



Evaluation of efficacy of a drug based on fipronil, ivermectin, and pyriproxyfen against ecto- and endoparasitic invasions of dogs and cats

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Article info

Received 25.12.2023

Received in revised form
03.02.2024

Accepted 18.02.2024

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Yuskiv, I. D., Tishyn, O. L., & Yuskiv, L. L. (2024). Evaluation of efficacy of a drug based on fipronil, ivermectin, and pyriproxyfen against ecto- and endoparasitic invasions of dogs and cats. *Regulatory Mechanisms in Biosystems*, 15(1), 113–118. doi:10.15421/022417

Ecto- and endoparasitic invasions of dogs and cats are treated using drugs based on fipronil, ivermectin, and pyriproxyfen. That is why our objective was performing a field study to measure the efficacy of a complex drug with different mechanisms of the actions of its active agents against ecto- and endoparasitic invasions of dogs and cats using the spot-on application method. The paper presents data of the efficiency of the complex drug PerFect TRIO, antiparasitic drops for spot-on use against ecto- and endoparasitic invasions of dogs and cats of different breeds, ages, sexes, and body mass. Parasitological study of dogs found parasitism by the fleas *Ctenocephalides canis* and *Pulex irritans* with the invasion intensity ranging 108 to 132 ectoparasite insects per animal, the average invasion intensity being 121.1 ± 3.17 ectoparasite insects per animal; Trichodectidae species such as *Trichodectes canis*, with the invasion intensity ranging 25 to 32 ectoparasite insects per 100 cm² of the body surface, averaging 28.6 ± 1.00 ectoparasite insects per 100 cm² of the body surface; and Trombidiformes such as *Cheyletiella yasguri*, the intensity being 24 to 41 ectoparasite mites per animal, the average intensity being 33.9 ± 2.52 mites per animal. According to the results of parasitological studies of cats, their bodies were parasitized by the fleas *Ctenocephalides felis* and *Pulex irritans*, with the invasion intensity ranging 58 to 80 ectoparasite insects per animal, with the average intensity of 67.7 ± 2.84 ectoparasite insects per animal, Trichodectidae species such as *Felicola subrostratus* with the invasion intensity of 17 to 26 ectoparasite insects per 100 cm² of the animal's body surface, averaging 21.3 ± 1.38 ectoparasite insects per 100 cm² of the body surface; the ear mite *Otodectes cynotis* with the intensity ranging 4 to 9 mites per animal, the average intensity being 6.1 ± 0.7 mites per animal; and the endoparasite – the digestive-tract nematode *Uncinaria stenocephala*, with the invasion intensity (II) of 28 to 41 eggs in one gram of feces (EGF), averaging 34.3 ± 2.06 EGF. In our study, local spot-on application of the tested drug PerFect TRIO, antiparasitic drops for dogs and cats, produced notable effects on wingless ectoparasite insects and acarians of the dogs. Therefore, the efficiency of the drug against Siphonaptera infestation of the dogs equaled 92.9% on the 8th h and 100% on the 24th and 72nd h; against Trichodectidae infestation, it was 91.5% effective on the first day and 100% on the second and seventh; against cheyletiellosis, it was 81.9% effective on the seventh day and 100% on days 14 and 21. As with the cats, parasitological studies found that the efficacy of the recommended doses of the tested drug against spontaneous Siphonaptera infestation was 92.0% on the 8th h and 100% on the 24th and 72nd h; against *Felicola* infestation, it was 90.6% effective on the first day and 100% on the second and seventh day; against otodectosis, the drug produced 81.4% efficacy on the 7th day and 100% on the 14th and 21st days; and against hookworm disease, the efficacy was 100% on days 7 and 14. Our field trials demonstrated that the tested antiparasitic drops for cats and dogs are easy-to-use, were well received by the animals regardless of breed, age, sex, body mass, and had no toxic impact on them, providing a broad range of treatment against ecto- and endoparasites of cats and dogs.

Keywords: parasitology; spot-on solution; flea infestation; Trichodectidae infestation; *Felicola* infestation; cheyletiellosis; *Otodectes cynotis*; uncinariasis; fipronil; ivermectin; pyriproxyfen; drug efficacy.

