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ASSESSING THE EFFICACY OF ANTIPARASITIC SPRAYS

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Summary. For the control of animal ectoparasites, a large number of prophylactic and therapeutic agents with different active ingredients and routes of administration have been proposed. The persistence of ectoparasitic infections in domestic animals has led to the search for more effective drugs and innovative forms of their production. The aim of the study was to establish and experimentally confirm the efficacy of innovative ectoparasiticidal sprays for dogs and cats for prophylactic and therapeutic purposes in parasitic infections. The antiparasitic agents used in the experiments were 'PROFILINE Spray', 'INSECTOSTOP Spray', and 'Barrier-Super Insecticidal Spray'. In accordance with the tasks set, the research was carried out using visual and microscopic methods in accordance with existing practical guidelines and current methodological recommendations. In the study of ectoparasite collections, ixodid ticks (*Ixodes ricinus*), dog fleas (*Ctenocephalus canis*), and cat fleas (*Ctenocephalus felis*) were found. It was shown that the knockdown effect in all ticks began with a state of disorientation, the ticks began to move chaotically 6 hours after exposure to the experimental agents, and after 12 hours there was a complete knockdown of all experimental ixodid ticks and fleas. The high efficacy of 'PROFILINE Spray', 'INSECTOSTOP Spray', and 'Barrier-Super Insecticidal Spray' against fleas (*Ctenocephalides* spp.), lice (*Trichodectes canis, Felicola subrostratus*), lice (*Linognathus setosus*), and ixodid ticks (*Ixodes* spp.) was experimentally proven. The efficacy of the products is 100% within 60 days of application to animals. According to the results of the studies, it was found that the investigated veterinary sprays can be used for the prevention and treatment of pets with fleas, lice, and ixodid ticks

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

Introduction. Ectoparasitic diseases of companion animals remain a pressing issue in veterinary science and practice today. These diseases represent a significant proportion of other parasitic pathologies and require a comprehensive and scientifically based solution. A relatively large number of prophylactic and therapeutic agents have been proposed to control ectoparasites, but not all of them are effective and parasites have developed resistance to some of them (Muhammad et al., 2021; Paliy et al., 2021a). This points to the need for a methodical search for more effective means and ways of using them.

Sprays are one of the modern forms of production of veterinary products, which in turn allows for more convenient use of products, their dosage, and use in limited quantities and on a limited area. At the same time, the efficacy of a veterinary product depends on the active ingredient and its quantity, as well as on excipients (Karasek et al., 2020; Paliy et al., 2021b).

Among the antiparasitic drugs used to control lice and fleas, fipronil and propoxur are the most widely used.

A new spot-on formulation containing fipronil (Eliminall®/Exproline vet™) Spot-on Solution for Dogs, Pfizer Animal Health, registered and manufactured by KRKA, d.d., Novo Mesto) was evaluated in three laboratory experiments and confirmed to be effective against fleas, ticks and lice on dogs for at least one month (Kužner et al., 2013). Effitix®, a topical ectoparasiticide containing fipronil-permethrin, provides rapid efficacy against *R. sanguineus* and *C. felis* that lasts for one month after a single application in dogs (Cvejić et al., 2017).

Frontline spray (0.25% (w/v) fipronil), Frontline spot-on for dogs (10% (w/v) fipronil) and Frontline Plus for dogs (10% (w/v) fipronil and 9% (S)-methoprene) have been confirmed in laboratory conditions to be effective against *Trichodectes canis* lice (Pollmeier et al., 2002) and spray 0.25% fipronil (Frontline Spray, Merial), 10% fipronil spot-on (Frontline Spot-on for Cats, Merial) and 10% fipronil / 12% (S)-methoprene (Frontline Plus for Cats, Merial) against *Felicola subrostratus* (Pollmeier et al., 2004).

A broad-spectrum product Effitix based on a combination of fipronil 6.1% and permethrin 54.5% has been developed that is safe and effective for dogs against ticks, fleas, and mosquitoes (Navarro et al., 2016).

Ticks have been shown to be sensitive to propoxur (Mullens et al., 2017; Thomas et al., 2018).

