with hematophagous feeding habits. These arthropods, with a wide range of hosts, transmit numerous diseases such as theileriosis, babesiosis, anaplasmosis, or lumpy skin disease most of them being zoonoses. The aim of this study was to identify tick species found on mouflons in Timiș County. Twelve ticks were collected at various developmental stages from a 4-year-old male from the Șarlota Hunting Complex (Timiș County). Species such as *Ixodes* and *Dermacentor* were found and identified by their morphological aspects. In conclusion, the study highlights the presence of ticks, potential disease vectors, on mouflons in Timis County, highlighting the importance of surveillance for both animal and public health. Understanding the morphological characteristics of these ticks helps to identify them in the context of developing strategies for effective control and prevention of tick-borne diseases in wildlife populations.

Keywords: Ticks, *Anaplasma* spp., *Babesia* spp., *Ehrlichia* spp., mouflon.

Ticks are obligatory vectors of terrestrial vertebrates, belonging to the order *Acari*, and they hold particular importance for veterinary medicine. Due to their hematophagous feeding behaviour, they transmit numerous blood-borne pathogens such as anaplasmosis, babesiosis, ehrlichiosis, etc., some of which are zoonoses (5, 12, 14, 17).

Ticks are divided into 3 principal families, each having different hosts: *Ixodidae*, *Argasidae*, and *Nuttalliellidae*. *Ixodidae* includes species that infest mammals, among the most common being *Ixodes* spp., *Dermacentor* spp., and *Rhipicephalus* spp. This family is represented by hard ticks. *Argasidae* comprises 4 genera, with the most important being the genus *Argas*. Soft ticks, which parasitize birds, belong to this family. The first two families encompass numerous species, reaching into the hundreds, unlike the third family, *Nuttalliellidae*, which includes only one species: *Nuttalliella namaqua* (3, 11, 23, 28).

According to the anatomical structure of mites, from a morphological standpoint, ticks present gnathosoma and idiosoma. The gnathosoma has in the structure the entire complex of components aiding in attachment to the host skin surface and facilitating blood ingestion, thus considered the mouthpart. Based on the length of the chelicerae present in the structure of this segment, ticks are divided into two categories: longirostral (*Ixodes*, *Hyalomma*) and brevirostral (*Dermacentor*). Idiosoma represents the body of the tick, with a chitinous shield, a reservoir for ingested blood, and four pairs of legs (2, 27, 28).

The life cycle of ticks is complex and comprises several stages, allowing them to feed on one or more hosts. For example, *Rhipicephalus annulatus* can grow on one host, *Hyalomma marginatum* on two hosts, and species of the genera *Dermacentor, Ixodes* and *Amblyomma* need three hosts (31). The female lays around 1000-3000 eggs from which larvae emerge. At this stage, a resemblance to insects is noted, as all ticks have only 3 pairs of legs. After the larval stage, the nymph develops, which will differentiate into one of the two sexes. During the life cycle, the male dies after fertilisation and the female will die after laying eggs (1, 28).

Being widely distributed in nature, especially in the wild where control methods are poor, among the numerous hosts of ticks is the mouflon. The mouflon (*Ovis aries musimon*) is a wild mammal, representing a subspecies of the primitive sheep whose origin comes from the Mediterranean islands of Corsica and Sardinia. Its habitat includes mountainous areas and deciduous forests, thus being introduced into European countries such as Spain, Germany, France, and even Romania, after several acclimatization attempts, in regions such as Dobrogea, the center of Muntenia, and the counties of Alba and Argeș (21, 25, 26).

Materials and methods

The study was conducted on a 4-year-old male mouflon. It was taken from the hunting complex Șarlota in Timiș County and brought for investigation to the Parasitology and Parasitic Diseases Clinic within the Faculty of Veterinary Medicine in Timișoara.

Following the inspection, a total of 12 ticks were observed, unevenly distributed in the abdominal region and the right thoracic limb region. The body of each tick was grasped with standard forceps and rotated to remove them from the mouflon's skin. After detachment from the ruminant's body, the ticks were initially placed on a single Petri dish. Following the collection of all 12 ticks, their identification was carried out one by one using a stereomicroscope. Morphological aspects were observed to achieve this identification (6). Subsequently, after examination under the stereomicroscope using multiple Petri dishes, they were grouped based on their gender.

Results and discussions

Based on morphological characters, out of a total of 12 ticks, 8 belong to the genus *Ixodes* and 4 to the genus *Dermacentor*.

Both females and males of the genus *Ixodes* have been identified, with females accounting for 5 out of 8. They were collected in the fasting or semi-engorged stage. These stages were observed according to the appearance of the dorsal part of the idiosome. Regardless of stage, the female is larger than the male. Fasting females show sclerotisation of the shield and blood reservoir, and after ingestion of a moderate amount of blood, they increase in volume. A characteristic of the semi-filled reservoir are the central longitudinal depressions, which disappear as the reservoir fills, giving it a globular appearance (Fig. 1). Males in the fasting stage are very small and the shield has an extension to the posterior end of the idiosome (Fig. 2). Because of this shield location and its hard consistency, the male does not require intense feeding, being also the cause of the inability to grow like the female. A similarity between the two sexes is the presence of a developed foreleg coxa, which plays an important role in firmly grasping the host (10, 29).

Fig. 1. *Ixodes* female, semi-engorged

Fig. 2. *Ixodes* male, fasting

Another morphological characteristic of ticks of the genus *Ixodes*, macroscopically visible, is their colour. These species have a light brown idiosome colour, especially when they are partially engorged, and dark brown when fully engorged (29).

The 4 ticks identified as belonging to the genus *Dermacentor* were males (Fig. 3). Species of this genus are larger than those of the genus *Ixodes*. Males have the same shield location, but the shield has a mosaic appearance with a pattern characteristic of the genus *Dermacentor*. Another particularity is the presence of 11 festoons on the dorsal surface at the caudal extremity (Fig. 3). The entire body of the tick as well as the shield is reddish in colour and the pattern is cream-coloured. The four pairs of legs are of considerable thickness compared to species of the genus *Ixodes*, and help to attach to the host. While the shield of *Ixodes* ticks gives the impression of a smooth appearance, that of *Dermacentor* species gives the impression of a wavy appearance. Due to the presence of this shield, these males will, like *Ixodes* males, consume small amounts of blood and are unable to reach the size of females (4, 7).

Different species within the genus *Dermacentor* have been identified. *D. reticulatus* and *D. marginatus* were the two species that were present. Semilunar porous regions and coxa 1 with uneven spurs are distinctive to the morphology of *D. marginatus*, whereas round porous areas and coxa 1 with equal spurs are specific to *D. reticulatus* (6).

Fig. 3. *Dermacentor* male

The tick species identified in this case report were *Ixodes ricinus*, *Dermacentor reticulatus* and *Dermacentor marginatus.*

Ticks are widespread in Europe, especially in woodland areas. They become active from early spring to late autumn, but as temperatures drop their activity is reduced. Due to this high prevalence and increased rate of breeding, these ectoparasites inevitably come into contact with animals (10, 13, 20).

Various studies state that ticks require both the existence of hosts and favourable environmental conditions, such as temperature and humidity to survive, as part of their life cycle is carried out in nature, on the soil surface, and not in contact with hosts. Increases in temperature values in recent years have proven to favour their survival but have increased the need to find hosts. Under these conditions, they have shortened their development period and at the same time increased their resistance to various factors, thus leading to lower mortality. Importantly, these changes have a negative effect when temperatures reach very high values. In terms of humidity, increasing humidity leads to increased activity. In dry environments, ticks have to return to wet soils to rehydrate. Rainfall has an antagonistic effect, leading to decreased activity (9, 13, 18).

According to the literature, the most widespread species in Romania is *Ixodes ricinus*. This species is ubiquitous and can infest a wide range of hosts, especially ruminants. *I. ricinus* is also of particular importance for human health, spreading numerous potentially zoonotic pathogens. The prevalence of this species ranges from 42% to 86% depending on the geographical area, with the highest percentage in forested areas. In addition to *Ixodes* species, *Dermacentor marginatus* is found in Romania, especially in western and southeastern areas. These two species are also widespread all over Europe as shown in Fig. 4 and 5 (32, 33). In other countries such as Germany, *Ixodes ricinus* was identified in 1,855 locations, *Dermacentor marginatus* was also reported in 77 locations and *Dermacentor reticulatus* in 96 locations. In northwest Spain, *I. ricinus* was also the most widespread species, being present in 17 different locations, and other species identified were *Dermacentor marginatus*, *Dermacentor reticulatus* and *Ixodes frontalis* (8, 15, 16).

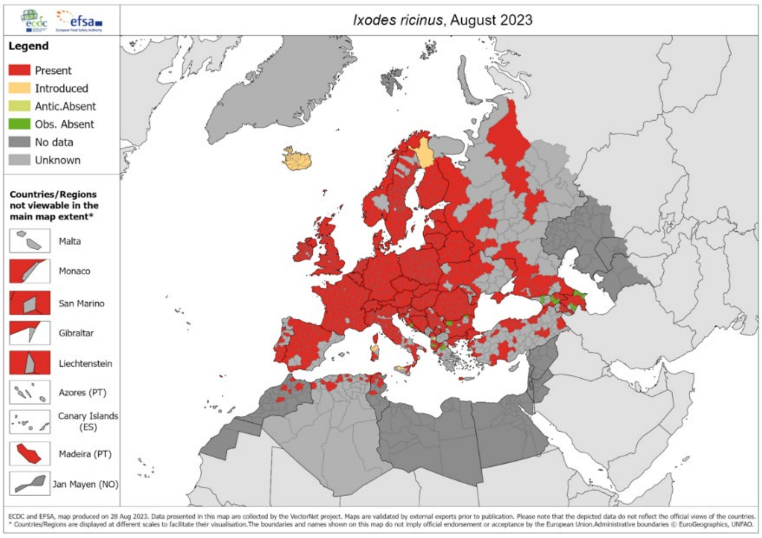

Fig. 4. Distribution of *I. ricinus* in Europe (32)

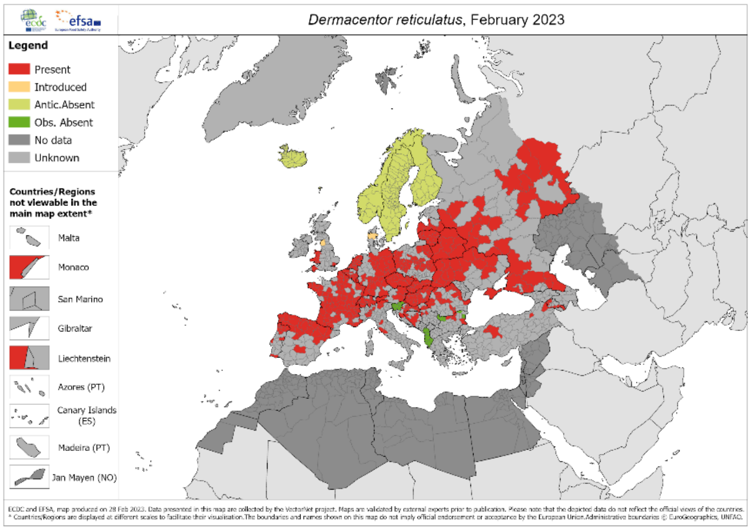

Fig. 5. Distribution of *D. reticulatus* in Europe (33)

Worldwide, ticks are a hotly debated topic because of their impact on human and animal health, both of which are potential hosts. As vectors, they spread a wide spectrum of pathogens (viruses or bacteria) and are the cause of many diseases such as babesiosis, anaplasmosis, erlichiosis, tick-borne encephalitis, etc. All these diseases affect the quality of life of animals and humans, in some cases with fatal consequences. For animals, tick-borne diseases can lead to significant economic losses. These global problems lead to a constant search for effective control methods and require continuous development of knowledge about ticks (18, 30).

Conclusions

In conclusion, the study highlights the presence of ticks, potential disease vectors, on mouflons in Timis County, highlighting the importance of surveillance for both animal and public health. Understanding the morphological characteristics of these ticks helps to identify them in the context of developing strategies for effective control and prevention of tick-borne diseases in wildlife populations.

References

1. **Anderson, J.F.,** The natural history of ticks, Medical Clinics of North America, 2002, 86, 2, 205-18.
2. **Anderson, J.F., Magnarelli, A.L.,** Biology of ticks, Infectious Disease Clinics of North America, 2008, 22, 195-215.

3. **Barker, S.C., Murrell, A.,** Systematics and evolution of ticks with a list of valid genus and species names, Parasitology, 2004, 129, 1, 15-36.
4. **Bartosik, K., Buczek, A., Buczek, W., Buczek, A.M., Kulina, D., Koman-Iżko, A.,** Host feeding behaviour of *Dermacentor reticulatus* males in relation to the transmission of pathogens, Annals of Agricultural and Environmental Medicine, 2019, 17, 26, 2, 227-230.
5. **Boulanger, N., Boyer, P., Talagrand-Reboul, E., Hansmann, Y.,** Ticks and tick-borne diseases, Médecine et Maladies Infectieuses, 2019, 49, 87-97.
6. **Estrada-Peña, A., Mihalca, A.D., Petney, T.N.,** Ticks of Europe and North Africa, 2017.
7. **Földvári, G., Široký, P., Szekeres, S., Majoros, G., Sprong, H.,** *Dermacentor reticulatus*: a vector on the rise, Parasites and Vectors, 2016, 1, 9, 1, 314.
8. **Giubega, S., Ilie, M.S., Luca, I., Florea, T., Dreghiciu, C., Oprescu, I., Morariu, S., Dărăbuș, G.,** Seroprevalence of anti-*Theileria equi* antibodies in horses from three geographically distinct areas of Romania, Pathogens 2022, 11, 669.
9. **Gray, S.J.,** *Ixodes ricinus* seasonal activity: Implications of global warming indicated by revisiting tick and weather data, International Journal of Medical Microbiology, 2008, 298, 19-24.
10. **Gray, J.,** Review The ecology of ticks transmitting Lyme borreliosis, Experimental and Applied Acarology, 1998, 22, 249-258.
11. **Horak, I.G., Camicas, J.L., Keirans, J.E.,** The *Argasidae*, *Ixodidae* and *Nuttalliellidae* (*Acari*: *Ixodida*): a world list of valid tick names, Ticks and Tick-Borne Pathogens, Springer, Dordrecht, 2003.
12. **Khan, A., Ahmed Muhammed, A., Nasreen, N., Iqbal, F., Cossio-Bayugar, R., Ali Sha, S.S., Alanazi, A.D., Zajac, Z.,** Tick-borne haemoparasitic diseases in small ruminants in Pakistan: Current knowledge and future perspectives, Saudi Journal of Biological Sciences, 2022, 29, 4, 2014-2025.
13. **Léger, E., Vourc'h, G., Vial, L.,** Changing distributions of ticks: causes and consequences, Experimental and Applied Acarology, 2013, 59, 219-244.
14. **Levin, L.M.,** Division of Vector-borne diseases, Centers for Disease Control and Prevention, 2022.
15. **Mihalca, A.D., Dumitrache, M.O., Magdaş, C.,** Synopsis of the hard ticks (*Acari: Ixodidae*) of Romania with update on host associations and geographical distribution, Experimental and Applied Acarology, 2012, 58, 183-206.
16. **Mihalca, A.D., Gherman, C.M., Magdaş, C.,** *Ixodes ricinus* is the dominant questing tick in forest habitats in Romania: the results from a countrywide dragging campaign, Experimental and Applied Acarology, 2012, 58, 175-182.

17. **Nasirian, H.,** Monitoring of hard tick parasitism in domestic ruminants: A scale evidence for policymakers, Veterinary Parasitology: Regional Studies and Reports, 2023, 41, 100878.
18. **Ogden, N.H., Lindsay, L.R.,** Effects of Climate and Climate Change on Vectors and Vector-Borne Diseases: Ticks Are Different, Trends in Parasitology, 2016, 32, 8, 646-656.
19. **Piesman, J, Eisen, L.,** Prevention of tick-borne diseases, Annual Review of Entomology, 2008, 53, 323-43.
20. **Popescu, D.,** Ecologia și distribuția căpușelor în Europa, Revista Europeană de Biologie și Ecologie, 2019, 25, 3, 45-60.
21. **Ptak Grazyna, C.M., Barboni, B., Muzzeddu, M., Cappai, P., Tischner, M., Loi, P.,** Preservation of the wild european mouflon: The first example of genetic management using a complete program of reproductive biotechnologies, Biology of Reproduction, 2002, 66, 796-801.
22. **Remesar, S., Fernández, P.D., Venzal, J.M., Pérez-Creo, A., Prieto, A., Estrada-Peña, A., López, C.M., Panadero, R., Fernández, G., Díez-Baños, P., Morrondo, P.,** Tick species diversity and population dynamics of *Ixodes ricinus* in Galicia (north-western Spain), Ticks and Tick-borne Diseases, 2019, 10, 132-137.
23. **Roshdy, M.A., Hoogstraal, H., Banaja, A.A.,** *Nuttalliella namaqua* (*Ixodoidea*: *Nuttalliellidae*): Spiracle structure and surface morphology, Z. Parasitenkd, 1983, 69, 817-821.
24. **Rubel, F., Brugger, K., Monazahian, M.,** The first German map of georeferenced ixodid tick locations, Parasites and Vectors, 2014, 7, 477.
25. **Rusu, Ş.,** Mouflon’s parasitofauna (*Ovis ammon musimon*, Pallas, 1811) from various natural and anthropised biotopes from the Republic of Moldova, Lucrări Ştiinţifice Seria Medicină Veterinară, 2020, 63, 2, 100-107.
26. **Schröder, O., Lieckfeldt, D., Lutz, W.,** Limited hybridization between domestic sheep and the European mouflon in Western Germany, European Journal of Wildlife Research, 2016, 62, 307-314.
27. **Sonenshine, E.D.,** Biology of ticks, Oxford University Press, 2014.
28. **Sonenshine, E.D.,** Ticks, (Second Edition), Academic Press, 2009.
29. **Starck, J.M., Mehnert, L., Biging, A.,** Morphological responses to feeding in ticks *(Ixodes ricinus)*, Zoological Letters, 2018, 20, 4.
30. **Zannou, O.M., Ouedraogo, A.S., Biguezoton, A.S., Abatih, E., Coral-Almeida, M., Farougou, S., Yao, K.P., Lempereur, L., Saegerman, C.,** Models for studying the distribution of ticks and Tick-Borne Diseases in animals: A Systematic Review and a Meta-Analysis with a Focus on Africa, Pathogens, 2021, 10, 893.
31. ***https://www.cdc.gov/dpdx/ticks/index.html

32.***https://www.ecdc.europa.eu/en/publications-data/ixodes-ricinus-current-known-distribution-august-2023
33.***https://www.ecdc.europa.eu/en/publications-data/dermacentor-reticulatus-current-known-distribution-february-2023

STUDY REGARDING THE MORPHOLOGICAL IDENTIFICATION OF *DEMODEX* MITE SPP. IN WOLF (*CANIS LUPUS*) FROM A HUNTING GROUND, ROMANIA

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Summary

The wolf (*Canis lupus*) is the most widespread species of large carnivores in Romania. It can be parasitized with endoparasites, but also with ectoparasites. Demodicosis is an ectoparasitosis caused by the mites *Demodex* spp. This study describes the identification of possible ectoparasites present in skin lesions, following the post-mortem examination of four *C. lupus* collected from a hunting ground from Romania, based on legal provisions. The study was carried out at the Discipline of Parasitology and Parasitic Diseases within the Faculty of Veterinary Medicine from Timișoara/ University of Life Sciences "King Mihai I" from Timișoara. Following the clinical examination, skin lesions characterized by alopecia, desquamation and lichenification were identified located on the head, limbs and tail. Corroborating the results of the clinical examination with the microscopic analysis of the skin scrapings, the diagnosis of demodicosis was established. This study describes the first morphological identification of *Demodex* spp. mites in wolves.