Introduction

Dogs and cats are a key part of the modern civilization, as demonstrated by the assessments that over a half of the planet's population keeps domestic animals. At the same time, dogs are the most popular, living in houses all around the globe (Pereira et al., 2016). Over one fourth of pet owners have cats (Downes et al., 2009; Mylostyvyi et al., 2023). Considering the emotional attachment of mankind to cats and dogs, the general attractiveness and close relationships between dogs, cats, and people, there is a potential for parasitic infestation, either through close contact with infested animals or contact with an infested environment.

Studies dealing with the spread and assessment of digestive-tract parasites in feces of stray dogs and cats keep pointing to high number of animals infested by myriad parasites that are pathogenic to people (Khademvatan et al., 2014; Szwabe & Blaszkowska, 2017).

Such a broad spread is mainly associated with absence of preventive measures or protocols of treating dogs and cats. At the same time, companion dogs and cats (www.esccap.org) contaminate the environment with feces, which are not collected and recycled, being a trophic source of transmission of parasitic diseases (Tamponi et al., 2020). Moreover, most owners are unaware of zoonotic parasites of their dogs and cats (Pereira et al., 2016).

Dogs and cats in Europe and Ukraine are often infested by a wide spectrum of endo- and ectoparasites. Ectoparasites include a large number of parasitic arthropods that taxonomically belong to the Insecta class (fleas, lice, Trichodectidae, mosquitoes, flies) and the Acari subclass (Acariformes, Trombidiformes, and Parasitiformes). Ectoparasites can cause damage of various kinds such as skin lesions and immunopathological reactions, carry pathogens, or transmit zoonotic diseases. Therefore controlling them is a part of keeping pets healthy (Beugnet et al., 2014; Kru-

chynenko, 2020; Yevstafieva et al., 2021, 2023). Endoparasites include an array of helminths (nematodes, cestodes, and trematodes), which by location of parasitism in the host are divided into the main groups of parasitic worms: intestinal helminths and extraintestinal helminths. Domestic dogs and cats can be also affected by numerous mixed infestations. Depending on intensity of invasion by parasites, on the one hand, and age, resistance, conditions of feeding and maintenance of the animals on the other hand, such diseases can cause various clinical symptoms in the animals, ranging from light gastrointestinal disorders and arrest in development to anemia or anorexia in the more severe cases, especially in puppies and kittens (Traversa, 2012; Kornyushin et al., 2013; Baneth, 2016; Beugnet et al., 2018; Martyschuk et al., 2022).

Over recent years, especially in large cities, the number of pets and feral animals, in particular dogs and cats, has surged, and hence increase in their ecto- and endoparasite morbidity. However, a new situation with natural focal invasions of animals, especially zoonoses, has formed in the early 21st century in Ukraine due to growing “populations” of feral animals in large cities and the emergence of numerous groups of street dogs that have adapted to certain ecological niches in natural biocoenoses of green areas of large and small cities, and also territories under various categories of protection. Due to continuous migrations of individual groups of such dogs and cats from natural biocoenoses to construction-free areas in cities and back, those animals have become a connective link that carries pathogens of dangerous diseases, in particular entomoses, acaroses, helminthiases, providing a quite stable exchange between natural and synanthropic sites of infestations. All of this conditions the relevance of in-depth research on mixed invasions (endo- and ectoparasites) of dogs and cats in different regions of Ukraine and the necessity of organizing continuous monitoring of epizootiological and epidemiological situation and effective treatment using endoparasitic and ectoparasitic drugs (Kornyushin et al., 2013; Ponomarenko et al., 2016; Boyko & Brygadyrenko, 2019a, 2019b, 2021; Boyko et al., 2020; Kruchynenko, 2020). The European Scientific Counsel Companion Animal Parasites (ESCCAP, www.esccap.org) developed recommendations regarding treatment and management of parasites of companion animals in order to protect the health of pets and population by reducing the risk of transmission of zoonotic parasites. Therefore, timely diagnostics, prophylaxis, and therapy of parasitic diseases, primarily in large cities, is relevant, considering such an important factor as environmental contamination with invasive elements and spread of anthroozoonotic diseases (Beugnet et al., 2014; Raza et al., 2018; Karpenko et al., 2022; Sameliuk et al., 2022; Elsheikha, 2023).