The aim of the study was to evaluate the efficacy of the use of antiparasitic sprays with different active ingredients for the prevention and treatment of dogs and cats.

Material and methods. The study to determine the efficacy of antiparasitic drugs on dogs and cats was conducted in the Laboratory of Veterinary Sanitation and

Parasitology of the National Scientific Center 'Institute of Experimental and Clinical Veterinary Medicine' and at the animal shelter (Balakliya).

Modern domestic antiparasitic drugs were used in the experiments:

— 'PROFILINE Spray' (100 ml of the drug contains the active ingredient: propoxur — 0.25 g; excipients: isopropyl alcohol, polyethylene glycol — 400);

— 'INSECTOSTOP Spray' (100 ml of the drug contains the active ingredient: fipronil — 0.3 g; excipients: isopropyl alcohol, polyethylene glycol — 400);

— 'Barrier-Super Insecticidal Spray' (1 ml of the product contains the active ingredient: fipronil — 80 mg; excipients: propylene glycol, isopropyl alcohol).

Scheme of the research:

— Clinical examination of the animals in the shelter, preliminary diagnosis, collection of ectoparasites and skin scrapings for laboratory examination, constant clinical monitoring of the physiological state of the experimental animals;

— Microscopic examination of samples for the detection of parasitic pathogens in biological material, their identification and determination of the prevalence of infection in dogs and cats;

— Formation of experimental groups of animals;

— External application of drugs, individually, directly on the skin, keeping the animals in the shelter, taking samples of scrapings for laboratory testing 5, 10, 30, and 45 days after the last application of drugs. Determination of the efficacy of the drug;

— Daily clinical examination of the health of the animals throughout the experiment.

The study included 18 outbred dogs of different age groups, weighing 3 to 20 kg, and 22 outbred cats of different age groups. The animals were kept in standardized cages at an air temperature of 24.0 ± 1.5 °C, relative humidity of 40–70%, and air movement of 0.2–0.5 m/s. The animals were fed according to the diet approved by the shelter.

In accordance with the objectives, the experiments were performed by visual and microscopic methods following practical guidelines (Yiskiv, 1998; Halat et al., 2009).

The intravital diagnosis of ectoparasitoses was performed and the number of ectoparasites was determined. Identification of ectoparasitic pathogens was carried out by microscopic method. The mean intensity of the infection was determined by counting ectoparasites per 10 cm^2 of animal skin area.

Sick animals were divided into groups that were separately treated with 'PROFILINE spray' (dogs, n = 6; cats, n = 8), 'INSECTOSTOP spray' (dogs, n = 6; cats, n = 7), and 'Barrier-Super Insecticidal Spray' (dogs, n = 6; cats, n = 7).

On 5^{th}, 10^{th}, 20^{th} and, 30^{th} days after treatment, the results of the study were recorded based on examinations of treated animals, counting live ectoparasites on them, and prevalence of infection after treatment and the efficacy of the drug were determined.

Animals were clinically examined before, during, and after treatment. External examination included assessment of general appearance, skin and coat condition, measurement of body temperature, pulse and respiratory rate, examination of the eyes, mouth and ear cavities, palpation of the skin and peripheral lymph nodes.

To collect ectoparasites from the skin, the animals were restrained in a supine position. The examination of the animals' skin started with the head. Then the neck, back, sides, abdomen and limbs were examined. The wool was parted and combed during the examination. Animals were examined first with the naked eye and then with a magnifying glass. Detected ectoparasites were removed from the skin of the animals with tweezers or a rubber gloved hand. The removed ectoparasites were placed in glass dishes filled with Barbagallo's fluid (3% aqueous formalin solution in saline) or 70% ethanol. Some of the ectoparasites were delivered alive to the laboratory in tubes or containers with wet filter paper inside. The filter paper strips were moistened with boiled wateR. The tubes and containers were covered with a layer of cloth and tied. A label was placed on each tube and container.

To test dust and litter from the facilities where the treated animals were kept, a 30 g sample was placed in a cylinder or conical flask, filled with water, and mixed thoroughly. Particles floating to the surface were removed and the water was carefully poured off, leaving a precipitate. The precipitate was mixed with 20 parts of saturated sodium chloride solution and allowed to stand for 20 minutes. Then, a sample (three drops) was taken from the surface of the flotation film with a wire loop, placed on a microscope slide, covered with a coverslip, and examined under a microscope.

