Part 3. Biosafety

UDC 619:616.995.42/.7:615.285.036:636.7/.8.083.8 DOI 10.36016/JVMBBS-2024-10-1-6

EFFECTIVENESS OF MODERN ANTIPARASITIC ANIMAL COLLARS

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Summary. Despite the successes achieved in the prevention and control of parasitic diseases in companion animals, the issue of developing and implementing innovative, highly effective antiparasitic agents in veterinary practice is still relevant today. This study aimed to evaluate the efficacy of modern antiparasitic collars for dogs and cats in the prevention and treatment of ectoparasitic infections. The antiparasitic agents used in the experiments were 'Flea and tick collar Comfort for cats', 'Antiparasitic collar TM Healthy Pet, Oberig', 'Flea and tick collar Comfort for dogs', and 'Antiparasitic collar TM Compliment, Oberig'. The active ingredient in the collars tested is diazinon. The experimental studies were conducted under current methodological recommendations and practical guidelines. The study of shelter pets and stray animals revealed their infestation with lice, fleas, and parasitic ticks of varying intensity. Experimentally, a high insecticidal effect of the products 'Flea and tick collar Comfort for cats' and 'Antiparasitic collar TM Healthy Pet, Oberig' was established, 'Flea and tick collar Comfort for dogs', 'Antiparasitic collar TM Compliment, Oberig' against fleas (*Ctenocephalides felis*, *Ctenocephalides canis*), chewing lice (*Felicola subrostratus*), sucking lice (*Linognathus setosus*), ticks (*Ixodes ricinus*, *Dermacentor* spp., *Rhipicephalus* spp.) The effectiveness of the drugs is 100%. It has been experimentally proven that the studied collars with the active ingredient diazinon can be used for preventive and therapeutic purposes for companion animals in case of infestation with fleas, chewing lice, sucking lice, and ticks

Keywords: diazinon, dogs, cats, fleas, lice, ticks

Introduction. One of the most common diseases in pets is ectoparasitosis, which is caused by the parasitism of fleas, sucking lice, chewing lice, and ticks on the body of animals. Due to climate change, there are changes in the life of animal parasites (Bogach et al., 2020). In this regard, it is necessary to constantly monitor the distribution of parasitic insects, their species composition, etc.

To date, there are many reports on how to address this problem in different countries (Gizaw et al., 2021). A study of 200 dogs and 137 cats in Ethiopia reported that 97% of dogs and 90.5% of cats were carriers of at least one species of ectoparasite. The dominant species in both cats and dogs is *Ctenocephalides felis* (Kumsa, Abiy and Abunna, 2019). In Albania, out of 181 dogs and 26 cats examined, most were found to be carriers of ectoparasites in both mono- and mixed infections. The arthropod ectoparasite fauna of dogs included two species of ticks (*Rhipicephalus sanguineus* and *Ixodes ricinus*), three species of mites (*Sarcoptes scabiei* var. *canis, Otodectes cynotis*, and *Demodex canis*), three species of fleas (*Ctenocephalides canis, Ctenocephalides felis*, and *Pulex irritans*), and one species of chewing lice (*Trichodectes canis*) (Xhaxhiu et al., 2009). Of 120 dogs examined in the Shimoga Region of Karnataka, 59 (49.1%) had ectoparasites (Krishna Murthy, Ananda and Adeppa, 2017). The prevalence of infection in domestic animals in Iran ranges from 68.5% to 100% in some regions. The most common ectoparasites in dogs are fleas, followed by sucking lice, ticks, and flies (Ebrahimzade, Fattahi and Ahoo, 2016). In Nigeria, 92.5% of 1,041 dogs tested had one or more ectoparasites (Jajere et al., 2023).

As dogs and cats live in shared environments with humans, they are likely to be key reservoirs of pathogens that infect humans in the same environment (Colella et al., 2020). Some ectoparasites transmit serious human diseases, so regular monitoring of them is a major challenge to control arthropods and the diseases they transmit (Abdullah et al., 2019; Liodaki et al., 2022).

It has been confirmed that ectoparasites were the main vectors of plague during the second pandemic (Dean et al., 2018). They are the cause of bartonellosis (Frye et al., 2015). Some ectoparasites are vectors of disease-causing bacteria and viruses that are treated with antibiotic and antiviral drugs, ultimately contributing to antimicrobial overuse (Carvalho da Silva et al., 2023).