On the pharmaceutical market, the most largely used drugs against ecto- and endoparasitic invasions of dogs and cats are endectocides – macrocyclic lactones and their derivatives (ivermectin, milbemycin, moxidectin, selamectin, eprinomectin, doramectin; against nematodes, insects, and acarians), which are used in combination with ectocides: phenylpyrazoles (fipronil, piriprol; against insects and acarians); pyrethroids (deltamethrin, flumethrin, permethrin; against insects and acarians); chloronicotinil nitroguanidines (imidacloprid, against insects); insect growth regulators (pyriproxyfen, S-methoprene, lufenuron; against eggs, larvae, and pupae of fleas) and isoxazolines (afoxolaner, sarolaner, lotilaner, fluralaner; against fleas and acarians) (Wiebe, 2015; Beugnet et al., 2018; Saari et al., 2019).

The producer of veterinary drugs offers the drug PerFect TRIO, antiparasitic drops for dogs and cats against ecto- and endoparasitic invasion of dogs and cats. The preparation is based on the combination of the compounds fipronil 100.0 mg/ivermectin 15.0 mg/pyriproxyfen 20.0 mg for external spot-on use. The combination of the active agents fipronil, ivermectin, and pyriproxyfen provides a broad range of antiparasitic action against fleas, Trichodectidae, lice, Sarcoptiformes and Trombidiformes, and nematodes of the digestive tract. The objective of the study was performing clinical tests on target animals to identify the effectiveness of the PerFect TRIO drops for external spot-on use in the doses recommended by the manufacturer against ecto- and endoparasites of dogs and cats.

Materials and methods

We used dogs and cats of different breeds, age, sex, and body mass, spontaneously infested with ectoparasites: the fleas *Ctenocephalides canis*, *Ctenocephalides felis*, *Pulex irritans*; Trichodectidae – *Trichodectes canis*,

Felicola subrostratus; Sarcoptiformes – *Otodectes cynotis*; and Trombidiformes – *Cheyletiella yasguri*; and endoparasites – the *Uncinaria stenocephala* nematode. The studies were carried out in veterinary clinics in Lviv and included animals from individuals and brought by of volunteers.

According to the general rules, based on the clinical-parasitic studies, we formed a group of 7 animals according to the analogue principle for each species of ecto- and endoparasites. Animals of the experimental groups were treated with PerFect TRIO, applying it spot-on on the skin in the regions inaccessible for licking, in the doses according to the manufacturer’s recommendations. When treating large dogs, the drug was applied on the skin in 34 places. In cases of otodectosis, the auricle and the ear canal were pre-cleaned from earwax, exudates, and eschars. The drug was used once, administering 2–3 drops into each ear (the drops were introduced into both ears). For an even distribution of the drug, the auricle was folded in halves and lightly massaged at the base. Drug leftover in the pipette (in calculation per animal’s body mass) was applied on the skin between the shoulder blades. To prevent sprinkling of the drug, the head of animal was fixated for several seconds.

In cases of ectoparasites, in compliance with our objectives, we performed parasitological studies of the animals visually and gathered the material for the laboratory studies to detect arthropods according to GCP standards and the Guiding Principles of the EU regarding testing and assessing efficiency of antiparasitic compounds for treatment and prophylaxis of fleas and acarians of dogs and cats (Marchiondo et al., 2007, 2013).

Intensity of invasions by fleas of *Ctenocephalides canis*, *Ctenocephalides felis*, and *Pulex irritans* was identified by counting ectoparasite insects on the skin and coating of the animals by grooming with a fine-toothed comb in five anatomic regions: dorsal median line (linea dorsalis medianum), sitting bone (tuber ischiadicum), left side (pars lateralis sinister “left”), right side (pars lateralis dexter “right”), inguinal region (regio inguinalis) for a certain time (usually, 5–20 minutes for dogs and 7 minutes for cats) (Dryden et al., 1994; Marchiondo et al., 2007). Intensity of invasions by *Trichodectes canis* and *Felicola subrostratus* was determined by grooming with a regular comb and visually counting the ectoparasite insects in 34 regions of the animal’s body, the total area accounting for 100 cm² (Woldstad et al., 2014).