Keywords: *Canis lupus, Demodex* spp., morfological identification.

Large carnivores in Romania are at the top of the trophic pyramid and represent the key elements in ecological relationships in natural ecosystems (25).

Canis lupus is the largest member of the Canidae family and is widespread in the USA, Europe and Asia. The distribution of *C. lupus* is closely related to climatic factors, environmental factors and food sources. In Romania, *C. lupus* populations are frequently found in the Carpathian basin and have an important role in regulating the biodiversity of the ecosystem through the prey-predator relationship. *Canis lupus* and the domestic dog (*Canis lupus familiaris*) share a common ancestor. In the last years, in Romania the *C. lupus* population increased by approximately 0.5% (24, 26).

Canis lupus is a natural reservoir of endoparasites and ectoparasites. The main ectoparasites that infest *C. lupus* are mites, fleas and ticks. Parasitism with mites of the genera *Sarcoptes* was most often diagnosed in *C. lupus* (1, 9, 16, 18, 19, 22).

Demodicosis is a parasitic, depilatory, nonpruritic dermatosis caused by mites of the genera *Demodex*. Demodectic mites are permanent parasites that live and develop in hair follicles, which is why demodicosis is one of the most important acariosis affecting carnivores (3, 10, 14, 20). The most identified species of *Demodex* in domestic carnivores were *D. canis*, *D. cornei* and *D. injai* (11, 12, 13).

The aim of the study was to identify the possible etiological agents responsible for the skin lesions in four *C. lupus*.

Materials and methods

Case presentation

The carcasses of four *C. lupus* that caused damage were harvested on the basis of legal exemptions approved by the Ministry of the Environment, Water and Forests, from a hunting ground from Romania. Their examination was carried out in the clinic of Parasitology and Parasitic Diseases within the Faculty of Veterinary Medicine/ University of Life Sciences "King Mihai I" from Timișoara.

The steps of the diagnosis were:

- individual general inspection of *C. lupus*
- skin lesions identification
- carrying out laboratory methods: skin scrapings

Skin scraping is a gold diagnostic method used to identify mites such as *Demodex*, *Sarcoptes* or *Notoedres*.

- microscopic examination of scrapings with a 10 X objective (5).

Results and discussions

Upon general examination, the carcasses of the *C. lupus* were severely emaciated and had widespread skin lesions.

The identified skin lesions were expressed by: alopecia, desquamation, lichenification and hyperkeratosis (Fig. 1. a, b, c, d).

The deep skin scrapings and their microscopic examination confirmed the presence of *Demodex* spp. mites identified on the basis of morphological features (Fig. 2).

The diagnosis established following the obtained results was demodicosis.

This study provides additional information regarding *Demodex* mite parasitism in wildlife. The main mites affecting domestic and wild carnivores are *Sarcoptes* spp., *Otodectes* spp., *Notoedres* spp. and *Demodex* spp. (4, 20).

Acariosis are very widespread diseases, affecting domestic and wild canids and having a negative impact on wildlife populations and public health (23).

Sarcoptes spp. are the mites that most often affect wild canids, the highest prevalence being reported in foxes (*Vulpes vulpes*), but also in *C. lupus* (7).

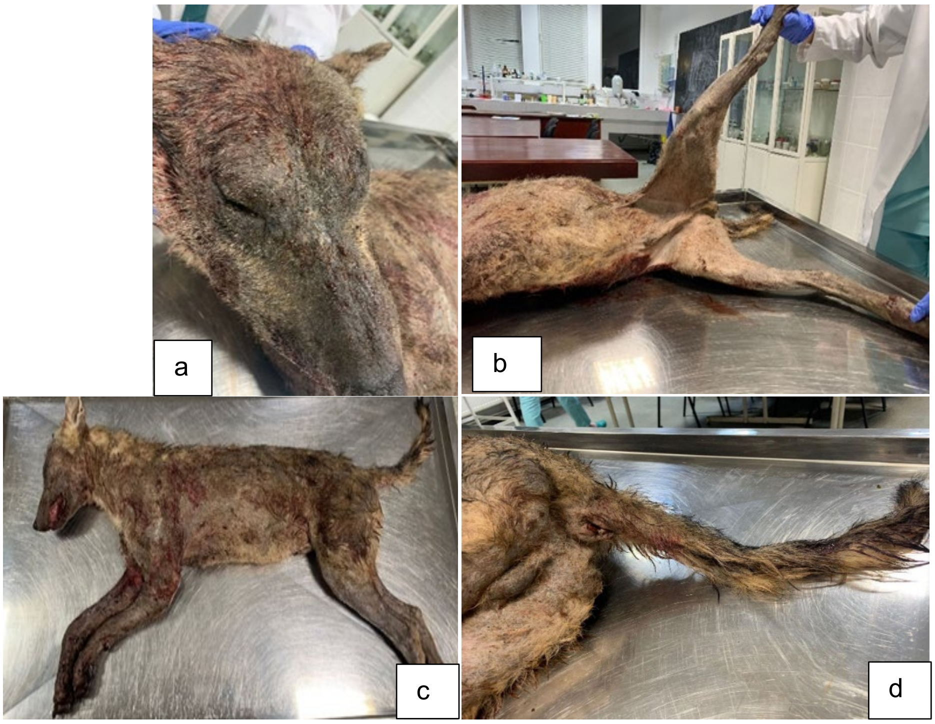

Fig. 1. a, b, c, d. Skin lesions; (a) – lichenification; (b) – alopecia; (c) – alopecia and hyperkeratosis; (d) – scaling and alopecia

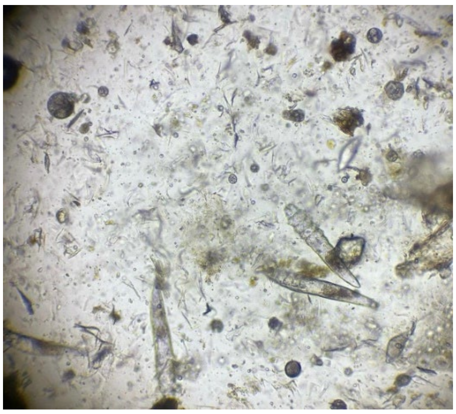

Fig. 2. *Demodex* spp. – egg and adults (10X objective)

In Spain, three *C. lupus* were examined post-mortem that presented skin lesions expressed by alopecia, lichenification, crusts and hyperkeratosis located in the limbs, inguinal area and in the lumbo-sacral and ischial regions. The diagnosis was established on the basis of deep skin scrapings, and following the microscopic examination of the scrapings, the *Sarcoptes* mite was identified (6).

A study carried out in 2023 highlights the first identification of the *Demodex injai* mite in the jackal (*Canis aureus*) in Romania (17).

Morphological identification associated with molecular analysis of *Demodex* mites in *C. lupus familiaris* in India revealed the presence of species *D. injai*, *D. canis* and *D cornei*, the largest of these three species being *D.injai* (2).

A study performed by Izdebska and Rolbiecki highlights the biodiversity of *Demodex* species and parasitized hosts. In this study, literature data from the period 1842-2020 were collected and 122 species of *Demodex* and their hosts were reported. The study aimed to identify the species of the genus *Demodex* and to diagnose the disease. In wolf no species of *Demodex* was identified (11).

Parasitism with *Demodex* spp. is frequently diagnosed in dogs (*C. lupus familiaris*), demodicosis being one of the most important acarioses. In wild canids, the identification of these mites has been reported in *V. vulpes*, *C. aureus* and *C. lupus* (1, 8, 14, 15, 23).

A study conducted in Poland reveals an increased prevalence of parasitism with *D. canis* in *C. lupus familiaris*, being identified in 42% of 39 cases, compared to *D. cornei* which was identified in 7% of cases (13).

A study carried out in Romania in 2002 revealed a prevalence of 23.67% of demodicosis, 80 *C. lupus familiaris* out of 338 were infested with *Demodex*, and 70 out of 80 *C. lupus familiaris* were less than one year old. Demodicosis was not reported in infant puppies (21).

Corroborating the data reported to date on demodicosis in wild canids, this study provides additional information on *Demodex* spp. parasitism in *C. lupus*.

Conclusions

This study reports the first morphological identification of *Demodex* spp. mites in wolves. Clinical examination, identification of skin lesions and accuracy of skin scraping are the key points in the diagnosis of demodicosis.

References

1. **Bermudez, S., Miranda, R.,** Distribution of ectoparasites of *Canis lupus familiaris* L. (*Carnivora: Canidae*) from Panama, Revista MVZ Córdoba, 2011, 1, 1, 2274-2282.
2. **Chaudhary, A.K., Dimri, U., Ajith, Y., Madesh, E., Yadav, S., Kavitha, K., Angmo, S.,** Comparative morphometric analysis for differentiation of three

Demodex mite species causing canine demodicosis, International Journal of Current Microbiology and Applied Sciences, 2020, 9, 12, 2151-2155.

3. **Cosoroaba, I.,** Parazitologie veterinară, Ed. Mirton, Timisoara, 2000.
4. **Darăbus, Gh., Oprescu, I., Morariu, S. Mederle, N., Ilie, M.,** Ghid practic în bolile parazitare, vol. I, Ed. Agroprint, Timişoara, 2013.
5. **Darăbus, Gh., Oprescu, I., Morariu, S., Mederle, N., Ilie, M., Imre, M.,** Parazitologie și Boli Parazitare, Ed. Mirton, Timişoara, 2022.
6. **Domínguez, G., Espí, A., Prieto, J.M., De La Torre, J.A.,** Sarcoptic mange in *Iberian wolves* (*Canis lupus signatus*) in northern Spain, Veterinary Record, 2008, 162, 754-755.
7. **Fuchs, B., Zimmermann, B., Wabakken, P., Bornstein, S., Mansson, J., Evans, A.L., Liberg, O., Sand, H., Kindberg, J., Ågren, E.O., Arnemo, J.M.,** Sarcoptic mange in the *Scandinavian wolf Canis lupus population*, BMC Veterinary Research, 2016, 12, 1, 156.
8. **Gherman, C., Cozma, V., Mircean, V., Brudacă, F., Rus, N., Detesan, A.,** Helminthic zoonoses in species of wild carnivores from the fauna of Romania, Scientia Parasitologica, 2002, 2.
9. **German, C.**, **Mihalca A.D.,** A synoptic overview of golden jackal parasites reveals high diversity of species, Parasites and Vectors, 2017, 10, 10-419.
10. **Gonzalez, R.R., Kotas, M.E., O'Leary, C.E., Singh, K., Damsky, W., Liao, C., Arouge, E., Tenvooren, I., Marquez, D.M., Schroeder, A.W., Cohen, J.N., Fassett, M.S., Lee, J., Daniel, S.D., Bittinger, K., Díaz, R.E., Fraser, J.S., Ali, N., Ansel, K.M., Spitzer, M.H., Liang, H.E., Locksley, R.M.,** Innate type 2 immunity controls hair follicle commensalism by *Demodex* mites, Immunity, 2022, 55, 10, 1891-1908.
11. **Izdebska, J., Rolbiecki, L.,** The biodiversity of demodecid mites (*Acariformes: Prostigmata*), specific parasites of mammals with a global checklist and a new finding for *Demodex sciurinus*, Diversity, 2020, 12, 261.
12. **Izdebska, J., Rolbiecki, L.,** The status of *Demodex cornei*: description of the species and developmental stages, and data on demodecid mites in the domestic dog *Canis lupus familiaris*, Medical and Veterinary Entomology, 2018, 32, 3, 346-357.
13. **Izdebska, J.N.,** *Demodex* sp. (*Acari*, *Demodecidae*) and demodecosis in dogs: characteristics, symptoms, occurrence, Bulletin of the Veterinary Institute in Pulawy, 2010, 54, 335-338.
14. **Mederle, N., Dărăbus, Gh., Oprescu, I., Morariu, S., Ilie, M., Indre, D., Mederle, O.,** Diagnosis of canine demodicosis, Scientia Parasitologica, 2010, 11, 1, 20-23.
15. **Mederle, N., Mederle, O., Morariu, S., Herman, V., Negrescu, A., Marin, A.M., Suici, T., Darăbus, Gh.,** Study regarding the role and medical and social implications of *Demodex* spp. Parasitism in dogs and humans, Revista Română Medicină Veterină, Timișoara, 2020, 30, 2, 39-46.

16. **Molnar, B., Ciucci, P., Mastrantonio, G., Betschart, B.,** Correlates of parasites and pseudoparasites in wolves (*Canis lupus*) across continents: A comparison among Yellowstone (USA), Abruzzo (IT) and Mercantour (FR) national parks, International Journal for Parasitology: Parasites and Wildlife, 2019, 12, 10, 196-206.
17. **Morariu, S., Morariu, F., Marin, A.M., Moraru, M.M.F., Popovici, D.C., Imre, M., Igna, V., Mederle, N.,** First morphological and molecular identification of *Demodex injai* in golden jackal (*Canis aureus* Linnaeus, 1758) in Romania, Pathogens, 2023, 12, 3, 412.
18. **Mörner, T., Eriksson, H., Bröjer, C., Nilsson, K., Uhlhorn, H., Agren, E., Segerstad, H., Jansson, DS, Gavier-Widén, D.,** Diseases and mortality in free-ranging brown bear (*Ursus arctos*), gray wolf (*Canis lupus*), and wolverine (*Gulo gulo*) in Sweden, Journal of Wildlife Diseases, 2005, 41, 2, 298-303.
19. **Moroni, B., Coenda, F., Garcia-Vozmediano, A., Nicoletti, A., Pregel, P., Mina, A., Tomassone, L., Rossi, L., Scaglione, F.E.,** Subcutaneous ticks in wild carnivores: any host-related differences?, Animals, 2022, 12, 23, 3411.
20. **Radbea, N.,** Demodicoza canină, Ed. Aura Timisoara, 2005.
21. **Radbea, N., Radbea, G., Cosoroabă, I., Dărăbuș, Gh., Oprescu, I., Morariu, S., Băniță C.,** Investigații epizootologice în demodicoza canină, Scientia Parasitologica, 2002, 1, 111-117.
22. **Segovia, J.M., Torres, J., Miquel, J., Llaneza, L., Feliu, C.,** Helminths in the wolf, *Canis lupus*, from north-western Spain, Journal of Helminthology, 2001, 75, 2, 183-192.
23. **Thomson, P., Carreño, N., Núñez, A.,** Main mites associated with dermatopathies present in dogs and other members of the Canidae family, Open Veterinary Journal, 2023, 13, 2, 131-142.
24. **Zaccaroni, M., Gazzola, A., Popescu, V.,Corradini, A.,** Wolf (*Canis lupus*) in Romania: winter feeding ecology and spatial interaction with lynx (*Lynx lynx*), Doctoral Thesis, Universita Degli Studi Firenze, 2016.
25. ***https://www.carnivoremari.ro
26. ***https://www.carnivoremari.ro/lupul

THERAPEUTIC APPROACHES IN CARDIOVASCULAR DIROFILARIOSIS IN DOG - CASES SERIES

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Summary

Dirofilariosis, a non-contagious nematodosis, is common to humans and domestic and wild animals, is spread by several species of mosquitoes, the intermediate host-key vector in the epidemiology of this parasitosis, and is localized in the pulmonary arteries and right heart. The aim of the present study was to demonstrate the negativity of rapid tests in three dogs following the application of „slow-kill” or „moxi-doxy” therapy. Blood samples were taken from each animal to examine and confirm or deny the presence of microfilariae in the blood (fresh blood smear) and antigens from adult females (CHW Ag Test Kit 2.0 – Bionote, Inc.). Examination forms were completed for all animals (owner identification data, animal identification data, epidemiological situation, anamnesis, medical history, skin, hair, apparent mucous membranes, etc.). This protocol is a life-saving procedure for animals, and its effectiveness has been demonstrated over several months, eliminating controversy about this therapeutic approach and providing rapid diagnostic test negativity and clinical cure of animals.

Keywords: dogs, *Dirofilaria immitis*, rapid test kit, ”slow-kill” „moxi-doxy”.

The presence of adult *Dirofilaria immitis* was first mentioned in the 17th century by Francesco Birago in his hunting dogs. Today, dirofilarioses is considered to be a group of parasitic diseases whose aetiological agent is species of the genus *Dirofilaria* and whose vectors, more than 70 species of mosquitoes (*Culex*, *Aedes*, *Ochlerotatus*, *Anopheles*, *Coquillettidia*, *Armigeres*, *Mansonia* and *Psorophora*), are involved in the transmission of the disease (3, 38).

Two species are frequently mentioned as being involved in causing the disease, both of which are considered highly pathogenic. *Dirofilaria immitis* is responsible for the induction of canine cardiopulmonary heartworm disease (HWD) and is also of pronounced zoonotic character (10, 11, 35, 38). There are numerous studies confirming the existence of a much larger proportion of *Dirofilaria immitis*, the species *Dirofilaria* (*Nochtiella*) *repens* has experienced a rapid rise in recent years, "travelling" from southern to northern Europe. This may also be due to the fact that adult forms of *D. repens* can survive in their natural hosts for up to four years (2, 15, 16, 27, 34).

The distribution of dirofilariosis is influenced by a number of factors, including: the increase in the vector population, the introduction of invasive species (*Aedes albopictus* and *Aedes koreicus*), massive movements of the canine population, the low rate of application of appropriate chemoprophylaxis, especially in endemic areas (4, 5, 13, 30, 31).

The most endemic area for canine heartworm disease is the Po River in northern Italy. The prevalence is between 22 and 80% (22, 33, 37).

According to literature data, the prevalence of *Dirofilaria immitis* in the Romanian canid population is 8.78%. On the other hand, the prevalence of *Dirofilaria immitis* and *Dirofilaria repens* in Romania is between 3.6% and 42% (2, 17, 18, 28, 39).

In Romania, Olteanu, 1996 (32) (35% *D. immitis*) Coman, 2007 (9) (23.07% *Dirofilaria* spp.) Ilie, 2009 (21) (*D. immitis – case study*), 2010 (20) (2.77% *Dirofilaria* spp.), 2015 (23) (6.8% *D. immitis*, 21% *D. repens*) Tudor, 2009 (41) (29.31% *D. immitis*) Ciocan, 2009 (7) (4.25% *D. immitis*), 2010 (6) (2.17% *D. repens*) and Fernoagă, 2010 (14) (*D. immitis – case study*) are authors that have brought contributions regarding the non-molecular, morphological or immunologic detection methods of *Dirofilaria* spp. infestations. Larger scale studies and case reports revealed the presence of *D. immitis* and *D. repens,* with various prevalence rates, infestations in dogs (8, 12, 19, 25, 29, 40) (3.5% *D. immitis*, 6.15% *D. repens*, 2.05% *D. immitis*, 3.3% *D. immitis,* 8.9% *D. immitis*, 19.44% *D. immitis*, 22.22% *D. repens, D. repens* – case report), cats (9, 36) (*D. repens* – case report, *D. immitis* – case report) and wild carnivores (24) (1.62% *D. repens*, 1.39% *D. immitis*) from different areas.

There are several diagnostic methods that can be applied to establish a definite diagnosis. Among these we mention: microscopic - direct examination of fresh blood drop, the modified Knott method, the filtration test, histochemical staining, specific antigen tests, ELISA and PCR (7, 18, 22).

Current treatments against heartworm include three active substances that belong to three different categories: an antibiotic, a macrocyclic lactone, and an arsenic-containing drug to destroy adult forms. This protocol lends itself in all cases, except when the disease is in an advanced stage, and surgical intervention is justified (26).

The high prevalence identified in canids may be due to a low percentage of diagnostic methods being applied to identify the helminthosis by veterinarians, but also to a lack of veterinary education in the human population.