The urgency of the problem of ectoparasite control leads to the development and widespread introduction of various antiparasitic drugs of different forms into practical veterinary medicine (Lavan et al., 2022; Paliy et al., 2023).

Future research should explore both distinctions between, and overlap across, ectoparasite defense systems and pathogen avoidance systems, as doing so will not only illuminate proximate motivational systems, including disgust but may also reveal important clinical and social consequences (Kupfer and Fessler, 2018). The value of rotation of acaricides should be investigated for a range of compounds under field conditions (Rodriguez-Vivas, Jonsson and Bhushan, 2018).

The objective of this study was to evaluate the use of anti-parasitic collars for pets with diseases caused by ectoparasites.

Materials and methods. Studies to determine the effectiveness of antiparasitic drugs on dogs and cats were conducted at the Laboratory of Veterinary Sanitation and Parasitology of the National Scientific Center 'Institute of Experimental and Clinical Veterinary Medicine'. Some studies were conducted at the animal shelter in Balakliya (Kharkiv Region).

Modern domestic antiparasitic products were used in the experiments:

— 'Flea and tick collar Comfort for cats'. Polymeric tape with a fixative with a specific odor of the components (1 g of the collar contains the active ingredient diazinon — 60 mg; excipients: polyvinyl chloride, dye).

— 'Antiparasitic collar TM Healthy Pet, Oberig'. Polymeric tape (1.0 g of the collar tape contains the active ingredient diazinon — 60 mg).

— 'Flea and tick collar Comfort for dogs'. Polymeric tape with a fixative with a specific odor of the components (1 g of the collar contains the active ingredient diazinon — 60 mg; excipients: polyvinyl chloride, dye).

— 'Antiparasitic collar TM Compliment, Oberig'. Polymeric tape (1.0 g of the collar tape contains the active ingredient diazinon — 60 mg).

Clinical trials of animal collars to study the therapeutic effect were conducted in the following areas:

— clinical examination of pets, preliminary diagnosis, sampling of ectoparasites from the skin of animals for laboratory testing, constant clinical monitoring of the physiological state of experimental animals;

— microscopic examination of samples to determine pathogens of ectoparasitic diseases in biological material, their identification, and to determine the prevalence of infections in animals;

— formation of experimental groups of animals;

— application of collars, individually, according to leaflets, keeping animals in the shelter, taking samples for laboratory testing on 5th, 10th, 30th, and 45th days after the application of collars, determining the effectiveness of their action;

— daily clinical examination of the health of experimental animals throughout the experiment.

A total of 40 cats and 36 dogs of varying body weights were examined for ectoparasites. Of these, 22 cats and 16 dogs were found to have ectoparasites. The experimental animals were housed in standardized aviaries and fed an approved diet. The studies were conducted using visual and microscopic methods following established methodologies (Kumsa, Abiy and Abunna, 2019; Colella et al., 2020).

At the preliminary stage, *in vivo* diagnostics of ectoparasitoses were performed, and the number of ectoparasites was determined. Identification of ectoparasitic pathogens was performed through microscopic examination. The mean intensity was determined by counting ectoparasites per 10 cm^2 of animal skin area.

The sick animals were divided into groups that were separately administered the following drugs: 'Flea and tick collar Comfort for cats' (n = 11), 'Antiparasitic collar TM Healthy Pet, Oberig' (n = 11), 'Flea and tick collar Comfort for dogs' (n = 8), 'Antiparasitic collar TM Compliment, Oberig' (n = 8). The animals were clinically examined before, during, and after the application of the collars. The collars were applied individually to each animal, following the instructions provided.

The study recorded results after 5, 10, 20, 30, and 45 days of collar application, based on examination of the treated animals, counting live ectoparasites on them, and determining the prevalence of infection after treatment and the effectiveness of the collar.

To collect ectoparasites from animal skin, the animals were fixed in a lying position. The examination of the skin began with the head, followed by the neck, back, sides, abdomen, and limbs. During the examination, the hair was parted and combed. Any detected ectoparasites were removed from the animal's skin using tweezers. The ectoparasites that were removed were preserved in either Barbagallo's fluid (a 3% aqueous formalin solution in saline) or 70% ethanol. Some of the ectoparasites were transported to the laboratory alive in tubes or containers with damp filter paper inside. The tubes and jars were covered with a cloth and labeled.