Otoscope study found live Sarcoptiformes mites *Otodectes cynotis* on the internal surface of the auricle and external ear canal of the animals. Intensity of invasion by skin-eating mites was determined by counting ectoparasites in the surface scrapings of the infested animals during microscopic study (vital method). Invasion by the Trombidiformes mite *Cheyletiella yasguri* was diagnosed based on clinical signs. First of all, we looked for dry scales on the back (in the area of dorsal part of the neck and torso along the spine), restlessness of the animals, suffering light itching. Monitoring of the skin of the animals was started from the head. Later, we examined the neck, spine, sides, stomach, and limbs. The hair was pulled to the sides during monitoring. First, we simply examined visually, and then using an otoscope. Intensity of invasion by the Trombidiformes mite *Cheyletiella yasguri* in the animals was identified using the duct-tape test (a 2 x 6 cm tape was attached to the hair and skin of an animal in selected places; the tape was put on a microscope slide and examined under a microscope) and by grooming for no less than 10 min using comb for fleas (the dorsal part of the lumbar-sacral part) (Zajac & Conboy, 2012; Beugnet et al., 2018).

Ectoparasites were identified using the microscopic method in accordance with the practical recommendations (Yuskiv, 1998; Zajac & Conboy, 2012; Taylor et al., 2016; Beugnet et al., 2018).

After treating the animals, efficacy of the drug against Siphonaptera infestation was measured on the 8th, 24th, and 72nd h, against Trichodectidae and *Felicola infestations* on the 1st, 2nd, 7th, 14th, and 28th days, by grooming with fine-tooth combs and carefully analyzing the coating of the dogs and cats for presence of wingless ectoparasite insects. Against otodectosis and cheyletiellosis, the efficacy of the drug was identified using the vital method for presence of the mites *Otodectes cynotis* and *Cheyletiella yasguri* on the 7th, 14th, and 21st days after treatment.

Efficacy of the drug against ectoparasites was identified according to the Abbott’s formula:

$$\text{The percentage of efficacy (\%)} = [(C - E) : C] \times 100,$$

where C = mean arithmetic value of the number of vital insects/ ectoparasite mites in the control group; E = mean arithmetic of the number of vital mites/ectoparasite mites in the treated group of animals (Marchiondo et al., 2007, 2013). Efficacy of the tested veterinary drug against endoparasites was measured according to the Guiding Principles of the EU VICH GL7, "Effectiveness of Anthelmintics. General Data", VICH GL20 "Effectiveness of Anthelmintics: Special Recommendation for Cats" and the scientific recommendations of WAAVP for assessing efficiency of anthelmintics for dogs and cats (Jacobs et al., 1994; Beugnet et al., 2022).

Fecal samples for the study were collected individually, in the amount of 10,015 g into clean plastic containers with a secure lid. Coprological studies were carried out using the sedimentation and flotation methods to diagnose infestation of the cats with helminths (Zajac & Conboy, 2012). Intensity of invasion of the animals by parasites was identified by counting eggs of helminths in samples of feces using the McMaster Counting Method and the method of Taylor et al. (2016). To detect eggs of digestive-tract nematodes, we used a saturated solution of sodium chloride with the specific weight of 1.20 (500 g of NaCl per 1.0 L of water). Number of eggs of helminths in one gram of feces (EGF) was estimated using Taylor's formula (Taylor et al., 2016).

The helminths and their embryos in the cats were identified microscopically according to the species guides for differential diagnostics (Zajac & Conboy, 2012; Taylor et al., 2016; Beugnet et al., 2018; Saari et al., 2019).

Prior to and after deworming of the animals, on days 7 and 14, we clinically examined the cats and performed helminth control. At the same time, we determined indices of parasitological infestation (extensiveness and intensiveness) of the cats with nematodes of the digestive tract before and after deworming and the drug efficacy was identified (Jacobs et al., 1994; Beugnet et al., 2022). All the groups were monitored around-the-

clock. Efficacy of the drug against endoparasites was assessed using the formula:

$$\text{The percentage of efficacy (\%)} = [(C - E) : C] \times 100,$$

where C = mean number of helminth eggs in control; E = mean number of helminth eggs in the treated animals (Jacobs et al., 1994).