The aim of the present study was to demonstrate the negativity of rapid tests in three dogs following the application of ”slow-kill” or ”moxi-doxy” therapy.

Materials and methods

The dogs were examined in the Parasitology and Parasitological diseases and Small Animal Pathology clinics of the Faculty of Veterinary Medicine, Timisoara, Romania.

The three dogs in the study were of different breeds, ages and sexes. From each, 3 ml of venous blood was collected in EDTA tubes for diagnosis.

First case. The patient investigated was a 4-year-old female Cane Corso dog, 40 kg, vaccinated according to protocol, unparasitized and with a temperature of 38.7 ºC. The female was spayed. The owner detailed the following: a month ago he was diagnosed with babesiosis and has been coughing up blood for a week. Water and food intake has been reduced, resulting in weight loss.

Second case. The investigated patient is a half-breed dog, 5 year old, male, 17 kg, neutered, neutered, vaccinated according to protocol, externally dewormed, 38 ºC.

Following discussion with the owner of the animal, he reported that his appetite had reduced and short walks were followed by long periods of severe dyspnea.

Third case. The patient investigated was a 6-year-old male German Shepherd dog, 30 kg, neutered, vaccinated according to protocol, dewormed, 38 ºC.

In this case, the patient's anamnesis was as follows: the owner noticed that the animal had been showing exercise intolerance for several weeks, although appetite was present. Following physical exertion he presents dyspnea.

Results and discussions

First case. Clinical examination and radiographic examination were performed. The list of hypothetical diagnoses/differential diagnosis included bacterial infection, viral infection, neoplasms and parasitosis: dirofilariosis and angyostrongylosis.

Clinical examination showed no changes in conformation, constitution, hair, skin, apparent mucous membranes, pulse, respiratory rate. Instead, the score for maintenance status was two.

Radiographic examination revealed numerous lung metastases (Fig. 1).

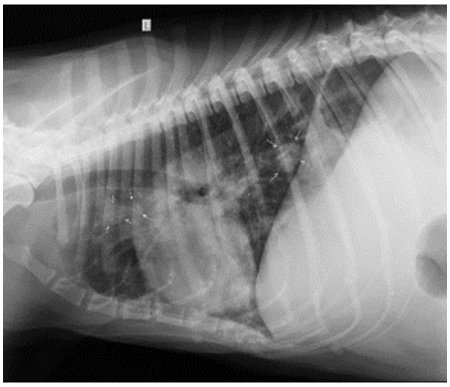

Fig. 1. Rx: pulmonary metastases

Second case. Clinical examination and radiographic examination were performed. The list of hypothetical diagnoses/differential diagnosis included bacterial infection, viral infection, neoplasms and parasitosis: dirofilariosis and angyostrongylosis.

Clinical examination of the animal revealed no changes in conformation, constitution, condition, skin, hair, apparent mucous membranes, respiratory rate, pulse.

Radiographic examination showed no changes in the lungs.

Third case. Clinical examination and radiographic examination were performed. The list of hypothetical diagnoses/differential diagnosis included bacterial infection, viral infection, neoplasms and parasitosis: dirofilariosis and angyostrongylosis.

On clinical examination, only slight dehydration was found, the rest of the data, conformation, constitution, state of maintenance, hair, skin, apparent mucous membranes, pulse, respiratory rate, being within physiological limits.

Radiographic examination confirmed the presence of numerous pulmonary metastases (Fig. 2).

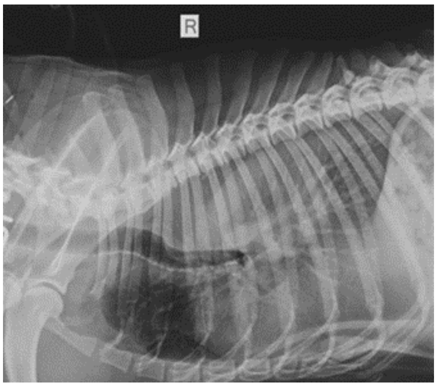

Fig. 2. Rx: pulmonary metastases

In none of the three cases, following the Baermann method, not find out *Angiostrongylus vasorum* infection.

The diagnosis of canine dirofilariosis, in all three cases, was based on the identification of microfilariae in direct examination of fresh blood drop. The presence of the microfilaria species involved was confirmed by the modified Knott's method (Fig. 3). Using the rapid antigen detection test (CHW Ag Test Kit 2.0 – Bionote, Inc.) *Dirofilaria immitis* was detected (Fig. 4).

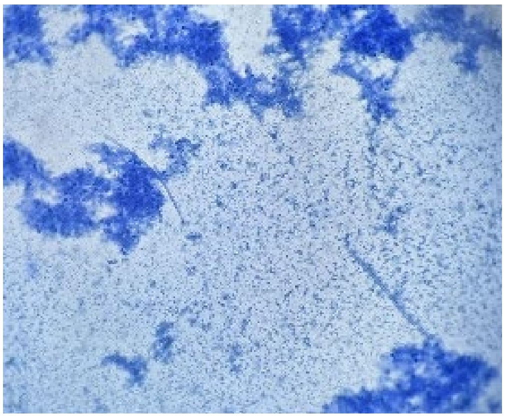

Fig. 3. *D. immitis* - Knott method

Fig. 4. CHW Ag Test Kit 2.0 - *Dirofilaria immitis*

Therapeutic protocol

The treatment applied to the animals is tailored and personalised for each individual animal, based on the modified American Heartworm Society's treatment protocol (44). The "slow-kill" or "moxi-doxy" therapy involved:

- ✓ days 1 - 28: administration of prednisone and doxycycline. In the case of prednisone the following steps were to be followed: days 1 - 7 (0,5 mg/kg) to be administered twice a day, morning and evening, days 8 - 14 to be administered once a day, morning only, days 15 - 21 to be administered once a day, morning only, but one day yes, one day no, days 21 - 28 to be administered once every three days, morning only.
- ✓ doxycycline will be administered in the morning and evening.
- ✓ on day three Advocate® (Imidacloprid, moxidectin) should be administered monthly.
- ✓ on day 17 - recheck for microfilariae and make any further recommendations.

- ✓ day 30 - recheck, rapid test and Advocate pipette.
- ✓ days 31 - 60 - doxycycline will be removed.
- ✓ day 60 - Advocate pipette.
- ✓ day 90 - recheck and further recommendations.

Following the above treatment, the situation of the animals in the study is as follows:

- first case: due to lung metastases, the dog died;
- the second case: the rapid antigen test was negative within 7 months of starting treatment;
- case three: although the animal had lung metastases, these did not affect its physiological status and the antigen test was negative (8 months).

A 2020 study cites the effectiveness of treatment based on doxycycline and moxidectin alone. The authors of the article mention how to use these two active substances: twice-yearly (i.e. every 6 months) doses of commercially available moxidectin extended-release injectable suspension and oral administration of doxycycline for 30 days. The results in this case confirm the efficacy of this adapted protocol, but also a good tolerance of the dogs (1).

The Palmetto Animal League recommends the use of "moxi-doxy" treatment because the killing of adult worms is slower, more specifically, over a period of 6-10 months. Strict adherence to the protocol, specifically: Moxidectin/Imidacloprid once a month and the antibiotic doxycycline twice a day for one month will result in negative antigen tests within 10 months in the vast majority of heartworm dogs. The lack of physical exertion that a dog normally does should also contribute to the success of this protocol, which can be replaced by walking on a leash. In the case of physical exertion, various complications can be discussed, such as: spontaneous pulmonary thromboembolism (42, 43).

Due to the lack of melarsamine arsenical treatment on the commercial market, Jacobson and DiGangi also resort to the alternative treatment option. They used doxycycline for 28 days, per os, and moxidectin in topical form in the labelled dose. The latter is repeated monthly until a rapid negative antigen test is achieved. Also included in this article are the various approaches to the controversies surrounding this type of therapeutic approach, as well as explicit recommendations of treatment regimens (26).

Conclusions

This protocol is a life-saving procedure for animals, and its effectiveness has been demonstrated over several months, eliminating controversy about this therapeutic approach and providing rapid diagnostic test negativity and clinical cure of animals.

References

1. **Alberigi, B., Fernandes, J.I., Paiva, J.P., Mendes-de-Almeida, F., Knackfuss, F., Merlo, A., Labarthe, N.,** Efficacy of semi-annual therapy of an extended-release injectable moxidectin suspension and oral doxycycline in *Dirofilaria immitis* naturally infected dogs, Parasites and Vectors, 2020,13, 1-8.
2. **Anvari, D., Narouei, E., Daryani, A., Sarvi, S., Moosazadeh, M., Ziaei Hezarjaribi, H., Narouei, M.R., Gholami, S.,** The global status of *Dirofilaria immitis* in dogs: a systematic review and meta-analysis based on published articles, Research in Veterinary Science, 2020, 131, 104-116.
3. **Aonuma, H., Yoshimura, A., Perera, N., Shinzawa, N., Bando, H., Oshiro, S., Nelson, B., Fukumoto, S., Kanuka, H.,** Loop-mediated isothermal amplification applied to filarial parasites detection in the mosquito vectors: *Dirofilaria immitis* as a study model, Parasites and Vectors, 2009, *2*, 1, 15.
4. **Brianti, E., Panarese, R., Napoli, E., De Benedetto, G., Gaglio, G., Bezerra-Santos, M.A., Mendoza-Roldan, J.A., Otranto, D.,** Dirofilaria immitis infection in the Pelagie archipelago: the southernmost hyperendemic focus in Europe, Transboundary and Emerging Diseases, 2022, 69, 1274-1280.
5. **Capelli, G., Genchi, C., Baneth, G., Bourdeau, P., Brianti, E., Cardoso, L., Fuehrer, H.P., Guannelli, A., Ionică, A.M., Maia, C., Modry, D., Montarsi, F., Krucken, J., Papadopoulos, E., Petrick, D., Pfeffer, M., Savic, S., Otranto, D., Poppert, S., Silaghi, C.,** Recent advances on *Dirofilaria repens* in dogs and humans in Europe, Parasites and Vectors, 2018, 11, 1-663.
6. **Ciocan, R., Dărăbuș, G., Ilie, M.S., Imre, M., Hotea, I.,** *Dirofilaria repens* infection prevalence in Hunedoara County, Lucrări Științifice Medicină Veterinară, Timișoara, 2010, 53, 12, 3, 597-600.
7. **Ciocan, R., Dărăbuş, Gh., Ilie, M.S., Hotea, I., Imre, K., Morariu, S., Morar, D., Balint, A., Indre, D.,** Preliminary observations of an epidemiological survey in dirofilariosis of dogs from Timis County, Lucrări Ştiințifice Medicină Veterinară, 2009, 42, 1, 109.
8. **Ciucă, L., Musella, V., Miron, L.D., Maurelli, M.P., Cringoli, G., Bosco, A., Rinaldi, L.,** Geographic distribution of canine heartworm (*Dirofilaria immitis*) infection in stray dogs of eastern Romania, Geospatial Health, 2016, 11, 3-499.
9. **Ciucă, L., Roman, C., Prisco, F., Miron, L., Acatrinei, D., Paciello, O., Maurelli, M.P., Vismarra, A., Cringoli, G., Rinaldi, L.,** First report of *Dirofilaria repens* infection in a microfilaraemic cat from Romania, Veterinary Parasitology: Regional studies and reports, 2020, 22-100497.
10. **Dantas-Torres, F., Otranto, D.,** Dirofilariosis in the Americas: a more virulent *Dirofilaria immitis*?, Parasites and Vectors, 2013, 6, 1-288.
11. **Dantas-Torres, F., Otranto, D.,** Overview on *Dirofilaria immitis* in the Americas, with notes on other filarial worms infecting dog, Veterinary Parasitology, 2020, 282-109113.

12. **Deak, G., Ionică, A.M., Szasz, I., Taulescu, M., Mihalca, A.D.,** A case of inguinal hernia associated with atypical *Dirofilaria repens* infection in a dog, Parasites and Vectors, 2021, 14, 1-125.
13. **Diakou, A., Kapantaidakis, E., Tamvakis, A., Giannakis, V., Strus, N.,** *Dirofilaria* infections in dogs in different areas of Greece, Parasites & Vectors, 2016, 9, 508.
14. **Fernoagă, C., Codreanu, M., Cornilă, M., Tudor, P., Grosu, F.,** Case study—canine heartworm disease, Symposium "Contribution of scientific research in progress of veterinary medicine", 2009, 83.
15. **Gabrielli, S., Mangano, V., Furzi, F., Oliva, A., Vita, S., Poscia, R., Fazii, P., Di Paolo, J., Marocco, R., Mastroianni, C.M., Bruschi, F., Mattiucci, S.,** Molecular identification of new cases of human dirofilariosis (*Dirofilaria repens*) in Italy, Pathogens, 2021, 10, 251.
16. **Genchi, C., Kramer, L.H.,** The prevalence of *Dirofilaria immitis* and *D. repens* in the Old World, Veterinary Parasitology, 2020, 280-108995.
17. **Giubega, S., Imre, M., Ilie, M.S., Imre, K., Luca, I., Florea, T., Dărăbuș, G., Morariu, S.,** Identity of microfilariae circulating in dogs from Western and South-Western Romania in the last decade, Pathogens, 2021, 10, 1400.
18. **Hamel, D., Silaghi, C., Lescai, D., Pfister, K.,** Epidemiological aspects on vector-borne infections in stray and pet dogs from Romania and Hungary with focus on *Babesia* spp., Parasitology Research, 2012, 110, 4, 1537-45.
19. **Ilie, M., Imre, K., Hotea, I., Imre, M., Dărăbuş, G., Morariu, S., Sorescu, I.D., Ilie, A.,** Preliminary observations on the distribution of canine haemoparasites in Timiş county, Scient Works C Series, 2010, 56, 74-78.
20. **Ilie, M.S., Dărăbuş, Gh., Ciocan, R., Hotea, I., Imre, K., Morarius, S., Mederle, N., Ilie, A., Morar, D.,** Dirofilariosis in dog case report, Lucrări Științifice Medicină Veterinară, Timişoara, 2009, 52, 1.
21. **Ilie, M.S., Imre, K., Hotea, I., Imre, M.,** Patogeni ai sângelui în continuă expansiune, Editura Agroprint şi Mirton, Timişoara, 2011.
22. **Ilie, M.S., Imre, M., Hotea, I., Oprescu, I., Tilibașa, E., Dărăbuș, Gh., Imre, K.,** Survey of canine dirofilariosis by non-molecular assays in Romania, Lucrări Științifice Medicină Veterinară, 2015, 48, 3 69-74.
23. **Ionică, A.M., Matei, I.A., D'Amico, G., Ababii, J., Daskalaki, A.A., Sándor, A.D., Enache, D.V., Gherman, C.M., Mihalca, A.D.,** Filarioid infections in wild carnivores: a multispecies survey in Romania, Parasites & Vectors, 2017, 10, 1, 332.
24. **Ionică, A.M., Matei, I.A., Mircean, V., Dumitrache, M.O., D'Amico, G., Győrke, A., Pantchev, N., Annoscia, G., Albrechtová, K., Otranto, D., Modrý, D., Mihalca, A.D.,** Current surveys on the prevalence and distribution of *Dirofilaria* spp. and *Acanthocheilonema reconditum* infections in dogs in Romania, Parasitology Research, 2015, 114, 3, 975-82.

25. **Jacobson, L.S., DiGangi, B.A.,** An accessible alternative to melarsomine: "Moxi-Doxy" for treatment of adult heartworm infection in dogs, Frontiers in Veterinary Science, 2021, 8, 702018.
26. **Kotnik, T., Rataj, A. V., Šoba, B.,** *Dirofilaria repens* in dogs and humans in Slovenia, Journal of Veterinary Research, 2022, 66, 1, 117-123.
27. **Laidoudi, Y., Otranto, D., Stolowy, N., Amrane, S., Santhakumari Manoj, R.R., Polette, L., Watier-Grillot, S., Mediannikov, O., Davoust, B., L'Ollivier, C.,** Human and animal dirofilariasis in Southeast of France, Microorganisms, 2021, 9, 1544.
28. **Mircean, V., Dumitrache, M.O., Györke, A., Pantchev, N., Jodies, R., Mihalca, A.D., Cozma, V.,** Seroprevalence and geographic distribution of *Dirofilaria immitis* and tick-borne infections (*Anaplasma phagocytophilum*, *Borrelia burgdorferi sensu lato*, and *Ehrlichia canis*) in dogs from Romania, Vector-Borne and Zoonotic Diseases, 2012, 12, 7, 595-604.
29. **Montarsi, F., Ciocchetta, S., Devine, G., Ravagnan, S., Mutinelli, F., Frangipane di Regalbono, A.,** Development of Dirofilaria immitis within the mosquito Aedes (Finlaya) koreicus, a new invasive species for Europe, Parasites and Vectors, 2015, 8, 177.
30. **Napoli, E., De Benedetto, G., Ciuca, L., Bosco, A., Lia, R. P., Veneziano, V., Bezerra Santos, M. A., Otranto, D., Rinaldi, L., Brianti, E.,** New distribution patterns of *Dirofilaria immitis* in Italy, Frontiers in Veterinary Science, 2023, 10, 1162403.
31. **Olteanu, G.,** Dirofilarosis in man and animals in Romania, In: Proceedings of VII European Multicolloquium of Parasitology, Trends in Parasitology, 38, 1-2, 1996, 360.
32. **Otranto, D., Capelli, G., Genchi, C.,** Changing distribution patterns of canine vector borne diseases in Italy: Leishmaniosis vs. dirofilariosis, Parasites and Vectors, 2009, 2, 1, 2.
33. **Otranto, D., Dantas-Torres, F., Brianti, E., Traversa, D., Petrić, D., Genchi, C., Capelli, G.,** Vector-borne helminths of dogs and humans in Europe, Parasites and Vectors, 2013, 6, 16.
34. **Pampiglione, S., Rivasi, F.,** Human dirofilariasis due to *Dirofilaria* (*Nochtiella*) *repens*: a review of world literature, Parassitologia, 1995, 37, 2-3, 149-193.
35. **Pană, D., Rădulescu, A., Mitrea, I.L., Ionita, M.,** First report on clinical feline heartworm (*Dirofilaria Immitis*) infection in Romania, Helminthologia, 2020, 57, 1, 49-56.
36. **Santoro, M., Miletti, G., Vangone, L., Spadari, L., Reccia, S., Fusco, G.,** Heartworm disease (*Dirofilaria immitis*) in two roaming dogs from the urban area of Castel Volturno, Southern Italy, Frontiers in Veterinary Science, 2019, 6, 270.
37. **Simón, F., López-Belmonte, J., Marcos-Atxutegi, C., Morchón, R., Martín-Pacho, J.R.,** What is happening outside North America regarding human dirofilariasis?, Veterinary Parasitology, 2005, 133, 181-189.