Prevalence (P) was defined as the ratio of the number of infected animals to the number of examined animals, expressed as a percentage:

$$P = \frac{X}{Y} \times 100$$

where: X — number of animals with detected ectoparasites;

Y — total number of animals.

Mean intensity (MI) of infection was determined by the number of ectoparasites per 10 cm^2 of animal skin area.

Effectiveness of the collar was calculated by the number of treated animals in percentage that were completely free of parasites.

Experiments on animals were conducted following the recommendations of the 'European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes' (CE, 1986) and Council Directive 2010/63/EU (CEC, 2010), and in accordance with Art. 26 of the Law of Ukraine No. 3447-IV of 21.02.2006 'About protection of animals from cruel treatment' (VRU, 2006) and basic bioethical principles (Simmonds, 2017). The research program was reviewed and approved by the Bioethics Committee of the National Scientific Center 'Institute of Experimental and Clinical Veterinary Medicine' under the current procedure.

Results and discussions. As a result of clinical examination of animals (n = 22) housed in the shelter, 14 cats affected by ectoparasites were found, the prevalence of the infection was 63.6%. Clinical examination of the affected cats revealed redness, inflammation of the skin, a clearly visible itch reflex, papules, scales, seborrhea, and noticeable bald spots. A stable infection of cats with fleas (85.7%) was found, mixed infection with fleas and chewing lice was detected in two cats, and mixed infection with sucking lice and chewing lice in two cats (Table 1).

Table 1 — Prevalence and mean intensity of infection in cats housed in a shelter (n = 14)

Ectoparasite species	P, %	MI, insects/10 cm^2
Ctenocephalides felis	85.7	5.5 ± 2.0
Felicola subrostratus	14.3	1.5 ± 0.5
Linognathus setosus	28.6	2.0 ± 1.5

The data presented in Table 1 show that the mean intensity of infection in cats with fleas was 5.5 ± 2.0 insects per 10 cm^2 of animal skin area, sucking lice — 2.0 ± 1.5 insects per 10 cm^2 of animal skin area, and chewing lice — 1.5 ± 0.5 insects per 10 cm^2 of animal skin area.

In addition, during the clinical examination of animals (n = 18) housed in the shelter, 10 dogs with manifestations of skin lesions by ectoparasites were found, with a prevalence rate of 55.6%. Clinical examination of the affected dogs revealed redness, inflammation of the skin, a well-defined itch reflex, papules, scales, and seborrhea. Bald spots are noticeable. The skin is scratched. The study revealed the infection of dogs with fleas (60%). Mixed infection was detected in 40% of the infected animals (two dogs were infected with chewing lice, two with chewing lice and sucking lice) (Table 2).

Table 2 — Prevalence and mean intensity in dogs housed in the shelter (n = 10)

Ectoparasite species	P, %	MI, insects/10 cm^2
Ctenocephalides canis	30.0	3.5 ± 2.0
Pulex irritans	30.0	3.5 ± 2.0
Trichodectes canis	40.0	1.5 ± 0.5
Linognathus setosus	20.0	2.0 ± 0.5

The results presented in Table 2 show that dogs were infected with two species of fleas (*Ctenocephalides canis* and *Pulex irritans*) (the mean intensity of infection in dogs with fleas was 3.5 ± 2.0 insects per 10 cm^2 of animal skin area, sucking lice — 2.0 ± 0.5 insects per 10 cm^2 of animal skin area, chewing lice — 1.5 ± 0.5 insects per 10 cm^2 of animal skin area.

After diagnosis, the infected cats were divided into two groups of seven animals each. Collars were applied to the animals individually as described in the leaflets. The animals were monitored for 45 days (Table 3).