All the procedures described in the study were performed according to the European Directive (Directive 2010/63/ES) regarding the procedures for protection of animals used for scientific purposes.

The results were analyzed in Statistica 6.0 (StatSoft Inc., USA). The data are presented in tables as $\bar{x} \pm SD$ (mean \pm standard deviation). Differences between the control and experimental groups were identified using ANOVA, where the differences were considered significant at $P < 0.05$ (taking into account Bonferroni's Correction).

Results

Clinical examination and parasitological study of the dogs and cats of various breeds, sex, and body mass, which were admitted to the veterinary clinics of Lviv, revealed infestation with the following entomoses: *Ctenocephalides canis*, *Ctenocephalides felis*, *Pulex irritans* (fleas), *Trichodectes canis*, *Felicola subrostratus* (chewing lice); acariases: *Cheyletiella yasguri* (Trombidiformes mite), *Otodectes cynotis* (Sarcoptiformes mites), and nematodosis of the digestive tract caused by *Uncinaria stenocephala* (strongyloidiasis of carnivores).

We performed clinical examinations and entomological studies of the dogs by grooming with fine-tooth comb for the indicated time in five anatomical regions of each animal. On their bodies, we found *C. canis* and *P. irritans*, the intensity ranging 108 to 132 fleas per animals, on average 121.1 ± 3.2 ectoparasite insects per animal (Table 1).

Table 1

Efficacy of the tested drug PerFect TRIO antiparasitic drops for dogs and cats against flea infestation of dogs and cats ($\bar{x} \pm SD$, $n = 7$)

Infestation intensity, species of fleas on the bodies of animals	Prior to treatment	After treatment			Efficacy, %		
		8 h	24 h	72 h	8 h	24 h	72 h
Experimental group (dogs), PerFect TRIO antiparasitic drops for dogs and cats	121.1 \pm 3.17	8.6 \pm 0.20	0.0	0.0	92.9	100.0	100.0
Experimental group (cats), PerFect TRIO antiparasitic drops for dogs and cats	67.7 \pm 2.84	5.4 \pm 0.20	0.0	0.0	92.0	100.0	100.0

The studies revealed that on the 8th h after treatment of the dogs, the intensity of invasion by fleas decreased. Therefore, the number of the fleas *Ctenocephalides canis* and *Pulex irritans* was 8 to 9 ectoparasite insects per animal, the average intensity being 8.6 ± 0.20 ectoparasite insects per animal. Starting from the first and third days after using the drug, parasitological studies found no fleas of *C. canis* and *P. irritans* on the coats of dogs of the experimental group (Table 1).

Therefore, the results indicate that the drug's efficacy against Siphonaptera infestation of the dogs equaled 92.9% on the 8th h after usage and 100% on the 24th and 7th h (Table 1).

The clinical examination and entomological studies of the dogs by grooming them revealed parasitism by *Trichodectes canis* with the invasion intensity of 25 to 32 ectoparasite insects per 100 cm² of the body surface, on average 28.6 ± 1.00 ectoparasites per 100 cm² of the body surface (Table 2). Clinically, the animals' infestation with Trichodectidae manifested in scratching, especially at night. The animals bit the regions of the skin and were anxious. There were scratches on the skin, leading to dermatitis.

The data presented in Table 2 show decline in the infestation intensity by the Trichodectidae insects as early as on the first day after drug application. At the same time, after using the drug, intensity of infestation of experimental-group animals with *T. canis* on the 1st day of the experiment equaled 2 to 3 ectoparasite insects per 100 cm² of body surface, the average intensity being 2.4 ± 0.2 ectoparasites per 100 cm² of the body surface.