38. **Sonnberger, K., Fuehrer, H.P., Sonnberger, B.W., Leschnik, M.,** The incidence of *Dirofilaria immitis* in shelter dogs and mosquitoes in Austria, Pathogens, 2021, 10, 550.
39. **Tomazatos, A., Cadar, D., Török, E., Maranda, I., Horváth, C., Keresztes, L., Spinu, M., Jansen, S., Jöst, H., Schmidt-Chanasit, J., Tannich, E., Lühken, R.,** Circulation of *Dirofilaria immitis* and *Dirofilaria repens* in the Danube Delta Biosphere Reserve, Romania, Parasites and Vectors, 2018, 11, 1, 392.
40. **Tudor, P., Brăslaşu, M.C., Brăslaşu, D.E., Tudor, N., Rădulescu, A.,** Epidemiological and clinical features regarding dirofilariosis in dog, Symposium on "Contribution of scientific research in progress of veterinary medicine", Bucureşti, 2009, 86.
41. **Younes, L., Barré-Cardi, H., Bedjaoui, S., Ayhan, N., Varloud, M., Mediannikov, O., Otranto, D., Davoust, B.,** *Dirofilaria immitis* and *Dirofilaria repens* in mosquitoes from Corsica Island, France, Parasites & Vectors, 2021, 14, 1, 427.
42. ***https://palmettoanimalleague.org/wpcontent/uploads/2021/05/2021-PAL-CLINIC-Heartworm-Protocol-Doc.pdf
43. ***https://d3ft8sckhnqim2.cloudfront.net/images/pdf/2020_AHS_Canine_Guidelines.pdf?1580934824
44. ***https://www.esccap.org/page/GL5+Control+of+VectorBorne+Diseases+in+Dogs+and+Cats/29/

THE IDENTIFICATION OF INTESTINAL PARASITES IN WILDCAT (*FELIS SILVESTRIS*) FROM THE HUNTING GROUNDS OF TIMIS COUNTY

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Summary

The wildcat (*Felis silvestris*) lives in the forest and rocky cliffs where it can easily hide, but also in swamps of wild rush beds, hardly reachable. In Romania, the species is found in alpine areas but also the Danube Floodplain. *Felis silvestris* populations are declining due to anthropogenic and phenological unfavorable conditions, and parasites may have an additional negative impact. In the present study, the occurrence of endoparasites in *F. silvestris* in Romania and the potential threats posed to *F. silvestris*, domestic animals, and humans in the study areas has been investigated. For a period of two years, 15 *F. silvestris* from two hunting grounds in Timis County have been necropsied at the Department of Parasitology, Faculty of Veterinary Medicine/University of Life Sciences "King Mihai I" from Timișoara. The coprological examination by the flotation method revealed parasitological infestation in all the examinated *F. silvestris*. Adult parasites such as nematodes and cestodes have been identified by necropsy, with the most prevalent being 14/15 adult cestodes (93.33%). Continued monitoring and research are needed to better understand the health effects of *F. silvestris* and the potential risks of transmission to domestic carnivores and humans, respectively.

Keywords: *F. silvestris*, endoparasites, Timis County.

The *F. silvestris* is the most widespread and common wild feline. In Europe, the species is sporadically distributed throughout the continent (24). In Romania, there are about 10.000 individuals spread from mountain areas to the seashore (27).

This species is protected by the Hunting and Game Fund Protection Law No 407/2006, Annex 2, and at the European level by Council Directive 92/43/EEC of 21 May 1992 on the conservation of natural habitats and of wild fauna and flora (25, 26). *Felis silvestris* are wild carnivores that can be infested with both ectoparasites and endoparasites (14, 15, 17).

Parasitic diseases reflect the health and balance of ecosystems, affecting not only individuals but also entire populations or communities (10). Knowledge of parasites affecting *F. silvestris* is scarce, due to the elusive nature of these animals and their minimal interactions with humans, making any sample collection difficult. However, in recent years, scientific interest in parasites affecting wildcats has increased significantly (22).

The cestodes have a cosmopolitan character due to the diversity of intermediate hosts and lack of specificity for definitive hosts, therefore wild carnivores are a source of environmental contamination and some of the species that parasitize to them may have a zoonotic character (1, 3, 9, 12).

The study aimed to evaluate the intestinal parasite fauna of *F. silvestris* from two hunting grounds in Timis County, using classical copro-parasitological methods and necropsy examination.

Materials and methods

The research was carried out over a period of two years, on a total of 15 *F. silvestris* (seven males and eight females) aged between 1 and 3 years. The collected animals were found dead (road kill) in two hunting grounds in Timis County.

The animals were examined at the Parasitic Diseases Clinic of the Faculty of Veterinary Medicine Timisoara/ University of Life Sciences ”King Mihai I” from Timisoara using the following methods:

- Qualitative method - identification of the parasitic load of the whole digestive tract with light eggs of nematodes, cestodes, and protozoan oocysts;
- Necropsy examination - according to the technical instructions of necropsy (2) (Fig. 1).

Fig. 1. Necropsy examination

Results and discussions

The results of the flotation method are as follows (Fig. 2):

- Positive *F. silvestris* - 15/15 (100%)
- Presence of morulated eggs - 11/15 (73.33%) (Fig. 3a)
- Presence of ascarid eggs - 6/15 (40%) (Fig. 3b)
- Presence of oocysts - 6/15 (40%)
- Presence of double bonds eggs (*Trichocephalus*) - 3/15 (20%) (Fig. 3c)

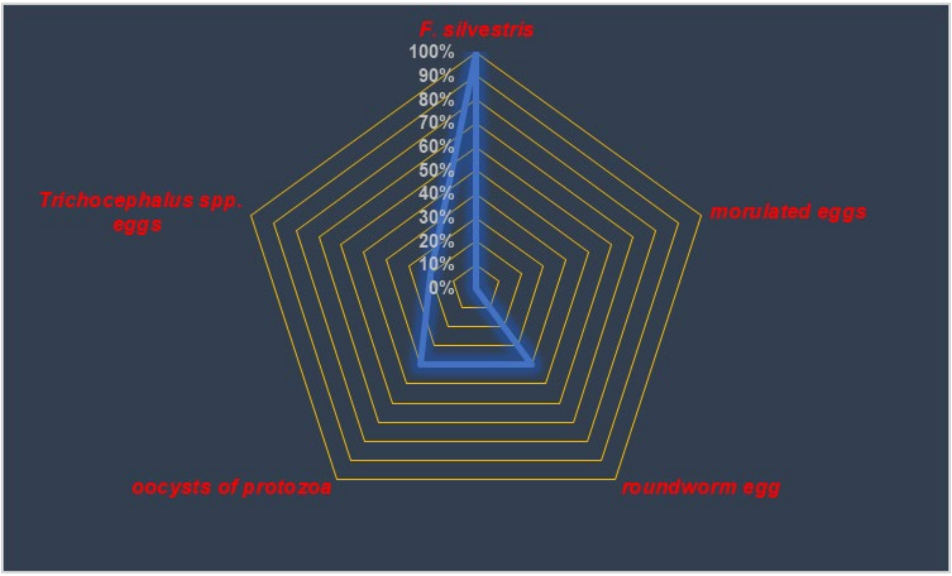

Fig. 2. Identified types of eggs by the flotation method

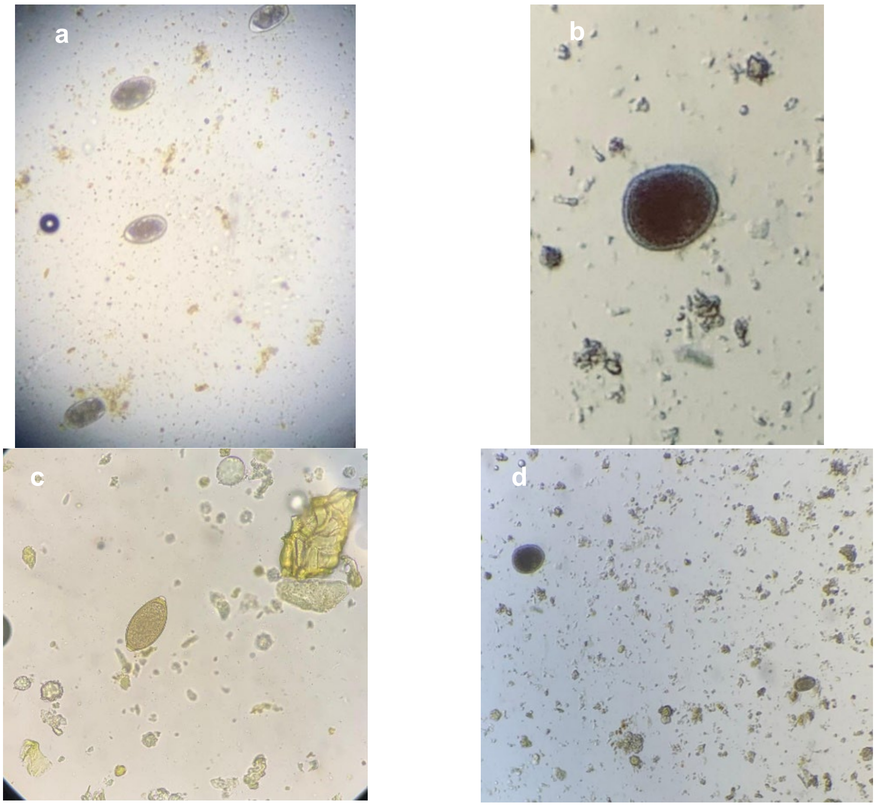

Fig. 3. Types of eggs: a - morulated eggs, b - ascarid eggs, c - double bonds eggs, d - morulated and ascarid eggs

Regarding the polyparasitism the results revealed (Fig. 4):

- Presence of morulated and double bonds eggs (*Trichocephalus*) - 3/15 (20%).
- Presence of morulated and ascarid eggs - 2/15 (13.33%) (Fig.3d)
- Presence of morulated eggs and oocysts - 3/15 (20%)
- Presence of ascarid eggs and oocysts - 3/15 (20%)
- Presence of morulated eggs, double bonds eggs (*Trichocephalus*) and oocysts - 1/15 (6.66%)

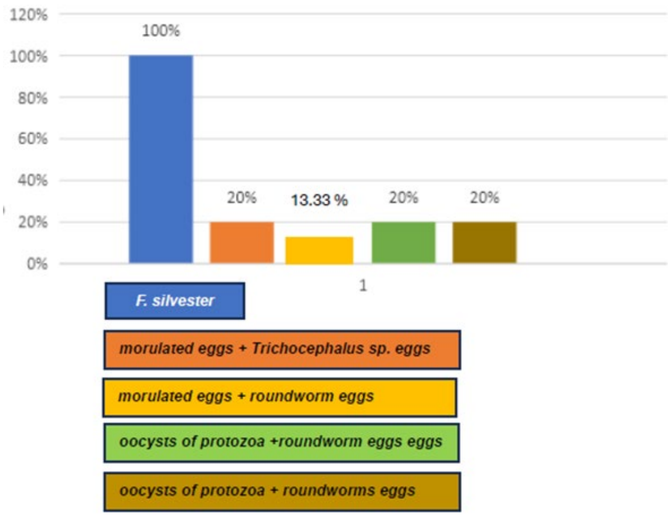

Fig. 4. The polyparasitism identified by the flotation method

The results of necropsy examination of the intestines are as follows (Fig. 5):

- Presence of cestodes - 14/15 (93.33%)
- Presence of roundworms - 9/15 (60%)
- Presence of intestinal strongyles - 11/15 (73.33%)

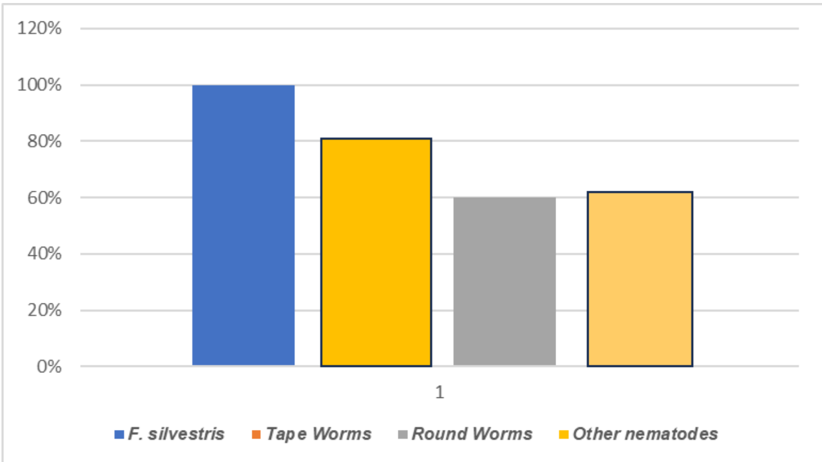

Fig. 5. Parasites identified by necropsy examination

Regarding the mono- and polyparasitism the results showed:

- Presence of cestodes - 1/15 (6.66%)
- Presence of intestinal strongyles - 1/15 (6.66%)
- Presence of cestodes, ascarids, and intestinal strongyles - 6/15 (40%)
- Presence of cestodes and roundworms - 3/15 (20%) (Fig. 6)
- Presence of cestodes and intestinal strongyles - 4/15 (26.66%)

Fig. 6. Presence of cestodes and ascarids in the small intestine

The results of this study showed a high prevalence of cestode parasitism 14/15 (93.33%) and intestinal strongyles (73.33%) followed by ascarids parasitism 9/15 (60%).

The results of the coproscopic examination are the with those of the necropsy of the intestine excepting cestode eggs which were not identified in the faeces.

This study provides additional information to the previous study carried out by Mederle et al., 2023, on the parasitic fauna of *F. silvestris* in Timis County in which they reported the presence of oocysts of Isospora, tapeworm eggs, eggs of *Toxocara cati*, *Ancylostoma* spp. and *Capillaria* spp. At necropsy, the genera *Mesocestoides*, *Taenia*, *Toxocara*/T*oxascaris,* and *Ancylostoma* were identified. Subsequent identification by polymerase chain reaction (PCR) revealed *Taenia taenieformis* and *Mesocestoides litteratus*, the last one with zoonotic potential (15).

Eckert et al. 2005 highlighted that the sensitivity of the flotation method is lower for the identification of cestode eggs (6), an aspect also founded in the present study.

This confirms that, in most cases, infested animals shed only a few eggs and release mature proglottids instead. Thus, copro-microscopic analysis is considered unreliable for the diagnosis of cestodes in *F. silvestris* (5).

Data from previous studies indicated the frequent presence of *T. taeniaeformis* in *F. silvestris* examined (8,14), data also supported by Diakou et al. in their study conducted in 2021 (5).

The very high prevalence of endoparasite infestations in *F. silvestris* observed in the present study is similar to the level of infestations identified by other authors in Europe (11, 12, 14, 17, 19, 20, 21).

It is important to investigate and monitor the parasite load of wildlife to develop strategies to control and prevent important zoonoses as well as emerging infections in wildlife, domestic animals, and humans (13, 4, 7, 15, 17, 23).

All *F. silvestris* (34) examined by Martinkovic et al. (14) were infected with at least one species of parasite, while the most diverse infection included six different species of parasites found in a single animal. The prevalence of parasite species was: *T. taeniaeformis* (55.9%), *Capillaria* sp. (50%), *T. cati* (50%), *Isospora* sp. (29.4%), *Strongyloides* sp. (23.5%), *Giardia* sp. (17.6%), *Ancylostoma tubaeformae* (14.7%), *Physaloptera* sp. (11.8%), *Hymenolepididae* (8.8%), *Alaria alata* (5. 9%), *Aelurostrongylus abstrusus* (5.9%), *Toxascaris leonina* (5.9%), *Trichinella* sp. (5.9%), *Mesocestoides lineatus* (5.9%), *Anoplocephalidae* (2. 9%), *Dipylidium caninum* (2.9%), *Trichuris* sp. (2.9%), *Isospora felis* (2.9%), *Eimeria* sp. (2.9%) and *Sarcocystis* sp. (2.9%) (14).

Krone et al. (12) necropsied 14 *F. silvestris* and 17 domestic cats (*Felis catus)*. Endoparasites were identified in 14 *F. silvestris* and 11 *F. catus*. A total of eight species of endoparasites were found in wildcats and six in *F. catus*. The nematodes *Toxocara mystax* and *T. leonina* and the cestode *T. taeniaeformis* have been the most prevalent parasites (12).

In the present study, the results of the copro-parasitological examination revealed the following: all *F. silvestris* were positive (15/15), the presence of morulated eggs (11/15), the presence of ascarid eggs (6/15), and the presence of oocysts (6/15), and necropsy examination revealed the presence of tapeworms (14/15), ascarids (9/15) and intestinal strongyles (11/15).

Conclusions

The coprological examination allowed to identification of eggs belong Nematoda and Protozoa and by necropsy examination, it were identified nematodes and cestodes. Simultaneous infections with protozoa, cestodes, and nematodes indicate a complex parasitism in the wildcats examined.

Continued monitoring and research are needed to better understand the health effects of *F. silvestris* and the potential risks of transmission to domestic carnivores and humans, respectively.

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References

1. **Conboy, G.,** Cestodes of dogs and cats in North America, Veterinary Clinics: Small Animals, 2009, 39, 6, 1075-1090.
2. **Dărăbuş, Gh., Oprescu, I., Morariu, S. Mederle, N., Ilie, M.,** Ghid practic în bolile parazitare, vol. I, Ed. Agroprint, Timişoara, 2013.
3. **Dărăbuş, Gh., Oprescu, I., Morariu, S. Mederle, N., Ilie, M., Imre, M.,** Parazitologie și Boli Parazitare, Ed. Mirton, Timişoara, 2022.
4. **Deplazes, P.,** Influence of urbanization on the epidemiology of intestinal helminths of the red fox (*Vulpes vulpes*) in Geneva, Switzerland, Parasitology Research, 2007, 101, 3, 605-611.
5. **Diakou, A., Migli, D., Dimzas, D., Morelli, S., Di Cesare, A., Dionisios, Y., Lymberakis, P., Traversa, D.,** Endoparasites of European Wildcats (Felis silvestris) in Greece, Pathogens, 2021, 10, 5, 594.
6. **Eckert, J., Friedhoff, K.T., Zahner, H., Deplazes, P.,** Lehrbuch der Parasitologie für die Tiermedizin, Enke Verlag Stuttgart, 2005, 147, 8, 632.
7. **Gaglio, G., Brianti, E., Brucato, G., Risitano, A.L., Otranto, D., Lia, R.P., Mallia, E., Anile, S., Giannetto, S.,** Helminths of wildcat (*Felis silvestris silvestris*) in Southern Italy, Parassitologia, 2010, 52, 1-2, 274-275.
8. **Galimberti, A., Romano, D.F., Genchi, M., Paoloni, D., Vercillo, F., Bizzarri, L., Sassera, D., Bandi, C., Genchi, C., Ragni, B., Casiraghi, M.,** Integrative taxonomy at work: DNA barcoding of taeniids harboured by wild and domestic cats, Molecular Ecology Resources, 2012, 12, 403-413.
9. **Gherman, C.M., Mihalca, A.D.,** A synoptic overview of golden jackal parasites reveals high diversity of species, Parasites & Vectors, 2017, 10, 419.
10. **Gressler, L.T., Gomes Noll, J.C., Barros de Freitas, Í., Gonzalez Monteiro, S.,** Multiparasitism in a wild cat (*Leopardus colocolo*) (Carnivora*: Felidae*) in southern Brazil, Brazilian Journal of Veterinary Parasitology, 2016, 25, 3, 374-377.
11. **Kirkova, Z., Raychev, E., Georgieva, D.,** studies on feeding habits and parasitological status of red fox, golden jackal, wild cat and stone marten in Sredna Gora, Bulgaria, Journal of Life Sciences, 2011, 5, 264-270.