Table 3 — Collars' effectiveness against fleas and lice on cats housed in a shelter (n = 14)

Animal group	Before applying collars		After applying collars									
	P, %	MI, average	5th day		10th day		20th day		30th day		45th day	
			P, %	MI, insects/10 cm^2	P, %	MI, insects/10 cm^2	P, %	MI, insects/10 cm^2	P, %	MI, insects/10 cm^2	P, %	MI, insects/10 cm^2
'Flea and tick collar Comfort for cats' (n = 7)	100	4.5	14.3	1.5	0	0	0	0	0	0	0	0
'Antiparasitic collar TM Healthy Pet, Oberig' (n = 7)	100	4.75	14.3	2.0	0	0	0	0	0	0	0	0

After applying the collars, two cats with characteristic features of the Siamese breed in appearance in the experimental and control groups showed short-term salivation for 10 minutes. From the second day and during clinical observation of the experimental and control animals, no complications or changes in clinical condition were observed after the application of the collars.

From the second to the fifth day after the application of the collars, dead adult fleas and lice were found on the treated animals. From the 10th day, no adult fleas and lice were found on the animal bodies, and up to the 10th day, live eggs of lice were found on the animal fur. From the 10th to the 45th day, no adult fleas and lice, and live eggs of ectoparasites were found on the bodies of animals.

At the same time, we divided the dogs into two groups of five animals each. The animals of the experimental and control groups were individually fitted with collars as described in the leaflets. The animals were monitored for 45 days (Table 4).

Two dogs showed short-term salivation after wearing the collars. From the second day and during clinical observation of experimental and control animals, no complications or changes in their clinical state were observed after the application of the collars.

From the second to the fifth day after the application of the collars, dead flea and lice adults were found on the treated animals. On the 10th day, no adults of fleas and lice were found on the bodies of animals. Up to the 10th day, live eggs of lice were found on the fur of animals. From the 30th to the 45th day, no adult fleas and lice, and live eggs of ectoparasites were found on the body of animals.

Table 4 — Collars' effectiveness against fleas and lice on dogs housed in a shelter (n = 10)

Animal group	Before applying collars		After applying collars									
	P, %	MI, average	5th day		10th day		20th day		30th day		45th day	
			P, %	MI, insects/10 cm²	P, %	MI, insects/10 cm²	P, %	MI, insects/10 cm²	P, %	MI, insects/10 cm²	P, %	MI, insects/10 cm²
'Flea and tick collar Comfort for dogs' (n = 5)	100	3.5	20.0	1.5	0	0	0	0	0	0	0	0
'Antiparasitic collar TM Compliment, Oberig' (n = 5)	100	3.75	20.0	1.5	0	0	0	0	0	0	0	0

As a result of clinical examination of cats (n = 18) that moved freely in the city (animals were sterilized, chipped, and moderately nourished), a noticeable itching reflex was detected in 8 animals, with areas covered with scales on the skin. The prevalence of infection in stray cats was 44.4% (Table 5).

The study revealed a flea infection prevalence of 100% among the stray cats. Ticks were found on four cats. Mixed infection in animals amounted to 50%. The mean intensity of flea infection in stray cats was 1.5 ± 0.5 individuals per 10 cm² of animal skin area, which is lower than in animals kept indoors.

Table 5 — Prevalence and mean intensity of infection in stray cats (n = 8)

Ectoparasite species	P, %	MI, individuals/10 cm²
Ctenocephalides felis	100.0	1.5 ± 0.5
Ixodes ricinus	37.5	1.5 ± 0.5
Rhipicephalus spp.	25.0	0.7 ± 0.3
Dermacentor spp.	12.5	0.5 ± 0.3

The experimental and control groups of animals were individually applied with collars as described in the leaflets. The animals were monitored for 45 days during feeding (Table 6).

Table 6 — Collars' effectiveness against fleas and ticks on stray cats (n = 8)

Animal group	Before applying collars		After applying collars									
	P, %	MI, average	5th day		10th day		20th day		30th day		45th day	
			P, %	MI, individuals/10 cm²	P, %	MI, individuals/10 cm²	P, %	MI, individuals/10 cm²	P, %	MI, individuals/10 cm²	P, %	MI, individuals/10 cm²
'Flea and tick collar Comfort for cats' (n = 4)	100	4.5	25	1.5	0	0	0	0	0	0	0	0
'Antiparasitic collar TM Healthy Pet, Oberig' (n = 4)	100	4.75	25	2.0	0	0	0	0	0	0	0	0

From the second to the fifth day after applying the collars, dead fleas were found on the treated animals. From the fifth to 45th day, no fleas were found on the bodies of the animals. Ticks were not found on the animals for 45 days.