Table 2

Efficacy of the tested drug PerFect TRIO antiparasitic drops for dogs and cats against trichodectoses infestation of dogs and *Felicola* infestation of cats ($\bar{x} \pm SD$, $n = 7$)

Infestation intensity	Prior to treatment	After treatment					Efficacy, %				
		Day 1	Day 2	Day 7	Day 14	Day 28	Day 1	Day 2	Day 7	Day 14	Day 28
Infestation intensity, species of <i>Trichodectes canis</i> on the bodies of animals	28.6 \pm 1.00	2.4 \pm 0.20	0.0	0.0	0.0	0.0	91.5	100	100	100	100
Infestation intensity, <i>Cheyletiella yasguri</i> mites on the bodies of animals	21.3 \pm 1.38	2.0 \pm 0.22	0.0	0.0	0.0	0.0	90.6	100	100	100	100

Table 3

Efficacy of the tested drug PerFect TRIO antiparasitic drops for dogs and cats against cheyletiellosis infestation of dogs and otodectosis of cats ($x \pm SD$, $n = 7$)

Infestation intensity	Prior to treatment	After treatment			Efficacy, %		
		Day 7	Day 14	Day 21	Day 7	Day 14	Day 21
Infestation intensity, <i>Cheyletiella yasguri</i> mites on the bodies of animals	33.9 ± 2.52	6.1 ± 0.51	0.0	0.0	81.9	100	100
Intensity of infestation with <i>Otodectes cynotis</i> mites in the ears of animals	6.1 ± 0.74	1.1 ± 0.14	0.0	0.0	81.4	100	100

The conducted clinical examination and entomological study of the cats by grooming with fine-tooth comb for the indicated time revealed parasitism by the fleas *C. felis* and *P. irritans* with the invasion intensity ranging 58 to 80 ectoparasite insects per animal, the average being 67.7 ± 2.84 ectoparasites per animal (Table 1).

On the 8th day after treating the cats with the drug according to the manufacturer's recommendations, we found decline in the number of parasites in the experimental group of animals. The invasion intensity of the fleas *C. felis* and *P. irritans* equaled 5 to 6 ectoparasite insects per animal, the average intensity being 5.4 ± 0.2 ectoparasites per animal. Starting from the first and third days after administration of the drug, entomological study found no individuals of *C. felis* and *P. irritans* on the coat of cats of the experimental group (Table 1).

Thus, the results we obtained indicate that the tested drug PerFect TRIO produced 92.0% effectiveness against Siphonaptera infestation of the cats was on the 8th h after usage and 100% on the 24th and 72nd h (Table 4).

The conducted clinical examination and entomological study of the cats by grooming revealed that the invasion intensity by *Felicola subrostratus* was 17 to 26 ectoparasite insects per 100 cm² of the body surface, the average being 21.3 ± 1.4 ectoparasites per 100 cm² of the body surface (Table 2). The clinical signs of infestation of the cats by Trichodectidae manifested similarly to the dogs.

According to the results of clinical examination and entomological study of the cats, PerFect TRIO decreased the intensity of Trichodectidae infestation. Therefore, the invasion intensity by *Felicola subrostratus* in the experimental group declined on the first day of the experiment from 1 to 3 ectoparasite animals per 100 cm² of the body surface, averaging 2.0 ± 0.2 ectoparasites per 100 cm² of the body surface. Examination of the coat and laboratory study of the animals two and seven days after applying the drug found no *F. subrostratus* (Table 2). Therefore, the results of our studies indicate that the drug's efficacy against *Felicola* infestation of the cats equaled 90.6% on day 1 and 100% on days 2, 7, 14, and 28 after usage (Table 2). Therefore, spot-on application of the tested drug in the doses recommended by manufacturer exerted powerful defensive action against *Felicola subrostratus* for 4 weeks.

The clinical examination and otoscopic study of the internal surface of the auricle and the outer ear canal revealed vital *Otodectes cynotis* individuals and otitis. The disease's main symptoms were exudes from the ears, accompanied by pain, itching, and redness of the auricle. The animals were anxious, often scratched their ears, causing abrasions on the auricle, and shook their head. The acarological study of the cats found parasitism by the ear mite *O. cynotis* with the general intensity ranging 4 to 9 specimens per animal, the average being 6.1 ± 0.7 specimens per animal (Table 3). The cats were treated with the drug as follows: internal surface of the auricle (two drops of the drug into each ear) and the coat, according to the manufacturer's recommendations. On the 7th day of the experiment, we saw decrease in the number of *O. cynotis* on the internal surface of the auricle and the outer ear canal in the experimental group of animals. Therefore, after treatment, the invasion intensity by the *Otodectes* mites was 1 to 2 specimens per animal, the average invasion intensity being 1.1 ± 0.1 mites per animal. The clinical examination and acarological study of the experimental group of animals on days 14 and 21 after treatment of the cats' auricles with the drug found no individuals of *O. cynotis* (Table 3). That is, the efficacy of PerFect TRIO against cats' otodectosis equaled 81.4% on day 7 after application and 100% on days 14 and 21.