12. **Krone, O., Guminsky, O., Meinig, H., Herrmann, M., Trinzen, M., Wibbelt, G.,** Endoparasite spectrum of wild cats (*Felis silvestris* Schreber, 1777) and domestic cats (*Felis catus* L.) from the Eifel, Pfalz region and Saarland, Germany, European Journal of Wildlife Research, 2008, 54, 95-100.
13. **Literák, I., Tenora, F., Letková, V., Goldová, M., Torres, J., Olson, P.,** *Mesocestoides litteratus* (Batsch, 1786) (Cestoda: Cyclophyllidea: Mesocestoididae) from the red fox: Morphological and 18S rDNA characterization of European isolates, Helminthologia, 2006, 43, 4, 191-195.
14. **Martinković, F., Sindičić, M., Lučinger, S., Štimac, I., Bujanić, M., Živičnjak, T., Stojčević D.J., Šprem, N., Popović, R., Konjević, D.,** Endoparasites of wildcats in Croatia, Veterinarski Arhiv, 2017, 87, 6, 713-729.
15. **Mederle, N., Dărabuș, G., Stancu, A., Pentea, M., Imre, M., Luca, I., Pavlovic, I., Zdravković, N.,** Intestinal endoparasitism in wild cat (*Felis silvestris*) from Banat area (Romania), Helminthologia, 2023, 60, 2, 161-165.
16. **Mituch, J.,** Die Helminthenfauna der Fleischfresser in der Slowakei, Folia Venatoria, 1972, 2, 161-172.
17. **Moraru, M.M.F., Popovici, D.C., Marin, A.M., Ghilean, B.M., Dreghiciu, I.C., Mederle, N.,** The endoparasitism identification in jackals from Timis County hunting grounds, Lucrări Științifice Medicină Veterinară, 2023, 56, 2, 136-142.
18. **Moraru, M.M.F., Popovici, D.C., Marin, A.M., Ghilean, B.M., Dreghiciu, I.C., Plotuna, A.M., Mederle, N.,** Study regarding the infestation with endoparasites in red foxes from Timis county hunting grounds; Lucrări Științifice Medicină Veterinară, 2023, 56, 2, 143-150.
19. **Napoli, E., Anile, S., Arrabito, C., Scornavacca, D., Mazzamuto, M.V., Gaglio, G, Otranto, D., Giannetto, S., Brianti, E.,** Survey on parasitic infections in wildcat (*Felis silvestris silvestris* Schreber, 1777) by scat collection, Parasitology Research, 2016, 115, 255-261.
20. **Papadopoulos, H., Himonas, C., Papazahariadou, M., Antoniadousotiriadou, K.,** Helminths of foxes and other wild carnivores from rural areas in Greece, Journal of Helminthology, 1997, 71, 227-231.
21. **Rodriguez, A., Carbonell, E.,** Gastrointestinal parasites of the Iberian lynx and other wild carnivores from central Spain, Acta Parasitologica, 1998, 43, 128-136.
22. **Thompson, R.C.A.,** Parasite zoonoses and wildlife: One Health, spillover and human activity,International Journal for Parasitology, 2013, 43, 1079-1088.
23. **Veronesi, F., Deak, G., Diakou, A.,** Wild Mesocarnivores as Reservoirs of Endoparasites Causing Important Zoonoses and Emerging Bridging Infections across Europe, Pathogens, 2023, 12, 2, 178.
24. **Yamaguchi, N., Kitchener, A., Driscoll, C., Nussberger, B.,** *Felis silvestris*, The IUCN Red List of Threatened Species 2015, e.T60354712A50652361.
25. ***https://eur-lex.europa.eu/legal-content/RO/TXT/PDF/?uri=CELEX:31992L0043
26. ***https://lege5.ro/App/Document/geydamjsgq/legea-vanatorii-si-a-protectiei-fondului-cinegetic-nr-407-2006

27.***https://www.mmediu.ro/articol/ministrul-barna-tanczos-a-prezentat-rezultatele-celui-mai-recent-studiu-care-estimeazapopulatia-de-urs-brun-din-romania/6108

DEMODEX MANGE AND *MALASSEZIA* FUNGI INVOLVED IN CUTANEOUS LESIONS OF DOG - CASE REPORT

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Summary

Demodicosis and malasseziosis are skin diseases caused by external parasitical agents. A dog with itching, scales, crusts, lichenification, hyperpigmentation, and alopecia on the abdomen, forelegs, and hind legs as well as on the ears and face were examined. The deep skin scrapping method has been performed but the results were negative. The skin biopsy has been processed for standard histological paraffin embedding and showed the presence of fragments of *Demodex sp* mites. Colonies of *Malassezia* sp. have been identified on microscopic examination of the tape smear. Based on these results, the dog was diagnosed with demodicosis and malasseziosis. Among the types of mixed skin infections that occur in dogs, the combination of demodicosis and malasseziosis infection is rare. Direct parasite detection in histological skin biopsies is a good tool for an undoubted diagnosis.

Keywords: Dog, diagnosis*, Demodex sp*., *Malassezia sp*.

Dogs are among the most popular pets, and their care requires significant attention. Pathology with cutaneous manifestations in dogs can have various origins: parasitic, bacterial, and fungal. *Demodex* sp. mite is one of the most common etiological agents affecting dogs (26).

Demodicosis is defined as an inflammatory skin infection of parasitic origin (18). It is caused by *Demodex* mites: *D. canis*, *D. injai,* and *D. cornei*. *Demodex* mites are a normal flora found in the skin of most apparently healthy dogs, and the disease occurs when these mites multiply and become pathogenic under the influence of various factors (low immune status, improper care, etc.) (5, 9, 13, 15).

Clinically, there are two forms of the disease, localized and generalized. The clinical picture of the disease is usually associated with erythema, pustules, scabs, hyperkeratosis, and alopecia and, pyodemodicosis occurs as a frequent complication (13, 23). The quickest method of diagnosing canine demodicosis is a microscopic examination of the skin scrapings (13, 17).

Malassezia spp. is considered an opportunistic flora, as it can cause superficial mycoses. Symptoms of *Malassezia* sp. infection in dogs are characterized by pruritus accompanied by erythema and crusting. Infected dogs usually have oily and bad-smelling skin with hyperpigmentation and lichenification (1, 12).

The mixed infections between *Demodex* spp. and *Malassezia* spp. are rare. Excessive sebum production and high humidity contribute to the growth of the fungus, but also to rapid mite multiplication (24). The clinical evolution of demodicosis can be misled by the presence of other aetiological agents, and establishing the correct diagnosis becomes a problem for the clinician (13).

This case report aimed to identify the aetiological agents involved in the skin lesions present in a four-year-old dog, using gold methods laboratory and standard histological techniques.

Materials and methods

A stray dog was brought to the Department of Parasitology, Faculty of Veterinary Medicine /University of Life Sciences ”King Mihai I” from Timișoara, with pruritus, alopecia, a bad smell, and hair loss around the eyes (Fig. 1). The 4-year-old dog came from a shelter in Resita. External deworming of the dog was carried out with the product Bravecto (fluralaner).

Fig. 1. Skin lesions; a - hair loss around the eyes, b - alopecia

The following diagnostic steps were performed (13):

- Clinical examination;
- Deep skin scrapings clarified with lactophenol and examined microscopically with 10 X objective (Fig. 2);
- Diff Quik stained skin impression and examined microscopically with 100X objective (Fig. 3);
- Skin samples from biopsy were processed for standard histological paraffin embedding (Paraffin Pastilles for Histology, Merck, Germany) and sectioned at 5μm thickness on a Cut 4062 microtome (Slee Mainz, Germany). Slides stained with the standard hematoxylin-eosin method were examined on an Olympus CX41 microscope (Olympus, Germany) equipped with an Olympus CX41RF digital camera and QuickPHOTO Micro 2.2 image analysis software (Promicra, Czech Republic) (Fig. 4) (10, 11,14).

Fig. 2. Skin scraping

Fig. 3. Diff Quik staining

Fig. 4. Skin biopsy/histopathological examination

Results and discussions

On clinical examination, lesions in the form of scales, scabs, lichenification, hyperpigmentation, alopecia on the abdomen, forelimbs and hindlimbs, and demodectic eyebrows were observed (Fig. 5).

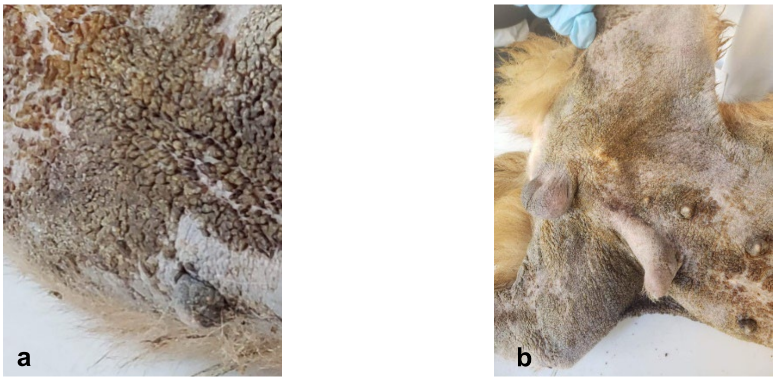

Fig. 5. Skin lesions; a - scaly lesions; b -hyperpigmentation, alopecia

Microscopic examination of the deep skin scrapings did not reveal the presence of the mites.

Microscopic examination of the skin impression identified ovoid and ellipsoidal yeast cells specific for *Malassesia* sp.

Histological examination of skin biopsy slides revealed the presence of mites in hair follicles (Fig. 6 -8). Due to the presence of the parasite, the body responds by extravasating leukocytes from the bloodstream into the adjacent connective tissue, triggering an inflammatory, defensive response (Fig. 9).

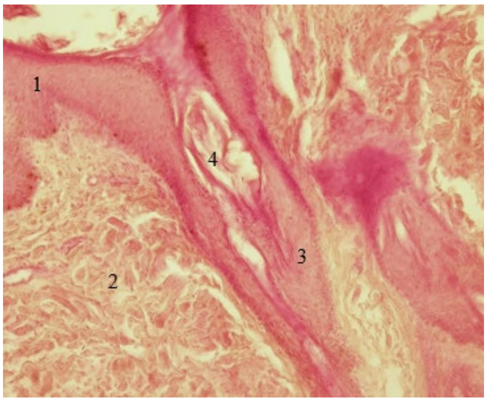

Fig. 6. Histological examination - Dog's skin section: 1. epidermis, 2, dermis, 3. hair follicle, 4. mite, H.E. stain, ob. 20X

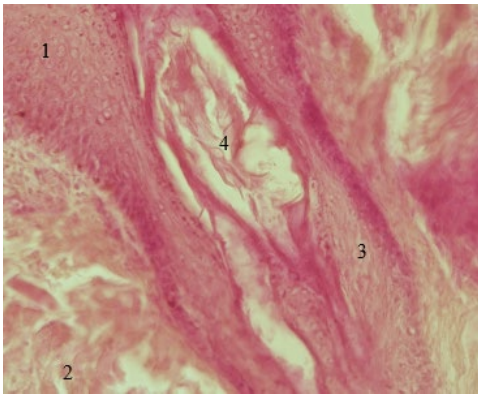

Fig. 7. Histological examination - Dog's skin section: 1. epidermis, 2, dermis, 3. hair follicle, 4. mite, H.E. stain, ob. 40X

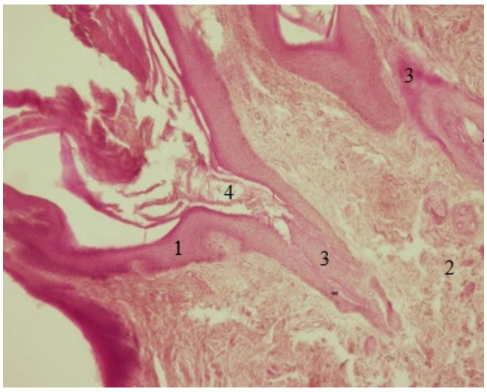

Fig. 8. Histological examination - Dog's skin section:1. epidermis, 2, dermis, 3. hair follicle, 4. mite, H.E. stain, ob. 10X

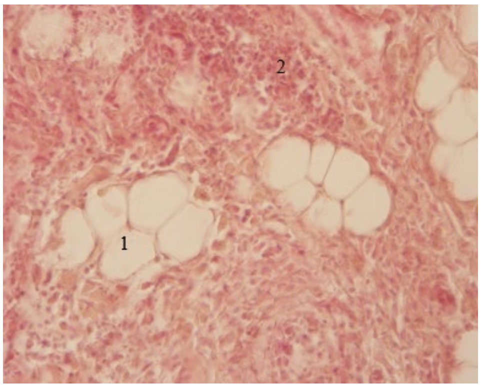

Fig. 9. Histological examination -Dog's skin section: 1. adipocytes, 2. leukocyte infiltrate, H.E. stain, ob. 40X

Demodicosis can lead to secondary infections (e.g. *Malassesia* sp. infection) (1) but, at the same time, the hyperseborrhea produced by the presence of the fungus favors the multiplication of *Demodex* sp. mites (16).

Histopathological examination of the skin can diagnose demodicosis when there is a clinical suspicion, but direct microscopy diagnosis is negative (2, 3, 13).

There are cases when diagnose through skin scraping techniques is difficult to perform because of the skin inflammation, the presence of other pathological agents (eg. nodular piodemodicosis), and special features of the skin specific to some bread (eg. the Shar-Peis dermis production of mucin is excessive)., The diagnosis through biopsy and histological examination is necessary when demodicosis is clinically suspected. Histological examination is used for confirmation of the mite presence which are easily identified within the hair follicles (10, 11, 14).

The study performed by Gartner et al. (8) on a group of 187 dogs diagnosed with demodicosis showed the absence of typical lesions in demodicosis (demodectic eyeballs) and the occurrence of hyperpigmentation and pruritus in dogs with dry demodicosis but without association with other pathogens (8). In the present study, the typical lesions for demodicosis were identified together with other pathogens (the fungus *Malassesia sp*.).

Demodicosis accompanied by *Malassesia* sp. infection has also been reported, particularly in dogs with a history of poor care, nutrition, and feeding (*24, 25*), as was the case of the dog presented in this study.

Similar to our study, the other histopathological research of the skin from dogs with demodicosis described the presence of *Demodex* mites in the hair follicles (19, 20, 21).

Twelve formalin-fixed, paraffin-embedded skin biopsies from dogs and one biopsy from a cat in which *Demodex* mites were histologically identified were also analyzed by real-time PCR technique. In all cases, multiple mites were present and histological lesions were characteristic of demodicosis (22).

Demodex mites are found on the skin of humans and dogs. They can also be present as skin commensals. A background of host immunosuppression is a common risk factor for overgrowth of *Demodex* mites in both humans and dogs. Symptoms and clinical impact are different in humans and dogs, probably related to variations in the pilosebaceous follicles in which the mites live and attract bacterial superinfection (6, 7).

The study performed by Das et al. (4) examined histopathological skin changes in dogs infected with *Rhipicephalus sanguineus, D. canis, Ctenocephalides canis,* and *Trichodectes canis*. In the case of *D. canis*, desquamation of the keratin layer and destruction of the superficial epidermal layer were observed, with the presence of the mite in the hair follicle surrounded by infiltrates of neutrophils and mononuclear cells (4).

In the present study, skin biopsy examination confirmed the presence of *Demodex* mite, and skin impression confirmed the presence of the fungus *Malassesia* spp.

Conclusions

Demodicosis in dogs can be associated with secondary infections such as *Malassezia* spp., especially in cases where there is a history of poor grooming, nutrition, and feeding.

Diagnosis of demodicosis can be difficult due to inflammation of the skin or the presence of other pathological conditions.

Histological examination of the skin is essential to confirm the diagnosis of demodicosis when direct microscopy is negative, but there is a clinical suspicion.

References

1. **Adiyati, P.N., Pribai, E.S.,** *Malassezia spp.* dan peranannya sebagai penyebab dermatitis pada hewan peliharaan, Journal of Veterinary, 2014, 15, 4, 570-581.
2. **Caswell, J.L., Yager, J.A., Parker, W.M., Moore, P.F.,** A prospective study of the immunophenotype and temporal changes in the histologic lesions of canine demodicosis, Veterinary Pathology, 1997, 34, 279-287.
3. **Chakrabarti, A., Misra, S.K.,** Studies on the pathology of *Demodex canis* in the internal organs of canines, Indian Journal of Animal Sciences, 1978, 48, 466-468.
4. **Das, G., Neog, R., Sarmah, P.C., Pathak, P., Goswami, S., Kalita, D.J., Bordoloi, G., Pathak, N., Dutta, K.J.,** Study on histopathology of dog skin naturally infested with ectoparasites, Veterinary Practitioner, 2022, 23, 1, 88.
5. **Dimri, U., Ranjan, R., Kumar, N., Sharma, M.C., Swarup, D., Sharma, B., Kataria, M.,** Changes in oxidative stress indices, zinc and copper

concentrations in blood in canine demodicosis, Veterinary Parasitology, 2008,154, 1-2, 98-102.
6. **Foley, R., Kelly, P., Gatault, S., Powell, F.,** *Demodex*: a skin resident in man and his best friend, European Academy of Dermatology and Venereology, 2020, 35, 1, 62-72.
7. **Forton, F., Seys, B.,** Density of *Demodex folliculorum* in rosacea: a case control study using standardized skin-surface biopsy, British Journal of Dermatology, 1993, 128, 650-659.
8. **Gartner, A., Dărăbuș, Gh., Badea, C., Hora, F., Tilibasa, E., Mederle, N.,** Clinical diagnosis in canine demodicosis. A new approach, Scientific Works, Series C Veterinary Medicine, 61, 2, 2015, 76-80.
9. **Gortel, K.,** Update on canine demodicosis, Veterinary Clinics of North America, Small Animal Practice, 2006, 36, 1, 229-241.
10. **Hnilica, K.A.,** Small Animal Dermatology: A Color Atlas and Therapeutic Guide, 3rd ed., St. Louis, Elsevier, 2011.
11. **Jackson, H.A., Marsella, R.,** BSAVA Manual of Canine and Feline Dermatology, 3rd ed., Quedgeley, Gloucester, England: British Small Animal Veterinary Association, 2012.
12. **Mederle, N., Dărăbuş, Gh.,** Epidemiological comparative inquiry in dermatomycosis of carnivores, Lucrări Științifice Medicină Veterinară Timișoara, 2007, 15, 160-164.
13. **Mederle, N., Dărăbuş, Gh., Oprescu, I., Morariu, S., Ilie, M., Indre, D., Mederle, O.,** Diagnosis of canine demodicosis, Scientia Parasitologica, 2010, 11, 1, 20-23.
14. **Miller, W.H., Griffin, C.E., Campbell, K.L.,** Muller & Kirk's Small Animal Dermatology, 7th ed., St. Louis, Elsevier; 2012.
15. **Morariu, S., Morariu, F., Marin, A.M., Moraru, M.M.F., Popovici, D.C., Imre, M., Igna, V., Mederle, N.,** First morphological and molecular identification of *Demodex injai* in golden jackal (*Canis aureus* Linnaeus, 1758) in Romania, Pathogens, 2023, 12, 412.
16. **Negrescu, A., Mederle, N., Marin, A.M., Dărăbuș Gh.,** Identification of *Demodex spp.* presence in dog infested with other parasites. Case report, Lucrări Științifice Medicină Veterinară Timișoara, 2022, 55, 3, 103-108.
17. **Paterson, T.E., Halliwell, R.E., Fields, P.J., Louw, M.L., Louw, J.P., Ball, G.S., Pinckney, R.D., McKibben, J.S.,** Treatment of canine-generalized demodicosis: A blind, randomized clinical trial comparing the efficacy of Advocate® (Bayer animal health) with ivermectin, Veterinary Dermatology, 2009, 20, 5-6, 447-455.
18. **Perego, R., Spada, E., Foppa, C., Proverbio, D.,** Critically appraised topic for the most effective and safe treatment for canine generalized demodicosis, BMC Veterinary Research, 2019, 15, 1, 17.
19. **Rabdea, N.,** Aspecte histopatologice în piodemoticoza canină, Revista Română de Medicină Veterinară, 2006, 2, 117-124.