As a result of the clinical examination of stray dogs (n = 18) that moved freely in the city (animals were sterilized, chipped, moderately nourished), 6 animals had a clearly visible itch reflex, scales were present on the skin. The prevalence of infection in stray dogs was 33.3% (Table 7).

The study found the infection of stray dogs with fleas and ticks to be 100%. At the same time, the mean intensity of flea infection in stray dogs was 1.25 ± 0.5 insects per 10 cm^2 of animal skin area, which is lower than in animals kept indoors.

Table 7 — Prevalence and mean intensity of infection in stray dogs (n = 6)

Ectoparasite species	P, %	MI, individuals/10 cm^2
Ctenocephalides canis	76.7	1.5 ± 0.5
Pulex irritans	33.3	1.0 ± 0.5
Ixodes ricinus	100.0	2.5 ± 0.5
Rhipicephalus spp.	33.3	0.5 ± 0.3
Dermacentor spp.	16.7	0.5±0.3

The infected animals were divided into two groups of three animals each. The experimental and control groups of animals were individually collared as described in the leaflets. The animals were monitored for 45 days during feeding (Table 8).

Table 8 — Collars' effectiveness against fleas and ticks on stray dogs (n = 6)

Animal group	Before applying collars		After applying collars									
	P, %	MI, average	5th day		10th day		20th day		30th day		45th day	
			P, %	MI, individuals/10 cm^2	P, %	MI, individuals/10 cm^2	P, %	MI, individuals/10 cm^2	P, %	MI, individuals/10 cm^2	P, %	MI, individuals/10 cm^2
'Flea and tick collar Comfort for dogs' (n = 3)	100	2.5	33.3	1.5	0	0	0	0	0	0	0	0
'Antiparasitic collar TM Compliment, Oberig' (n = 3)	100	2.75	33.3	1.5	0	0	0	0	0	0	0	0

From the second to the fifth day after the application of the collars, dead fleas were found on the treated animals. After the fifth day and up to the 45th day, no fleas were found on the bodies of the animals. Ticks were not found on the animals for 45 days.

Thus, it was proved that the effectiveness of 'Flea and tick collar Comfort for cats' and 'Antiparasitic collar TM Healthy Pet, Oberig' in production conditions for cats affected by fleas, lice, and ticks was 100%. It was found that the effectiveness of the 'Flea and tick collar Comfort for dogs' and 'Antiparasitic collar TM Compliment, Oberig' in production conditions for the infection of dogs with fleas, lice, and ticks was 100%.

During our monitoring studies, we found a stable flea infection rate of 85.7% to 100% in cats. At the same time, mixed infection with fleas and chewing lice, as well as sucking lice and chewing lice, was detected in animals. The prevalence of infection in dogs ranged from 33.3% to 55.6%. The infection of the examined animals with fleas was 100%.

Other researchers have found that dogs are most often parasitized by fleas *Ctenocephalides felis* (95%), *Pulex irritans* (20.5%), *Echidnophaga gallinacea* (9%), *Xenopsylla cheopis* (0.5%), as well as ticks *Haemaphysalis leachi* (17.5%), *Amblyomma variegatum* (8.5%). A smaller percentage is represented by *Rhipicephalus sanguineus* (8%), *Rhipicephalus pulchellus* (5.5%) and *Rhipicephalus* (*Boophilus*) *decoloratus* (2.5%), as well as lice *Heterodoxus spiniger* (5%), *Linognathus setosus* (1.5%) and *Trichodectes canis* (0.5%). Along with this, fleas *Ctenocephalides felis* (61.7%), *Echidnophaga gallinacea* (24.1%), *Pulex irritans* (1.5%), *Xenopsylla cheopis* (0.7%), as well as ticks *Haemaphysalis leachi* (10.9%), *Amblyomma variegatum* (1.5%), and *Rhipicephalus sanguineus* (0.7%) (Kumsa, Abiy and Abunna, 2019).