Clinical examination and parasitological study of the cats revealed infestation with *Uncinaria stenocephala*, with the general invasion intensity ranging 28 to 41 eggs in 1 g of feces, averaging 34.3 ± 2.1 EGF (Table 4). After deworming of the cats with the studied drug in the manufacturer-recommended doses, coprological studies on the 7th day found no eggs of

U. stenocephala. Likewise, on day 14 after using the tested drug, no eggs of *U. stenocephala* were found (Table 4). Accordingly, PerFect TRIO produced 100% efficacy against hookworm disease on day 7 (Table 4). After treating siphonapterosis, Trichodectidae infestation, cheyletiellosis of the dogs and siphonapterosis, *Felicola* infestation, otodectosis, and hookworm disease of the cats, the general clinical studies indicated that singular spot-on application of PerFect TRIO in the doses recommended by manufacturer caused no side-effects or toxic impact on the animals. In our clinical trials on dogs and cats, PerFect TRIO displayed a broad range of strong insecticidal, acaricidal, and nematocidal actions, while causing no skin irritations, dermatitises, seborrhoeic dermatitis, allergic, or other unfavorable effects.

Table 4

Efficacy of the tested drug PerFect TRIO antiparasitic drops for dogs and cats against uncinariasis (hookworm disease) of cats ($x \pm SD$, $n = 7$)

Invasion intensity, eggs per 1 g of feces	Experimental group, PerFect TRIO antiparasitic drops for dogs and cats
Prior to treatment	34.3 ± 2.06
After treatment	
Day 7	0.0
Day 14	0.0
Efficacy, %	
Day 7	100.0
Day 14	100.0

Discussion

The skin is the largest organ that covers the entire body of mammals from head to toes. It is affected by various diseases, mostly caused mostly by arthropods. Despite the fact that some pathological states could have no direct effect on the visceral organs, continuous and strong irritation of the skin makes the animals anxious and restless. Any arthropod-caused skin disease of an animal must be immediately examined and treatment must be prescribed as soon as possible. In veterinary practice, first aid for treating arthropod infestation (fleas, chewing lice, lice, mites) is an essential component of prophylaxis strategy for the healthcare of dogs and cats. This is associated with high spread of flea infestations and other ectoparasite insects, all-year-round risk of acarians, threat of flea allergy dermatitis (FAD), pathogens carried by insects and mites, and also fleas infesting homes (Little et al., 2018; Abdullah et al., 2020).

The new drug on the pharmaceutical market – PerFect TRIO, antiparasitic drops for dogs and cats with the active compounds fipronil, ivermectin, and pyriproxyfen, used locally spot-on with one-month interval, was safe and highly effective against natural invasions of dogs and cats by ectoparasite insects.

However, as reported, fipronil (Effipro[®]) demonstrated a quick elimination of fleas. Therefore, after spot-on use of the drug, according to the manual on the label, the drug's efficacy against fleas was 0.0% to 43.4% after one hour of infestation, and > 99% after 12 h of infestation (Halos et al., 2016). Local treatment of the dogs with 10% fipronil produced > 95% elimination of the ectoparasite fleas for 35 days after the animals had been infested with 100 or 300 non-fed fleas, and on the 42nd day, the efficiency decreased to 68% (Coelho et al., 2015).

It has to be noted that spot-on usage of therapeutic doses of PerFect TRIO with the active compound fipronil on dogs and cats provided 92.0% to 92.9% efficacy against *Ctenocephalides canis*, *C. felis*, and *Pulex irritans* on the 8th h, and 100% on the 24th and 72nd h. Clinical trials confirmed that spot-on applied fipronil (Spotline[®]) was effective against fleas (*C. felis*, *C. canis*) on cats and fleas (*Ctenocephalides canis*, *C. felis*, *P. irritans*) and Ixodidae ticks (*Rhipicephalus sanguineus*) on dogs (Tüzer