20. **Rabdea, N., Dărăbuș, Gh., Oprescu, I., Ilie, M., Nicola, A., Mederle, O.,** Aspecte histopatologice în demoticoza generalizată la câine, Scientia Parasitologica, 2005, 6, 1-2, 100-1003.
21. **Rabdea, N., Mederle, O., Dărăbus, Gh., Oprescu, I., Morariu, S., Ilie, M.,** Immunohistochemical results in canine piodemoticosis, Lucrări Științifice Medicină Veterinară, USAMV ”Ion Ionescu de la Brad” Iași, 2006, 49, 8, 404-406.
22. **Ravera, I., Altet, L., Francino, O., Bardagí, M., Sánchez, A., Ferrer, L.,** Development of a real-time PCR to detect Demodex canis DNA in different tissue samples, Parasitology Research, 2011, 108, 305-308.
23. **Scott, D.W, Miller, W.M, Griffin, C.E.,** Parasitic skin diseases., Di Berardino C, 6th ed., Philadelphia, W.B. Saunders Company, 2001.
24. **Srikala, D., Kumar, K.S., Kumar, V.V.V.A., Nagaraj, P., Ayodhya, S., Rao, D.S.T.,** Management of mixed infestation of malasseziosis and demodicosis in dogs, Intas Polivet, 2010, 11, 1, 74-76.
25. **Tarallo, V.D., Lia, R.P., Sasanelli, M., Cafarchia, C., Otranto, D.,** Efficacy of Amitraz plus Metaflumizone for the treatment of canine demodicosis associated with *Malassezia pachydermatis*, Parasite Vectors, 2009, 2, 13, 1-5.
26. **Wirawan, I.G., Widiastuti, S.K., Batan, I.W.,** Laporan Kasus: Demodicosis Pada Anjing Lokal Bali, Indonesia Medicus Veterinus, 2019, 8, 1, 9-18.

EVALUATION OF ENDOPARASITISM IN FALLOW DEER (*DAMA DAMA L.*) FROM OLT COUNTY (ROMANIA) HUNTING GROUNDS

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Summary
The fallow deer (*Dama dama* L.) is a species of solenodon (ruminant) of the Cervidae family in the Romanian fauna, particularly of the genus *Dama*. This species does not live alone, in the habitat together with other species between which various relationships or interactions are established. This interaction between two or more species takes various forms: competition, commensalism, mutualism, predation, or parasitism. From this point of view, the *D. dama* is no exception. In this context, the purpose of the study was to identify the possible presence of endoparasites in *D. Dama* from two hunting grounds in Olt County, using classical coproparasitological methods, macroscopic and microscopic examination of gastrointestinal mass and organs from hunted animals. Of the 14 samples of *D. dama,* gastrointestinal nematodes (14.28%), and *Gongylonema* spp. (28.57%) were identified. In conclusion, the presence of endoparasites is associated with their impact on the health of the *D. dama*, but also with the possible risk of infection of the environment and, implicitly, of other cervids and domestic ruminants.
Keywords: Endoparasitism, *D. dama*, Olt County.

The *D. dama* is a species of selenodon (ruminant) in the Order Artiodactyla, of the even-toed ungulates, which totals more than 200 species globally, taxonomically classified into 10 distinct families. This species has been present in wild habitats in Romania since the Neolithic period and covers more than 65% of Romania's surface (1).

A herbivorous species par excellence, the *D. dama* is a species with low demands on the quality of the food available, which is why it is found in forest or mixed habitats with lower-quality forage. Of course, it prefers quality food, which is why it is also found in various crops: legumes (alfalfa, clover, borage), cereals (wheat, triticale, rye, sunflower, maize) where it causes damage, but where it also interferes with various domestic animals (3).

Parasitism is a very complex phenomenon involving on the one hand the host and the parasite and on the other hand the interactions between them (24). The nature of these interactions is dynamic and it can easily be anticipated that both the host and the parasite will try to adapt to the situation, without being able to control the duration and end of this process (10).

The emergence of new parasite species or spatial variations in parasite-host interactions (especially new, unusual hosts) is a major factor on which monitoring of this co-evolutionary process depends (24, 29).

Trichostrongylosis, a gastrointestinal helminthosis of ruminants, is clinically manifested by diarrhea, anemia, and cachexia. The disease has a seasonal course, with clinical manifestations in late summer and autumn. Infection occurs in pastures with feed and water, affecting mainly young animals. These parasitic diseases cause significant economic damage through their implications in terms of reduced production and non-value of forage, morbidity, and mortality (9, 25).

Gongylonema spp. is a widespread nematode throughout the world and affects domestic and wild mammals, birds, and occasionally humans. It is produced by species of the *Gongylonema* genus that are located in the mucosa of the upper digestive tract, including the tongue and especially the esophagus, producing white or red zigzag tracks in the mucosa (26).

The present study aimed to assess the impact of endoparasitism on the health status and management of populations of *D. Dama* from two hunting grounds in Olt County, using classical coproparasitological methods, macroscopic and microscopic examination of gastrointestinal mass and organs from hunted animals.

Materials and methods

The study was carried out over one year, on some 14 *D. dama* (eight males, respectively six females) aged between one year and 10 years from two hunting grounds in Olt County Romania (Fig. 1). The animals were hunted by the annual harvest quotas set by the Ministry of Environment, Water and Forestry. The establishment of these quotas was carried out based on hunting management criteria and followed the extraction of fallow deer specimens by sex, specimen quality, and age categories (21). Afterward, organs (lung, liver, and nervous system) and gastrointestinal tracts were taken from each individual and examined in the Parasitic Diseases Clinic of the Faculty of Veterinary Medicine/University of Life Sciences "King Mihai I" from Timisoara, Romania.

Coprological and necropsy examination

Fecal samples were collected from the gut of each individual, and stored at 4°C until processing. The following methods were used (8):

- Qualitative method - identification of the parasitic load of the whole digestive tract with light eggs of nematodes, cestodes, and protozoan oocysts.
- Polyvalent method (of successive washes) - identification of the presence of trematode eggs.
- Larvoscopic method - highlighting parasitism with pulmonary nematodes.

Necropsy examination was according to the technical instructions of necropsy (8) (Fig. 2).

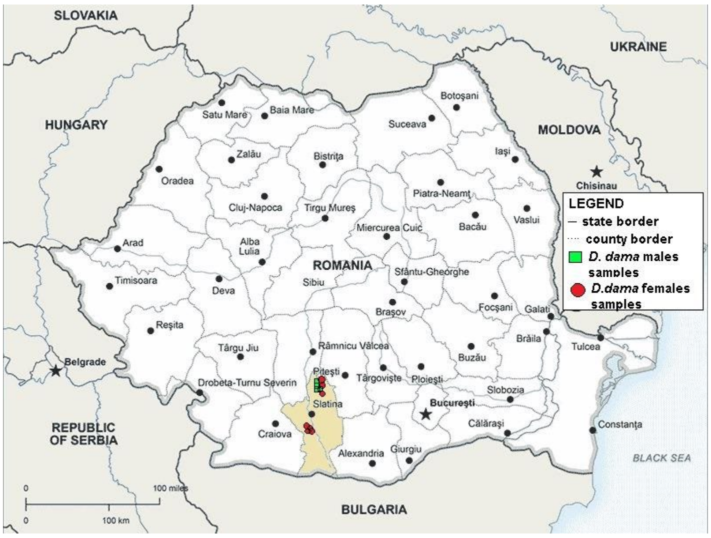

Fig. 1. Map showing the geographical areas where *D. dama* were collected; green squares show the sites where male sample animals were found and circles show the sites where female animals were found

Fig. 2. Necropsy examination

Results and discussions

The results of the coprological examination performed by the flotation method revealed the presence of the following parasitic elements: morulated eggs (gastrointestinal nematodes, *Nematodirus spp.*) and eggs of *Gongylonema* in six out of 14 samples examined (42.86%) (Fig. 3, Fig. 4).

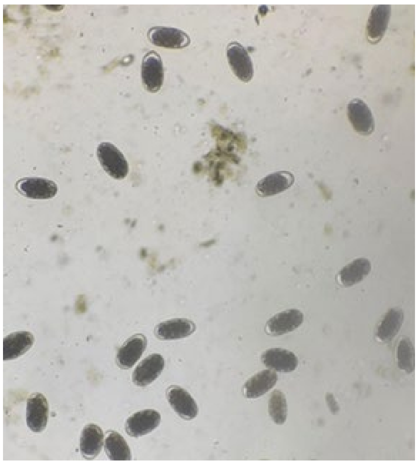

Fig. 3. Nematodes eggs

Fig. 4. *Nematodirus spp*. egg

The results of the coprological examination performed by the sedimentation method revealed the absence of parasitic elements belonging to the genera *Fasciola*, *Paramphistomum,* and *Dicrocelium*.

Pulmonary nematode larvae were not evident in the Baermann/Vajda examinations.

Out of the 14 esophageal samples, the nematode *Gongylonema* was identified in four of the *D. dama* (28.57%) (Fig. 5).

The results of the necropsy examination of the gastrointestinal mass revealed the presence of different genres of gastrointestinal nematodes (GIN) in two out of 14 samples examined (14.28%) with the following localization: in abomasum – *Haemonchus* spp. and *Ostertagia* spp., and in the small intestine – *Nematodirus* spp. (Fig. 6-8).

In the present study, the presence of gastrointestinal nematodes (GIN) and the nematode *Gongynolema* was identified by necropsy, and eggs of GIN and *Gongylonema* spp. were identified by the flotation method.

Survival strategies of parasites and the impact of endoparasitism on the health of domestic and wild animals are the subject of bibliographic references from Romania and abroad (2, 4, 7, 11, 13, 15, 16, 17, 18).

The first study reported by Hora et al. in cervides in the western part of Romania evokes the presence of digestive and respiratory nematodes in *D. dama*: *Haemonchus contortus, Nematodirus filicollis, Oesophagostomum venulosum* and *Dictyocaulus* spp. Only one species of trematode, *Dicrocelium dentriticum*, has been identified in roe deer (*Capreolus capreolus*) and red deer (*Cervus elaphus*) (12).

Infection with *Sarcosystis* spp in the musculature has been reported in *C. capreolus* from Romanian hunting grounds (14), as well as the presence of pulmonary (*Dictyocaulus filaria*) and digestive nematodes (*Trichostrongylus, Haemonchus, Chabertia, Trichocephalus*) (5, 20).

Fig. 5. Esophagus of *D. dama* parasitized with *Gongylonema* spp.

Fig. 6. *Ostertagia* spp. – male (posterior extremity)

Fig. 7. Gastrointestinal nematodes

Fig. 8. *Haemonchus spp.*

Darabus et al. (6) identified parasitism with *Eimeria* spp. and *Nematodirus* spp. in *D. dama*, in the Timisoara Zoo (Romania). Dicrocelial trematodes, paramphistomes, and gastrointestinal nematodes have been identified in *D. dama* from Romania (23, 25, 27). Recently, infections of *Fascioloides magna* and *Gongylonema pulchrum* in *D. dama* have been reported for the first time in Romania (24, 26).

D. dama from Hungary were parasitized with the trematode *Dicrocoelium dendriticum* and nine nematode species: *Dictyocaulus viviparus, Haemonchus contortus, Ostertagia leptospicularis, Skrjabinagia kolchida, Trichostrongylus axei, Capillaria bovis, Nematodirus filicollis, Nematodirus filicollis, Nematodirus roscidus* (30).

In the present study, the identification of different species of gastrointestinal nematodes as well as the nematode *Gongylonema* joins the results of the previously presented studies.

The presence of endoparasites is associated with their impact on the health of the *D. dama*, but also with the possible risk of infection of the environment and, implicitly, of other cervids and domestic ruminants (19, 22, 28).

Conclusions

In *D. dama* from Olt County (Romania), the presence of gastrointestinal nematodes (NGI), respectively the nematode *Gongynolema*, was identified through the necropsy examination, and the flotation method, eggs of NGI and *Gongylonema* spp were identified.

The identification of endoparasites in *D. dama* from Olt County (Romania) justifies, once again, the importance of their surveillance and monitoring, as well as the abundance and distribution of intermediate and final hosts.

Parasitological control strategies applied to host animals, parasites, and the environment could mitigate the potential negative effects of existing and invasive parasites on the biodiversity, health, and economy of deer populations in Romania.

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References

1. **Bokonyi, S.,** Angaben zum friihholozänen Vorkommen des Damhirschen Cervus (*Dama*) dama L. 1758 in Europa, Saugetierwissenschaftlichen Mitteilungen, 1971, 19, 3, 206-217.

2. **Borkovcova, M., Langrova, I., Totkova, A.,** Endoparasitoses of fallow deer (*Dama dama*) in game-park in South Moravia (Czech Republic), Helminthologia, 2013, 50, 15-19.
3. **Cotta, V., Bodea, M.,** Vânatul României, Editura Agrosilvică, 1969.
4. **Dar, J.A., Fazili, M.F., Bhat, B.A., Ahmad, R., Wani, I.N.,** Prevalence of gastrointestinal helminth parasites of Himalayan goral (*Naemorhedus goral*) in Kazinag National Park, Jammu and Kashmir, India, Indian Journal of Animal Research, 2022, 56, 5, 587-593.
5. **Dărăbuș, G., Afrenie, M., Herman, V., Ilie, M.S., Indre, D.,** Nematoda parasitism in deer (*Capreolus capreolus*) - necropsic case study, Lucrări Științifice Medicină Veterinară Iași, 2009, 52, 11, 529-531.
6. **Dărăbuş, G., Afrenie, M., Ilie, M.S., Indre, D.,** The researches regarding digestive parasitism in herbivorous from Zoological Garden Timisoara, Lucrări Științifice Medicină Veterinară Timișoara, 2009, XLII, 1.
7. **Dărăbuş, Gh., Morariu, S., Mederle, N., Ilie, M.S., Luca, I., Cireşan, A.C.,** A comparative study regarding the efficacy of anthelmintic treatment for gastrointestinal parasitism in sheep, Revista Română de Medicină Veterinară, 2021, 31, 1, 77-80.
8. **Dărăbuş, Gh., Oprescu, I., Morariu, S. Mederle, N., Ilie, M.,** Ghid practic în bolile parazitare, vol. I, Ed. Agroprint, Timişoara, 2013.
9. **Dărăbuş, Gh., Oprescu, I., Morariu, S. Mederle, N., Ilie, M., Imre, M.,** Parazitologie și Boli Parazitare, Ed. Mirton, Timişoara, 2022.
10. **Feis, M.E., Goedknegt, A.M., Thieltges, D.W., Buschbaum, C., Wegner, K.M.,** Biological invasions and host–parasite coevolution: different coevolutionary trajectories along separate parasite invasion fronts, Zoology, 2016, 119, 366-374.
11. **Hora, F.Ș., Mederle, N., Badea, C., Tilibașa, E.M., Ilie, M.S., Dărăbuș, Gh.,** Digestive parasite fauna in hare (*Lepus europaeus*) in Western Romania, Scientific Works Series C Veterinary Medicine, 2015, 61, 1, 138-141.
12. **Hora, Ș., Genchi, C., Ferrari, N., Morariu, S., Mederle, N., Dărăbuș, G.,** Frequency of gastrointestinal and pulmonary helminth infections in wild deer from western Romania Veterinary Parasitology: Regional Studies and Reports, 2017, 8, 75-77.
13. **Iglodyova, A., Lazar, P., Curlik, J., Karolova, R., Ciberej, J., Bockova, E., Strkolcova, G.,** Observations on autochtonous liver flukes in wild ruminants in Slovakia, Helminthologia, 2017, 54, 307-313.
14. **Imre, K., Sala, C., Morar, A., Ilie, M.S., Imre, M., Oniță, P., Dărăbuș, G.,** Occurence of *Sarcocystis* spp. in roe deer (*Capreolus capreolus*) from western Romania - preliminary results, Lucrări Științifice Medicină Veterinară Timișoara, 2012, 45, 126-130.
15. **Indre, D., Dărăbuș, Gh., Oprescu, I., Morariu, S., Mederle, N., Ilie, M.S., Măndiță, D.N.,** The identification of gastrointestinal nematodes species in

sheep in five localities from Timis County, Lucrări Științifice Medicină Veterinară Timișoara, 2009, 42, 1.

16. **Magdalek, J., Bourgoin, G., Vadlejch, J.,** Non-native nematode *Ashworthius sidemi* currently dominates the abomasal parasite community of cervid hosts in the Czech Republic, Frontiers in Veterinary Science, 2022, 9, 862092.
17. **Marin, A.M., Moraru, M.M.F., Popovici, D.C., Sîrbu, B.A.M., Cireșan, C.A., Pavlović, I., Albu Kaya, M.G., Mederle, N.,** Study regarding the endoparasitism in goats from Dolj County, Lucrări Științifice Medicină Veterinară, Timișoara, 2022, 55, 2, 142-151.
18. **Mederle N.,** Incidența fasciolozei la ovinele și bovinele din județul Argeș, Revista Română de Medicină Veterinară, 2008, 18, 3, 128-135.
19. **Muller, M., Weber, A., Reith, B., Kratzer, R.,** Diseases of fallow deer from enclosures in Northern Bavaria. Results of post mortem examinations from 1988 to 2002, Tierarztliche Umschau, 2013, 58, 9, 476-481.
20. **Oprescu, I., Dărăbuș, G., Morariu, S., Mederle, N., Ilie, M.S., Hora, F., Rujici, D.,** Prevalence of helminthosis in hunting species *Capreolus capreolus* (Roe deer) and *Sus scrofa L.* (wild boar) in Câmpia Village sylvatic ecosystem, Caras-Severin County, Lucrari Stiintifice, Medicina Veterinara, Timisoara, 2015, 48, 124-133.
21. Ordinul Ministrului Mediului, Apelor și Pădurilor nr. 1571/07.06.2022 Privind Aprobarea Cotelor de Recoltă Pentru Unele specii de Faună de Interes Cinegetic.
22. **Pilarczyk, B., Tomza-Marciniak, A., Udala, J., Kuba, J.,** The prevalence and control of gastrointestinal nematodes in farmed fallow deer (*Dama dama* L.), Veterinarski Arhiv, 2015, 85, 415-423.
23. **Popovici D.C., Mederle N., Marin A.M., Moraru M.M.F., Albu Kaya M.G., Ionescu O.,** Evaluation of the parasitic load in fallow deer (*Dama dama* L.) from Arad County, Lucrări Științifice Medicină Veterinară, Timișoara, 2022, 55, 2, 172-179.
24. **Popovici, D.C., Dărăbuș, G., Marin, A.M., Ionescu, O., Moraru, M.M.F., Imre, M., Tîrziu, E., Mederle, N.,** Identification and molecular characterization of giant liver fluke (*Fascioloides magna*) infection in european fallow deer (*Dama dama*) in Romania-First Report, Microorganisms, 2024, 12, 3, 527.
25. **Popovici, D.C., Ionescu, O., Marin, A.M., Moraru, M.M.F., Robu, M., Badea, C., Mederle, N.,** Study regarding the infestation with gastrointestinal nematodes in fallow deer (*Dama dama* l.) from Timiș county, Lucrări Științifice Medicină Veterinară Timișoara, 2023, 56, 3, 89-95.
26. **Popovici, D.C., Marin, A.M., Ionescu, O., Moraru, M.M.F., Kaya, D.A., Imre, M., Mederle, N.,** First molecular data of *Gongylonema pulchrum* (Rhabditida: Gongylonematidae) in european fallow deer *Dama dama* from Romania, Pathogens, 2024, 13, 2, 175.
27. **Popovici, D.C., Mederle, N., Marin, A.M., Moraru, M.M.F., Ghilean, B.M., Kaya, D.A., Ionescu, O.,** Evaluation of endoparasitism in fallow deer (*Dama*

dama L.) from Bihor County (Romania) hunting grounds, Lucrări Științifice Medicină Veterinară Timișoara, 2023, 56, 2, 213-219.

28. **Schäffer, D., von Borell, E.,** Animal welfare-conforming fallow deer farming in Germany-a review, Deutsche tierärztliche Wochenschrift, 2002, 109, 9, 400-411.
29. **Sinclair, R.E., Fryxell, J.,** Wildlife ecology, conservation, and management, Garsington Road, Oxford, UK, 2006.
30. **Takács, A.,** Data on the parasitological status of a fallow deer population living in a free hunting area, Magyar Állatorvosok Lapja, 2000, 122, 10, 618-620.