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, microfilariae;
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.

Efficacy (E) of the drug was calculated by the number of treated animals in percentage that were completely free of parasites.

In order to determine the acaricidal effect of the drugs by topical application, experimental groups of

ectoparasites were formed. They were treated separately with the test agents. The experiments were carried out in 250 cm beakers. The beakers were covered with gauze and sealed with rubber rings. The beakers were left in the room at a temperature of 20–25 °C.

The 'knockdown effect' in each beaker was determined after 6, 12, 24 hours (WHO, Geneva, 1974).

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 Commission of the National Scientific Center 'Institute of Experimental and Clinical Veterinary Medicine' in the current order.

Results and discussions. Ectoparasite collections included ixodid ticks (*Ixodes ricinus*), dog fleas (*Ctenocephalides canis*), and cat fleas (*Ctenocephalus felis*). The mean intensity (MI) of *Ixodes* ticks was 1–3 individuals per 10 cm^2 of skin area in dogs and 1–2 per 10 cm^2 of skin area in cats, and the prevalence (P) was 100%. The P of fleas in dogs was 100%, in cats — up to 100%, MI in dogs and cats — 7–10 fleas per 10 cm^2 of skin area, MI in dogs and cats — 1–2 lice per 10 cm^2 of skin area, MI in dogs — 1–2 lice per 10 cm^2 of skin area.

The onset of the 'knockdown effect' state was determined in the laboratory on ectoparasites collected from animals. The results of the determination of the rate of the onset of the 'knockdown effect' of ixodid ticks and fleas under the influence of the experimental agents are given in Table 1.

Table 1 — Efficacy of the drugs on ectoparasites when applied topically

Parasite species	Number of parasites	'Knockdown effect', after / hours		
		6	12	24
PROFILINE spray				
Ixodes ricinus	10	5	5	–
Ctenocephalus felis	10	8	2	–
Ctenocephalides canis	10	7	3	–
INSECTOSTOP spray				
Ixodes ricinus	10	5	5	–
Ctenocephalus felis	10	7	3	–
Ctenocephalides canis	10	7	3	–
Barrier-Super Insecticidal Spray				
Ixodes ricinus	10	5	5	–
Ctenocephalus felis	10	7	3	–
Ctenocephalides canis	10	6	4	–

The results presented in Table 1 show that the 'knockdown' effect in all ticks began with a state of disorientation, ticks began to move chaotically 6 hours after the effect of the products, and after 12 hours there was a complete 'knockdown' of all experimental Ixodid ticks and fleas.

The results of the determination of the therapeutic efficacy of the products studied are presented in Table 2.

Table 2 — Study of therapeutic efficacy of antiparasitic drugs

Drug	Parasite species	Infected		
		Before treatment		After treatment
		P,%	MI, insects/ 10 cm^2	Efficacy of the drug after 10 days,%
Dogs (n = 18)				
PROFI-LINE spray	*Ctenocephalus canis* *Linognathus setotus* *Trichodectes canis* *Ixodes ricinus*	100	7–8 1–2 1–2 1–3	100 (not detected)
INSECTO-STOP spray	*Ctenocephalus canis* *Linognathus setotus* *Trichodectes canis* *Ixodes ricinus*	100	1–8	100 (not detected)
Barrier-Super Insecticidal Spray	*Ctenocephalus canis* *Linognathus setotus* *Trichodectes canis* *Ixodes ricinus*	100	1–8	100 (not detected)
Cats (n = 22)				
PROFI-LINE spray	*Ctenocephalus felis* *Felicola subrostratus* *Ixodes ricinus*	100	7–10 1–2 1–2	100 (not detected)
INSECTO-STOP spray	*Ctenocephalus felis* *Felicola subrostratus* *Ixodes ricinus*	100	1–10	100 (not detected)
Barrier-Super Insecticidal Spray	*Ctenocephalus felis* *Felicola subrostratus* *Ixodes ricinus*	100	1–10	100 (not detected)

According to the results of the application of experimental products against ectoparasites of animals, it was found that already on the 2nd day after their application dead ticks, fleas and lice were found on the treated animals. Dead ectoparasites were found on animals on the 5th day, and fleas and lice were not observed on the body of animals on the 10th day. No fleas, lice or ticks were found on the body of the animals during the 30-day observation period. No complications or changes in clinical state were observed during treatment and clinical observation of experimental and control animals after administration of the drugs.