In Albania, in dogs, the infection rate was 23.8% for *Rhipicephalus sanguineus*, 0.6% for *Ixodes ricinus*, 4.4% for *Sarcoptes scabiei* var. *canis*, 6.7% for *Otodectes cynotis*, 0.6% for *Demodex canis*, 75.7% for *Ctenocephalides canis*, 5.0% for *Ctenocephalides felis*, 8.3% for *Pulex irritans* and 6.6% for *Trichodectes canis*. Mixed infection with two or three species of ectoparasites was recorded in 38.1% of dogs. Fleas infected 75.7% of dogs, and ticks parasitized 24.3% of dogs. However, during the examination of cats, infection with only one species of ectoparasites *Ctenocephalides felis* was found in cats (Xhaxhiu et al., 2009).

Of the 59 infected domestic dogs, 22 (37.28%) were positive for fleas, 18 (30.5%) for ticks, 9 (15.2%) for lice, 7 (11.8%) for scaroptosis, and 3 (5.0%) for demodicosis. Two flea species were identified as *Ctenocephalides canis* (59%) and *Ctenocephalides felis* (41%). Tick and louse species were identified as *Riphicephalus sanguineus* and *Trichodectus canis*, respectively. The prevalence of ectoparasites was higher among stray and adult dogs compared to domestic dogs and puppies, respectively (Krishna Murthy, Ananda and Adeppa, 2017).

In Iran, arthropods isolated from domestic animals included fleas (77.5%), lice (50%), ticks (8.6%), and flies (6.8%). Among the ectoparasites of dogs, four species of fleas were found: *Ctenocephalides canis* (29.8%), *C. felis* (19.9%), *Pulex iritans* (2.9%), and *Xenopsiella cheopis* (0.7%). One species of lice, *Trichodectes canis* (41.3%), one species of tick, *Rhipicephalus sanguinus* (0.7%), and one species of fly, *Hippobosca* sp. (1.1%), were also identified (Ebrahimzade, Fattahi and Ahoo, 2016).

Both our studies and other reports have found a high species diversity and high frequency of ectoparasites on dogs and cats (Kumsa, Abiy and Abunna, 2019).

There is measurable resistance ectoparasites to most of the compounds that are commercially available, and this can be expected to increase. There is a need to develop and validate the efficacy of strategies for ectoparasite control that will delay the emergence of resistance (Rodriguez-Vivas, Jonsson and Bhushan, 2018).

Organophosphorus compounds are promising for the control of animal ectoparasites (El-Maghraby et al., 2022). An organophosphate pesticide that is widely used in agriculture for insect control and in veterinary medicine for ectoparasite control is diazinon (Jafari et al., 2012; Rahimi Anbarkeh et al., 2019; Legesse et al., 2022). The mechanism of action of diazinon is based on the inhibition of acetylcholinesterase, an enzyme necessary for the functioning of the insect nervous system. The insecticide has a half-life of 2 to 6 weeks. Diazinon promotes lipid accumulation and activates the adipogenic signaling pathway in an *in vitro* model (Smith, Yu, X. and Yin, 2018).

Our studies have proven the prospects of using antiparasitic drugs with the active ingredient diazinon when applied to pets. Our other studies have shown the high insecticidal efficacy of collars with the active ingredient fipronil (Paliy et al., 2021).

Thus, our results expand the range of existing antiparasitic collars for companion animals.

Conclusions. Based on experimental studies, it was found that 'Flea and tick collar Comfort for cats', 'Antiparasitic collar TM Healthy Pet, Oberig', 'Flea and tick collar Comfort for dogs', and 'Antiparasitic collar TM Compliment, Oberig' are well tolerated by animals and do not cause side effects or changes in the clinical state of animals.

The high insecticidal effect of the experimental collars against fleas (*Ctenocephalides felis, Ctenocephalides canis*), chewing lice (*Felicola subrostratus*), sucking lice (*Linognathus setosus*), ticks (*Ixodes ricinus*, *Dermacentor* spp., *Rhipicephalus* spp.) was established.

The effectiveness of the 'Flea and tick collar Comfort for cats', 'Antiparasitic collar TM Healthy Pet, Oberig', 'Flea and tick collar Comfort for dogs', and 'Antiparasitic collar TM Compliment, Oberig' in production conditions for the infection of pets with fleas, lice and ticks is 100%.

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