TRICHOPHYTON VERRUCOSUM INFECTION IN A CATTLE FARM FROM TIMISOARA

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Summary

Microsporum, *Trichophyton* and *Epidermophyton*. In bovine trichophytosis, the aetiological agent responsible for the appearance of clinical signs is *Trichophyton verrucosum*. This disease is a zoonosis with serious economic and sanitary consequences for animal and human health. Trichophytosis is transmitted through direct contact with carrier animals, but also through the contaminated environment. The purpose of this study was to identify the nature of the aetiological agents and to establish the epidemiological situation of the cattle in a farm from the North of Timisoara. The studied animals were divided into age categories (0-2, 2-4, 4-6 and 6-12 months old). We evaluated the cattle clinically and through laboratory methods. To establish the diagnosis we identified specific lesions characterized by: alopecia, white scabs and trichophytic plaques. We performed skin scrapes from the lesions and we inoculated one dermatophyte test medium (DTM) which was subsequently incubated. The skin scrapings were negative, thus we excluded the presence of mites, and the color of the culture medium turned red following the incubation period, demonstrating the presence of dermatophytes. Of the 39 examined cattle, 14 (35.9%) showed clinical signs. The age category with the highest percentage in expressing clinical signs was 4-6 months (85.7%), and the category with the lowest percentage was 0-2 months, in which none of the calves showed clinical signs. The transmissibility, the impairment of the technological flow, the serious economic and health consequences involved in this pathology may pose a danger to animals and humans. Rigorous farm hygiene, treatment of animals and early diagnosis are the methods by which proper parasitological control can be achieved in cattle trichophytosis.
Keywords: Dermatophytoses, *Trichophyton verrucosum*, bovine.

Dermatophytes are pathogenic fungi that cause superficial mycoses (dermatophytosis, also called ringworm) in humans and animals. This class includes phylogenetically and ecologically related, Gram-positive, aerobic, filamentous pathogens belonging to the *Arthrodermataceae* family (order *Onygenales*), capable of using keratin as their sole source of nutrients. In recent decades, dermatophytes have been classified into three distinct genera: *Microsporum, Trichophyton, Epidermophyton*, but since the advent and use of phylogenetics, their numbers have increased considerably. Although the number of species has increased over time, reaching more than 40 identified species, the most important pathogenic species are still part of the aforementioned genera (8, 13, 17, 21).

Dermatophytoses are commonly diagnosed in farm animals, pets and wildlife. Infections produced by these fungi are usually self-limiting, showing good and prompt response after treatment application. There is also the possibility that the disease manifestations are severe, systemic, especially in immunosuppressed animals (3, 20).

Trichophyton spp. has been reported as one of the most frequent factors of morbidity in calves, and *Trichophyton verrucosum* species is the dermatophyte isolated almost exclusively from cattle, although other species such as *Microsporum gypseum, T. mentagrophytes, T. rubrum and T. simii* have also been reported (15).

This dermatophytosis can have an acute or chronic course. Cattle become infected through direct contact with diseased animals, but also with asymptomatic carriers. The disease can also be transmitted indirectly through contact with the contaminated environment, where spores persist for up to four years (16, 22).

Young animals are more susceptible and at the same time more affected in infection with these fungi compared to adults, due to the fact that juveniles do not have specific immunity sufficiently developed (3, 4).

Trichophytosis is also a public health concern because it is a zoonosis. Transmission to humans is common in rural areas and is considered an occupational disease for agricultural workers in direct contact with infected animals. Typically, the affected areas are the scalp, nails and skin (14, 18, 19).

The infection is often apparent, with alopecic areas covered with thin farinaceous desquamations, or with thick crusty lamellar scales difficult to pull away from the skin. Lesions are mainly distributed on the head and neck, but in more severe cases, the whole body can be affected (1, 3, 16).

This disease has serious economic and health consequences, which are often ignored: depreciation of skins, decreased yields, high costs for parasitological control, movement restrictions that may alter the technological flow and zoonotic character (6).

The aim of this study was to establish the epidemiological situation and identify the nature of the pathogens involved in the occurrence of specific lesions in cattle on a farm located in the northern part of Timișoara.

Materials and methods

The study was carried out in November 2023 on a cattle farm in the north of Timisoara.

A total of 39 cattle (12 males and 27 females), Romanian Black Baltata breed, aged between 1 month and 12 months, were examined. The group of cattle was divided into the following age categories: 0-2 months (3 calves), 2-4 months (9 calves), 4-6 months (7 calves), 6-12 months (20 heifers), which were subjected to clinical and laboratory examinations.

The following steps were performed to establish the diagnosis and pathogen detection: rigorous clinical examination of each individual and complementary laboratory examinations: skin scraping, trichogram, scotch tape test, inoculation on dermatophyte-specific culture medium (DTM) of hairs and incubation at 27°C for 5-

10 days, ending with microscopic examination of the pathogens involved in the appearance of lesions (Fig. 1, Fig. 2) (7).

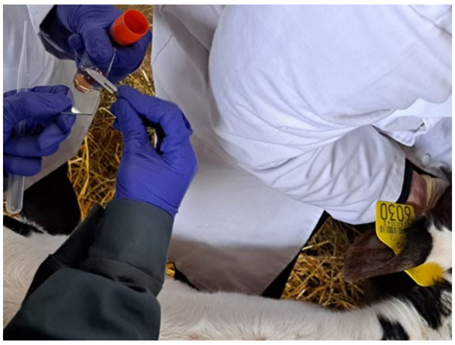


Fig. 1. Skin scraping (original)

Fig. 2. Inoculation of culture media (original)

Results and discussions

Clinical examination revealed the presence of lesions specific for *T. verrucosum* infection in 14 cattle out of 39 examined.

The presence of alopecic areas, white crusts and trichophytic plaques on the head, around the eyes, the nape area, the dorsal region and on the limbs was observed (Fig. 3, 4).

Fig. 3. Calf with facial lesions (original)

Fig. 4. Calf with lesions on the head and withers (original)

Microscopic analysis of skin scrapings did not reveal the presence of mites. No changes were found on the surface of the examined hairs. After 3 days of incubation, the colour of the culture medium turned red, confirming the presence of dermatophytes in the inoculated medium (Fig. 5). After the sixth day of incubation, slightly folded, heaped glabrous grey white colonies grew on the surface of the culture medium (Fig. 6). Microscopic examination of the culture revealed the characteristic presence of septated hyphae with chlamydoconidias arranged in chains with single microconidia specific to the genus *Trichophyton* (Fig. 7).

Fig. 5. Color change of DTM (original)

Fig. 6. Characteristic *T. verrucosum* colonies on DTM (original)

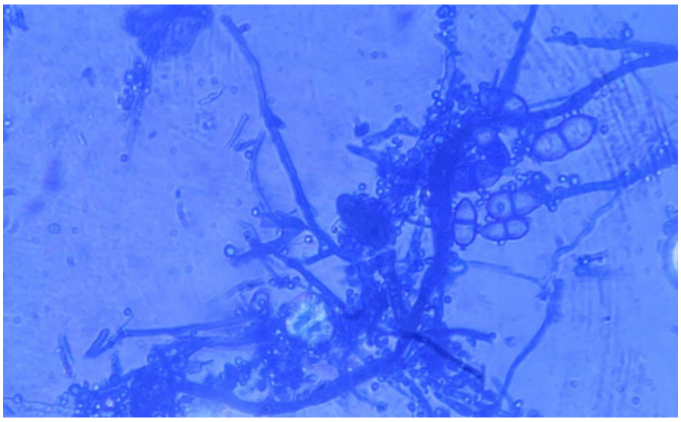


Fig. 7. Septated hyphae with chlamydocondias arranged in chains (original)

Of the total number of cattle examined (19 calves and 20 heifers), 14 showed clinical signs (35.9%), of which 9 calves (47.36%) and 5 heifers (25%) (Fig. 8).

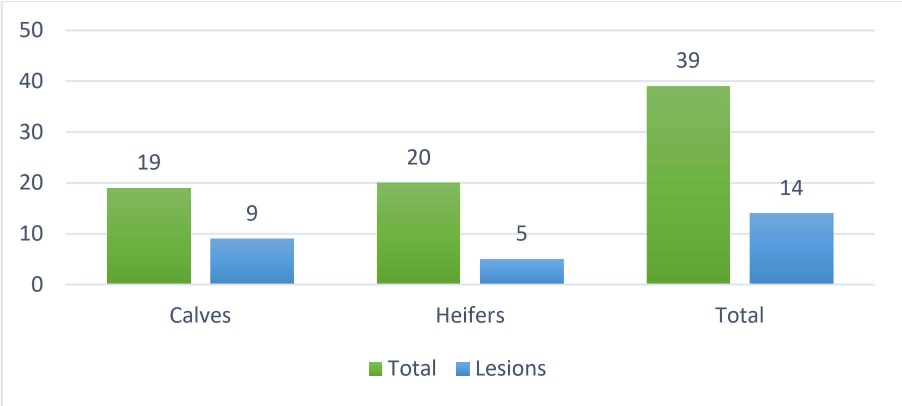
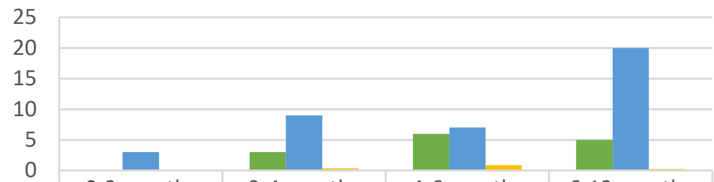


Fig. 8. Prevalence according to age of animals with lesions

Examination of animals with clinical signs based on age category revealed the following results (Fig. 6):

- o 0/3 positive calves in the 0-2 months category (0%)
- o 3/9 positive calves in the 2-4 months category (33.3%)
- o 6/7 positive calves in the 4-6 months category (85.7%)
- o 5/20 positive heifers in the 6-12 months category (25%)

The results obtained from the examination of cattle by age categories confirm that young cattle are more susceptible to *T. verrucosum* infection. Out of 3 examined calves from the 0-2 months age category, none showed clinical signs; this is explained by the fact that each individual in this category was reared in an individual pen and did not have contact with other animals. In the 2-4 months age category, only 3 calves out of the 9 calves examined showed clinical signs (33.3%), while for the 4-6 months age category, the number of individuals with skin lesions increased significantly, as follows: out of the 7 examined calves, 6 showed clinical signs (85.7%). It can be observed that the incidence of ringworm is much higher in the 4-6 months age category compared to the rest of the age categories (Fig. 9).



	0-2 months	2-4 months	4-6 months	6-12 months
Lesions	0	3	6	5
Total	3	9	7	20
%	0%	33.30%	85.70%	25%

Fig. 9. Cattle with skin lesions divided according to the age group

A study conducted in Iran identified the presence of specific trichophytic lesions in 188 cattle out of a total of 1150 examined (16.34%), with 19.33% positive cases in cattle under 2 years of age and 10.75% for those over 2 years of age. This, as in our study, confirms that young cattle are more susceptible than adults and that the development of the immune system plays a key role in the appearance of clinical signs of trichophytosis in cattle (2).

In Umbria (Central Italy), Agnetti et al. examined over 395 animals and 31 farmers, and positive results were present in 71.7% of calves under 6 months of age and 11% in cattle over 6 months of age. The *T. verrucosum* pathogen was the most prevalent pathogen identified, being present in 18 of the 20 farms surveyed. The high zoonotic potential of this disease was also confirmed by the presence of positive results in 14 farmers working on these farms with skin lesions (1).

Another study was conducted in Pakistan using both routine laboratory methods and PCR analysis on samples of cattle and sheep showing characteristic trichophytic lesions. The study confirmed that cattle were the most susceptible to infection (1.6%) compared to small ruminants, where the disease was confirmed in 0.1% of the animals examined. The study was carried out over two seasons and reported that the prevalence of trichophytosis was much higher in young cattle in the intensive system during the cold season (9).

In the winter in the cattle sheds, the concentration of animals per unit area and humidity are increased, which makes trichophytosis outbreaks in cattle more frequent and more difficult to control. However, disease prevalence is also high in summer in poorly maintained animals kept in unhygienic and unventilated housing (6).

In Nigeria, Dalis et al. (5) observed that all 14 calves with specific trichophytic lesions, located in the eye area and on the neck, were positive after laboratory analysis of samples taken. It is considered important to study the prevalence of the disease in the cattle population and to institute preventive and control measures for the disease (5).

In Romania, Hanganu et al. (10) identified the presence of *T. verrucosum* in 100% of the individuals examined for the age category 0-3 months, and for the category 3-6 months, 85.7% of the calves were positive, which associates the described data with those reported in our study. Hora et al. examined 320 adult and young cattle, from Arad county, reared in extensive system, over a period of 2 years. Of the 320 cattle examined 32.1% were positive. Based on studies conducted in Romania, it is confirmed that this skin pathology has an increased incidence, this aspect being correlated with weather conditions, animal movement and zoohygienic conditions (10, 11).

A study in Norway reports that this territory was endemic for *Trycophyton*, but since 1980, when they started using the vaccine with the implementation of vaccination campaigns, the annual incidence of cases has decreased considerably, from 1.7% in 1980 to 0.043% in 2004, and in 2009 no positive cases were reported to the authorities. Immunisation through vaccination has greatly benefited endemic countries and has helped to reduce the number of cases of trichiasis to zero (12). Good results have also been obtained in Romania by Tîrziu and Decun, who in 1999 administered the Tricovac vaccine to 1497 cattle, finding in all cases a very good tolerance and preventive and curative effects of about 95% (23).

Conclusions

This study analysed the epidemiological situation of a farm in the Timisoara and identified the types of pathogens responsible for the occurrence of lesions. The diagnosis of trichophytosis was based on epidemiological data, clinical aspects, and positive laboratory findings. The transmissibility, the disruption of the technological flow, the serious economic and health consequences of this disease may pose a danger to animals and humans. Rigorous farm hygiene, treatment of animals and early diagnosis are the methods by which adequate parasitological control in bovine trichophytosis can be achieved.

References

1. **Agnetti, F., Righi, C., Scoccia, E., Felici, A., Crotti, S., Moretta, I., Moretti, A., Maresca, C., Troiani, L., Papini, M.**, *Trichophyton verrucosum* infection in cattle farms of Umbria (Central Italy) and transmission to humans, Mycoses, 2014, 57, 7, 400-405.
2. **Akbarmehr, J.,** The prevalence of cattle ringworm in native dairy farms of Sarab city (East Azarbayjan province) Iran, Advanced Journal of Microbiology Research, 2011, 13, 2, 1-4.
3. **Chermette, R., Ferreiro. L., Guillot, J.,** Dermatophytoses in animals, Mycopathologia, 2008, 166, 385-405.
4. **Constantin, N., Constantinoiu, C., Cosoroabă, I., Cozma, V., Dărăbuș, G., Didă, I., Ducă, I., Gherman, C.M., Iacob, O., Ilie, M.S., Ioniță, M., Magdaș,**

C., Mederle, N., Mihalca, A., Militaru, D., Mircean, V., Miron, L., Mitrea, I.L., Morariu, S., Oprescu, I., Șuteu, E., Tudor, P., Tratat de Medicină Veterinară, Vol. VI, Risoprint, 2014.

5. **Dalis, J.S., Kazeem, H. M., Kwaga, J. K. P., Kwanashie, C.N.,** An outbreak of ringworm caused by *Trichophyton verrucosum* in a group of calves in Vom, Nigeria, African Journal of Microbiology Research, 2014, 8, 783-787.
6. **Dărăbuș, Gh., Morariu, S., Oprescu, I., Mederle, N.,** Parazitologie și boli parazitare, Ed. Mirton, Timișoara, 2016.
7. **Dărăbuş, Gh., Oprescu, I., Morariu, S., Mederle, N., Ilie, M.,** Ghid practic în bolile parazitare, vol. I, Ed. Agroprint, Timişoara, 2013.
8. **de Hoog, G.S., Dukik, K., Monod, M., Packeu, A., Stubbe, D., Hendrickx, M., Kupsch, C., Stielow, J.B., Freeke, J., Göker, M., Rezaei-Matehkolaei, A., Mirhendi, H., Gräser, Y.,** Toward a novel multilocus phylogenetic taxonomy for the dermatophytes, Mycopathologia, 2017, 182, 5-31.
9. **Hameed, K., Riaz, Ch.F., Nawaz, M.A., Naqvi, S.M.S., Gräser, Y., Kupsch, C., Pasquetti, M., Rossi, L., Molinar-Mina, A.R., Tizzani, P., Chiavassa, E., Peano, A.,** *Trichophyton verrucosum* infection in livestock in the Chitral district of Pakistan, The Journal of Infection in Developing Countries, 2017, 11, 4, 326-333.
10. **Hanganu, M., Ghilean, B.M., Marin, A.M., Moraru, M.M.F., Novac M.P., Robu, M., Mederle, N.,** The infection with *Trichophyton* spp. to cattle from a farm in timiș county, Lucrări Științifice Medicină Veterinară, 2023, 56, 3, 52-59.
11. **Hora, F.S., Dărăbuş, Gh., Badea, C., Mederle, O., Mederle, N.,** Epidemiological, clinical and therapeutic aspects of the infestation with *Trichophyton* spp. (Fungi: *Eurotiomycetes: Arthrodermataceae*), Scientia Parazitologica, 2015, 16, 1-2, 28-32.
12. **Lund, A., Bratberg, A.M., Næss, B., Gudding, R.,** Control of bovine ringworm by vaccination in Norway, Veterinary Immunology and Immunopathology, 2013, 158, 1-2, 37-45.
13. **Mahendra, P.,** Dermatophytosis in an adult cattle due to *Trichophyton verrucosum*, Animal Husbandry, Dairy and Veterinary Science, 2017, 1, 1-3.
14. **Maslen, M.,** Human cases of cattle ringworm due to *Trichophyton verrucosum* in Victoria, Australia, Australasian Journal of Dermatology, 2000, 41, 90-94.
15. **Mitra, S.K., Sikdar, A., Das, P.,** Dermatophytes isolated from selected ruminants in India, Mycopathologia, 1998, 142, 13-16.
16. **Moretti, A., Boncio, L., Pasquali, P., Piergili-Fioretti, D.,** Epidemiological aspects of dermatophyte infections in horses and in cattle, Journal of Veterinary Medicine, 1998, 45, 205-208.
17. **Mousa, W.S., Eman, A.,** Review: overview on bovine dermatophytosis, International Journal of Veterinary Sciences and Animal Husbandry, 2018, 3, 2, 16-19.

18. **Papini, R., Nardoni, S., Fanelli, A., Mancianti, F.,** High infection rate of *Trichophyton verrucosum* in calves from Central Italy, Zoonoses Public Health, 2009, 56, 2, 59-64.
19. **Rippon, J.W.,** Medical mycology: The pathogenic fungi and the pathogenic actin omycetes, W.B. Saunders, 1988.
20. **Rouzaud, C., Hay, R., Chosidow, O., Dupin, N., Puel, A., Lortholary, O., Lanternier, F.,** Severe dermatophytosis and acquired or innate immunodeficiency: a review, Journal of Fungi, 2015, 2, 4.
21. **Sugiyama, M., Summerbell, R., Mikawa, T.,** Molecular phylogeny of onygenalean fungi based on small subunit (SSU) and large subunit (LSU) ribosomal DNA sequences, Studies in Mycology, 2002, 47, 5-23.
22. **Swai, E.S., Sanka, P.N.,** Bovine dermatophytosis caused by *Trychophyton Verrucosum*: A case report, Veteterinary World, 2012, 5, 297-300.
23. **Tîrziu, E., Decun, M.,** Cattle enzootic ringworm prophylaxis and control with Tricovac, Revista Română de Medicină Veterinară, 1999, 9, 4, 375-381.