Efficacy of PROFILINE Spray, INSECTOSTOP Spray, and Insecticidal Barrier-Super Insecticidal Spray in dogs and cats infected with ectoparasites (ixodid ticks, fleas, lice) is 100%.

The results of the examination of bedding samples from the facilities where the animals treated with the test products were kept for the presence of ectoparasite larvae, nymphs and adults are presented in Table 3.

Table 3 — Examination of animal facility litter for larvae, nymphs, and adults of ectoparasites

Number of larvae and adults of ectoparasites in the litter (100 g) in the place of animal housing	
Before treatment	after the treatment of animals with the drug on the 10th day
PROFILINE spray	
17.0 ± 1.5	not detected
INSECTOSTOP spray	
10.0 ± 1.5	not detected
Barrier-Super Insecticidal Spray	
8.5 ± 1.5	not detected

As can be seen from the materials presented in Table 3, no larvae or adults of ectoparasites were found in the litter after treatment with insecticidal sprays on 10th day. The litter in the enclosures is changed every 3 days, which prevents reinfection of the animals. There is limited knowledge on ectoparasiticide resistance in Europe, as documentation on this subject is scattered and incidental. For most ectoparasite species in European countries there is no comprehensive database that provides an overview regarding the resistance situation against commonly used ectoparasiticides and possible trends over time (EMA, 2023).

Other studies have confirmed that treatment with fipronil spot-on at the recommended commercial dose rapidly reduced existing flea, tick and lice infections in dogs. Treatment provided up to 8 weeks of flea reinfection control and up to 4 weeks of tick and lice control (Kužner et al., 2013). Topical fipronil has been shown to be effective in the treatment and control of sucking and biting lice infections in laboratory rats (Diaz, 2005).

Considering all stages of the flea life cycle, the combination product with fipronil for topical application provided a high level of overall flea control, with a therapeutic effect against adult fleas and inhibition of intermediate flea stages within 12 weeks (Young et al., 2004).

All groups of dogs receiving a single topical treatment with the combination of fipronil and permethrin had significantly ($p < 0.005$) fewer fleas than untreated controls 24 and 48 hours after treatment The reduction in *C. felis* ranged from 98.4 to 100% at all time points in all studies (Fankhauser et al., 2015).

It should be noted that the use of insecticides in sublethal doses leads to the development of resistance in parasites (McNair, 2015; Zhang et al., 2019). Therefore, it is necessary to determine the effective doses of new drugs and test them before their widespread introduction into production.

Thus, our results are consistent with those of other researchers regarding the high antiectoparasitic efficacy of products containing the active ingredients propoxur and fipronil.

Conclusions. Studies on PROFILINE Spray, INSECTOSTOP Spray, and Barrier-Super Insecticidal Spray have shown that they are well tolerated by animals and do not cause any side effects or changes in the clinical state of the animals.

Laboratory studies showed a high insecticidal efficacy of the experimental products against fleas (*C. canis, C. felis*) and ticks (*Ixodes* spp.).

Clinical studies have shown high activity of the sprays against fleas (*Ctenocephalides* spp.), lice (*Trichodectes canis, Felicola subrostratus*), lice (*Linognathus setosus*) and ixodid ticks (*Ixodes* spp). The efficacy of the products is 100% within 60 days after application to animals in case of infection (*C. canis, C. felis, Ctenocephalides* spp., *Trichodectes canis, Felicola subrostratus, Linognathus setosus*) and 45 days in case of *Ixodes* spp.

According to the results of the studies, it was found that PROFILINE Spray, INSECTOSTOP Spray, and Barrier-Super Insecticidal Spray can be used for the prevention and treatment of pets affected by fleas, lice, ticks and ixodid ticks.

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