PREVALENCE OF GASTROINTESTINAL PARASITES IDENTIFIED IN GERMAN GIANT RABBITS ON A FARM IN TIMIS COUNTY

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Summary

Parasitic infections are one of the main causes of mortality in rabbit breeding. The aim of this study was to determine the prevalence of gastrointestinal parasites in rabbits of the German Uriah breed from a farm in Făget, Timiș county. During the study 288 faecal samples were collected and divided into four age categories ≤ 2 months, 2 - 4 months, 4 - 6 months and > 12 months. The samples were examined using the flotation method. Statistical analysis was performed using the Fisher - GraphPad QuickCalcs test (2024). The results of this study showed an overall prevalence of gastrointestinal parasites of 68.1%. The identified parasite species belong to two classes: *Protozoa* (*Eimeria* spp. 66.8%) and *Nematoda* (*Passalurus ambiguus*, 33.2%). Rabbits aged ≤ 2 months ($P < 0.0001$), but also those aged 2 to 4 months ($P < 0.0001$) were the most affected by gastrointestinal parasites, compared to the other two age categories, 4 - 6 months and > 12 months, respectively. The results of this study reveal that young rabbits are more susceptible to parasitic infections with *Eimeria* spp. and *Passalurus ambiguus* than adult rabbits.

Keywords: rabbits, breeding, prevalence, gastrointestinal parasites, Timis county

Domestic rabbits (*Oryctolagus cuniculus*) are small mammals of the Leporidae family, native to Europe, especially Spain and Portugal, and now introduced and widespread on all continents of the world except Antarctica (4, 20).

According to data, global rabbit meat production reaches 1.8 million tonnes per year, with about 50% of production focused in Asia, with China being the main producer of rabbit meat, followed by other major producers such as Italy, Spain and France (26, 28). Regardless of how important rabbit production is, farmers face a lot of challenges resulting from the existence of gastrointestinal parasites. It should be noted that internal parasites are represented by helminths and protozoa. Helminths that invade the digestive tract of rabbits are *Passalurus ambiguus* and *Taenia taeniformis*, while protozoa that frequently enter the digestive tract are *Eimeria* spp. and *Encephalitozoon cuniculi* (22). Widely described in numerous publications (1, 3, 6, 9 – 11, 13, 15, 17, 21, 23, 25) coccidia, due to their direct and short life cycle, can spread rapidly in traditional breeding units but also in large meat rabbit farms, leading to significant economic losses. Infections with gastrointestinal nematodes can lead to serious, often fatal, disease with clinical symptoms such as anaemia in the case of infection with haematophagous species and discomfort in the case of infection with *Passalurus ambiguus*.

The main aim of this study was to determine the degree of parasitic infection in a rabbit population of the German Giant breed from a rabbit farm in Timis county.

Materials and methods

The research was carried out on rabbits of the German Giant breed from September 2022 to February 2023 in a rabbit farm in Făget, Timiș county. Rabbits were reared in a semi-intensive system, in individual cages on 3 levels (Fig. 1). The rabbits were aged between 2 months and 1 year. The last treatment administered (Sulfacoccirom - oral solution; 1.4 ml product/1 litre of water for two days) was in February 2022, which was administered preventively.

Fig. 1. Distribution of rabbits in individual cages

This study was based on the collection of faecal samples only, therefore no Bioethics Committee approval was required.

A total of 288 faecal samples were collected from under the rabbit cages with the prior consent of the breeder. The faeces were packed in sterile plastic stool sample containers and appropriately labelled. Samples were then stored in a cool box at 4°C, transported and examined at the Parasitology and Parasitic Diseases Clinic within the FVMT.

To detect the presence of possible parasitic elements, the flotation method with supersaturated NaCl solution was used, following the technique described by Willis (24).

Assessment of any statistically significant differences between the data collected and the prevalence rate of infection was performed using the Fisher - GraphPad QuickCalcs (2024) test (27).

Differences were considered statistically significant when the P-value was less than 0.05.

Results and discussions

From a total of 288 faecal samples examined (Table 1), 196 (68.1%) were found to be infected with at least one gastrointestinal parasite species, namely oocysts of *Eimeria* spp. (131/196; 66.8%) and eggs of *Passalurus ambiguus* (65/195; 33.2%) (Fig. 2-3).

Polyparasitism was present in 54 of 196 faecal samples examined, representing a prevalence of 27.6% (Fig. 4).

Table 1

Species of parasites identified during the study

Collected samples (n=196/288) 68,1%		**Identified species**		
		Eimeria spp.	*Passalurus ambiguus*	Negative
Age	≤ 2 months	31 (23.7%)	12 (18.5%)	
	2 - 4 months	83 (63.4%)	38 (58.5%)	
	4 - 6 months	13 (9.9%)	15 (23.1%)	
	> 12 months	4 (3.1%)	0	
Total		**131**	**65**	**92**

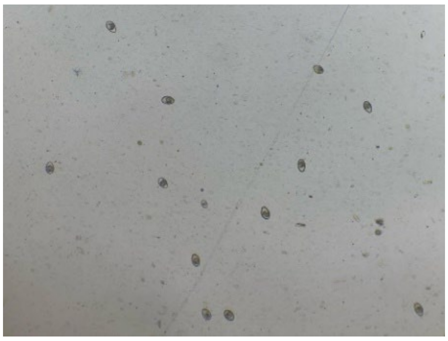

Fig. 2. *Eimeria* spp. oocysts

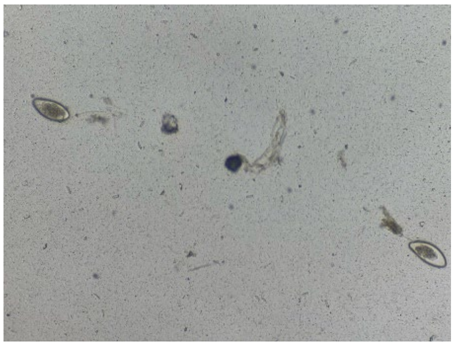

Fig. 3. *Passalurus ambiguus* eggs

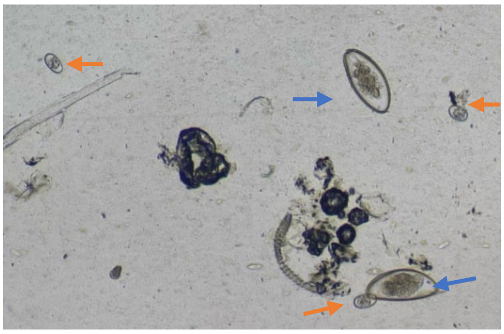

Fig. 4. Polyparasitism with *Eimeria* spp. (orange arrow) and *Passalurus ambiguus* (blue arrow)

Rabbits aged ≤ 2 months, but also those aged 2 to 4 months were the most affected by gastrointestinal parasites (Table 1). The prevalence of gastrointestinal parasites according to age categories is shown in Fig. 5.

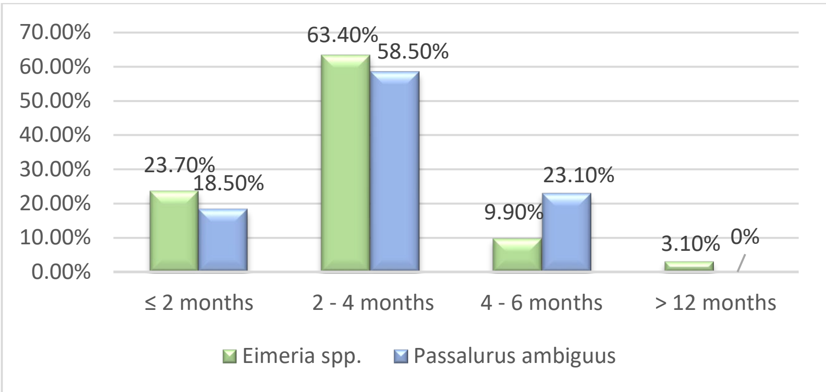

Fig. 5. Prevalence of gastrointestinal parasites in rabbits according to age

The P-value determined by Fisher´s test comparing the prevalence of gastrointestinal parasite species identified in the study according to age categories is shown in Table 2.

Significantly higher prevalence rates ($P < 0.0001$) for *Eimeria* spp. infection were observed in rabbits aged ≤ 2 months compared to rabbits aged 2 - 4 months ($P < 0.0001$), but also in rabbits aged >12 months ($P < 0.0001$). Significantly higher prevalence rates were also observed in rabbits aged 2 - 4 months compared to rabbits aged 4 - 6 months ($P < 0.0001$) and >12 months ($P < 0.0001$), respectively.

Table 2

Comparative P-value of the prevalence of gastrointestinal parasites by age group obtained using Fisher´s test

Crt. no.	Statistical analysis		Observations
	Identified parasites	Age	
1.	*Eimeria* spp.	≤ 2 months vs 2 – 4 luni	The *p* value is equal to 0,0001. According to conventional criteria, this difference is considered statistically significant.
2.		≤ 2 months vs 4 – 6 months	The *p* value is equal to 0,0046. According to conventional criteria, this difference is considered statistically insignificant.
3.		≤ 2 months vs >12 months	The *p* value is equal to 0,0001. According to conventional criteria, this difference is considered statistically significant.
4.		2 – 4 months vs 4 – 6 months	The *p* value is equal to 0,0001. According to conventional criteria, this difference is considered statistically significant.
5.		2 – 4 months vs >12 months	The *p* value is equal to 0,0001. According to conventional criteria, this difference is considered statistically significant.
6.		4 – 6 months vs >12 months	The p value is equal to 0,0419. According to conventional criteria, this difference is considered statistically insignificant.
7.	*Passalurus ambiguus*	≤ 2 months vs 2 – 4 months	The p value is equal to 0,0001. According to conventional criteria, this difference is considered statistically significant.
8.		≤ 2 months vs 4 – 6 months	The p value is equal to 0,6660. According to conventional criteria, this difference is considered statistically insignificant.
9.		≤ 2 months vs >12 months	The p value is equal to 0,0003. According to conventional criteria, this difference is considered statistically significant.
10.		2 – 4 months vs 4 – 6 months	The p value is equal to 0,0001. According to conventional criteria, this difference is considered statistically significant.
11.		2 – 4 months vs >12 months	The p value is equal to 0,0001. According to conventional criteria, this difference is considered statistically significant.
12.		4 – 6 months vs >12 months	The p value is equal to 0,0001. According to conventional criteria, this difference is considered statistically significant.

Significantly higher prevalence rate was also observed for *Passalurus ambiguus* infection in rabbits aged ≤ 2 months compared to rabbits aged 2-4 months ($P < 0.0001$) and >12 months ($P < 0.0003$), respectively. Highly significant differences were also found in the age category 2 - 4 months compared to the age category 4 - 6 months ($P < 0.0001$) and >12 months ($P < 0.0001$), but significantly greater differences were also observed in the age category 4 - 6 months compared to the age category >12 months ($P < 0.0001$).

Coccidiosis is the main cause of intestinal disorders in rabbits, mainly affecting young rabbits after weaning (16). The present study revealed a high prevalence of *Eimeria* spp. infection in young rabbits after weaning (63.4%). The high prevalence can be explained by the role mothers play in transmitting the infection to their young (7, 18).

In the present study *Passalurus ambiguus* had a high prevalence, 58.5% in the age category 2 - 4 months. The prevalence of this parasite varies with age and season (2, 14). In Egypt, *P. ambiguus* is one of the most prevalent helminths identified in domestic rabbits, with up to 40% of samples being infected, with young animals being more commonly infected than adults (2).

In a study by Dzesinyuy et al. (5) conducted on 130 rabbits from a farm in Cameroon, an overall prevalence of gastrointestinal parasites of 48.5% was observed. Three species of gastrointestinal parasites were identified: *Eimeria* spp. with a prevalence of 88.89%, *Trichostrongylus* spp. 6.35% and *Passalurus ambiguus* 4.67% (5).

In 2023, Patrick et al. (19) conducted a study on 100 rabbits of different ages, breeds and sexes from a farm in Malaysia. Six gastrointestinal parasites were observed and identified, namely: *Eimeria* spp., *Toxoplasma* spp., *Diphyllobothrium* spp., *Taenia* spp., *Ascaris* spp. and *Fasciola hepatica*. The overall prevalence was 98%, for adults the prevalence was 97.56% and for youth the prevalence was 88.87% (19).

Tanjung et al. (22) conducted a study in 2019 to determine gastrointestinal parasite species and their prevalence in rabbits raised on a farm in Indonesia. Rabbits were divided into two age categories, juveniles (< 3 months) and adults (> 3 months). Fecal samples were processed by the flotation method. The results showed that 3 parasite species were identified in juvenile rabbits, namely *Eimeria* spp. (40%), *Strongyloides* spp. (20%) and *Passalurus ambiguus* (13.33%). In adult rabbits, monoparasitism with *Eimeria* spp. was observed with a prevalence of 12% (22).

Between 2011 and 2013, Kornas et al. conducted a study to determine the gastrointestinal parasites present in New Zealand White rabbits at the Experimental Research Station of the University of Agriculture in Krakovia. The study revealed infection with *Eimeria* spp., *Passalurus ambiguus*, *Trichuris leporis* and *Graphidium strigosum* (8).

It was observed that polyparasitism with *Passalurus ambiguus* and *Eimeria* spp. causes a decrease in the number of births in co-infected animals. This suggests that gastrointestinal parasites directly or indirectly affect reproduction in females (12).

Conclusions

Overall prevalence of gastrointestinal parasites identified in this study: *Eimeria* spp. and *Passalurus ambiguus* was 68.1%.

The highest prevalence rates were observed in young rabbits aged ≤ 2 months, but also in rabbits aged 2-4 months.

In caged rabbits, infection with parasites of the genus *Eimeria* and the pinworm *Passalurus ambiguus* is most common.

References

1. **Abdel-Baki, A.A.S., Al-Quraishy, S.,** Prevalence of coccidia (*Eimeria* spp.) infection in domestic rabbits, *Oryctolagus cuniculus*, in Riyadh, Saudi Arabia, Pakistan Journal of Zoology, 2013, 45, 5, 1329-1333.
2. **Ashmawy, K., El-Sokkary, M., Abu-Akkada, S., Dewair, A.,** Incidence of *Passalurus ambiguus* in domestic rabbits in Behera province, Alexandria Journal of Veterinary Sciences, 2010, 30, 1, 115-120.
3. **Bhat, T.K., Jithendran, K.P., Kurade, N.P.,** Rabbit coccidiosis and its control: A review, World Rabbit Science, 1996, 4, 1, 37-41.
4. **Duszynski, D.W., Couch, L.,** The biology and identification of the coccidia (*Apicomplexa*) of rabbits of the world (1st ed.), Elsevier, 2013.
5. **Dzesinyuy, T.S., Anchang, S.M., Mahamat, O.,** Gastrointestinal parasites and relationship with faecal IgA and cortisol in farmed rabbits in Bamenda, North West, Cameroon, South Asian Journal of Parasitology, 2023, 6, 2, 51-61.
6. **González-Redondo, P., Finzi, A., Negretti, P., Micci, M.,** Incidence of coccidiosis in different rabbit keeping systems, Arquivo Brasileiro de Medicina Veterinaria e Zootecnia, 2008, 60, 5, 1267-1270.
7. **Henneb, M., Aissi, M.,** Etude cinétique de l'excrétion oocystale chez la lapine et sa descendance et identification des différentes espèces de coccidies, Proc. 15èmesJournées de la Recherche Cunicole, Le Mans, France, 2013, 221-224.
8. **Kornas, S., Kowal, J., Wierzbowska, I., Basiaga, M., Nosal, P., Niedbala, P.,** The Alice - " Follow the White Rabbit"-parasites of farm rabbits based on coproscopy, Annals of parasitology, 2015, 61, 4.
9. **Laha, R., Das, M., Goswami, A.,** Coccidiosis in rabbits in a subtropical hilly region, Indian Journal of Animal Research, 2015, 49, 2, 231-233.
10. **Lebas, F., Coudert, P., de Rochambeau, H. and Thebault, R.C.,** The Rabbit: Husbandry, Health and Production, FAO Animal Production and Health Series, 21, Rome, 1996.
11. **Licois, D.,** Domestic rabbit enteropathies, Proceedings 8th World Rabbit Congress, Pueblo, Mexico, 2004, 385-403.
12. **Mavrogianni, V., Papadopoulos, E., Fragkou, I., Gougoulis, D., Valasi, I., Orfanou, D., Fthenakis, G.,** Administration of a long acting antiparasitic to pre-pubertal ewe lambs in Greece results in earlier reproductive activity reproductive and improved performance, Veterinary Parasitology, 2011, 177, 1-

2, 139-144.

13. **Maziz-Bettahar, S., Aissi, M., Ainbaziz, H., Bachene, M.S., Zenia, S., Ghisani, F.,** Prevalence of coccidian infection in rabbit farms in North Algeria, Veterinary World, 2018, 11, 11, 1569-1573.
14. **Nosal, P., Petryszak, A., Nowosad, B., Sobolewska, M.,** Gastrointestinal parasites of rabbits in coproscopic investigations, Wiadomosci Parazytologiczne, 2006, 52, 4, 327-330.
15. **Okumu, P.O., Gathumbi, P.K., Karanja, D.N., Mande, J.D., Wanyoike, M.M., Gachuiri, C.K., Kiarie, N., Mwanza, R.N. and Borter, D.K.,** Prevalence, pathology and risk fac tors for coccidiosis in domestic rabbits (*Oryctolagus cuniculus*) in selected regions in Kenya, Veterinary Quarterly, 2014, 34, 4, 205-210.
16. **Paklandl, M., Hlaskova, L.,** The reproduction of *Eimeria flavescens* and *Eimeria intestinalis* in suckling rabbits, Parasitology Research, 2007, 101, 1435-1437.
17. **Paklandl, M.,** Coccidia of rabbit: A review, Folia Parasitologica, 2009, 56, 3, 153-166.
18. **Papeschi, C., Fichi, G., Perrucci, S.,** Oocyst excretion pattern of three intestinal *Eimeria* species in female rabbits, World Rabbit Science, 2013, 21, 77-83.
19. **Patrick, J.A.,** Gastrointestinal Infection of rabbits in Ranau Rabbit Farm, Ranau, Sabah Malaysia, Science Letters, 2023, 17, 1, 145-155.
20. **Smith, A.T., Boyer, A.F.,** *Oryctolagus cuniculus*, The IUCN Red List of Threatened Species, 2008.
21. **Szkucik, K., Pyz-Łukasik, R., Szczepaniak, K.O., Paszkiewicz, W.,** Occurrence of gastrointestinal parasites in slaughter rabbits, Parasitology Research, 2014, 113, 1, 59-64.
22. **Tanjung, M., Rangkuti, P.M.,** Species and prevalence of rabbit gastrointestinal parasites in Berastagi Farm Karo District, North Sumatra, Indonesia, Proceedings of the International Conference on Natural Resources and Technology, 2019, 193-198.
23. **Varga, I.,** Large-scale management and parasite populations: Coccidia in rabbit, Veterinary Parasitology, 1982, 11, 1, 69-84.
24. **Willis, H.H.,** A simple levitation method for the detection of hookworm ova, Medical Journal of Australia, 1921, 8, 375-6.
25. **Yin, G., Goraya, M.U., Huang, J., Suo, X., Huang, Z., Liu, X.,** Survey of coccidial infection of rabbits in Sichuan Province, Southwest China, SpringerPlus, 2016, 5, 1, 870.
26. **Zoltan, J., Karoly, B., Marta, P., Istvan, P.Z., Laszlo, M.,** Global rabbit meat production with a special focus on the role of China, Lucrări Științifice Management Agricol, 2017, 19, 3, 31-36.
27. ***https://www.graphpad.com/quickcalcs/
28. *****FAOSTAT**, The statistics division of the FAO, 2012.

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