et al., 2010). At the same time, high efficacy level against fleas of domestic animals was seen for 28 days after single application of fipronil (Dryden et al., 2000). Despite increase in the number of ectoparasitocides for domestic animals and their usage, morbidity of cats and dogs by arthropods in Europe is still high. Considering high rates of fleas' reproduction, the most efficient modern drugs for their control and prevention of infestations are those diminishing their reproduction, particularly systemic insect growth regulators (IGR), which provide a powerful ovicide action. Pyriproxyfen is an insect growth regulator (IGR), which affects flea eggs and other juvenile stages of fleas, and plays an important role in control of fleas of cats and dogs (Rust, 2017). Pyriproxyfen is absorbed by adult fleas during contact with the coat of treated animals. Just a 0.0001 mg/kg concentration of pyriproxyfen is enough to control flea larvae. It was found that the development of larvae and pupation of fleas is completely inhibited by 0.01 µg/kg concentration of the active ingredient on the cats' coat. Pyriproxyfen in 0.01 mg/kg concentration sterilized fleas on the cat coat for six hours (Meola et al., 2000; Stanneck et al., 2003). Insect growth regulators have multi-time effect on immature and adult fleas, thus enhancing the effectiveness of combined treatment.

Combination treatment with adulticides (fipronil, imidacloprid, nitenpiram, selamectin) and IGRs disrupts the life cycle of fleas and controls them in houses. Synergic combinations allow reduction in concentrations of adulticide and IGRs. Therefore, such a combination of pyriproxyfen and fipronil in Effipro[®] Duo eliminated fleas and acarians and provided three months of protection from reproduction of fleas in the environment (Dryden, 2009; Siak & Burrows, 2013).

It has to be noted that single spot-on application of fipronil (spot-on Frontline[®]) exhibited high effectiveness (100%) against invasion by *Cheyletiella* spp. (Scarampella et al., 2005), and also the chewing louse *Felicola subrostratus*, both in laboratory conditions and field studies (effectiveness of spot-on Frontline[®] was > 98% on day 2 and 100% on days 28 and 42) (Pollmeier et al., 2004). Results of those studies demonstrated that spot-on fipronil usage is an effective method to treat ectoparasite mites (*Cheyletiella* spp.) and wingless insects such as chewing lice (*F. subrostratus*).

The presented results indicate that spot-on application of the active compounds fipronil, ivermectin, and pyriproxyfen in the PerFect TRIO drops were effective and safe for treatment of invasions by the chewing lice *Trichodectes canis* and *Felicola subrostratus* and the ectoparasitic mites *Cheyletiella yasguri*. The parasitological studies revealed that the effectiveness of a single application was 91.5% against the chewing louse *Trichodectes canis* on the first day and 100% on second and seventh, 90.6% against the chewing louse *Felicola subrostratus* on the first day and 100% on the second and seventh days, and 81.9% against the ectoparasite mite *Cheyletiella yasguri* on the seventh day and 100% on days 14 and 21. The data indicate that timely application of ectoparasitocides according to the veterinary recommendations is important for optimal efficacy and effective control of ectoparasites. The antiparasitic drug PerFect TRIO Drops with the active compounds fipronil, ivermectin, and pyriproxyfen, which was used locally spot-on with a one month interval, was safe and highly efficient against natural invasions by ectoparasite fleas, ectoparasitic chewing lice, and *Cheyletiella* mites in dogs and cats.

Furthermore, the Insectostop antiparasitic drug for cats and dogs, the active compound of which is also fipronil, was also reported to be effective against the fleas *Ctenocephalides canis* / *C. felis*, *Pulex irritans*, Ixodidae ticks *Ixodes ricinus*, *Dermacentor reticulatus*, and *Otodectes cynotis*. Therefore, its efficacy against siphonapterosis of cats and dogs was 87.9% to 92.4% 8 h after application and 100% on the first and third days. At the same time, the efficacy of Insectostop against ixodiasis of dogs equaled 82.9% on the 8th h after application and 97.6% and 100% on the 24th and 72nd h, respectively. Against dog and cat otodectosis, the drug's efficacy equaled 75.6% to 76.9% on the 7th day, 98.1% to 100.0% on the 14th day, and 100.0% on the 21st day (Tishyn et al., 2021).

Nonetheless, the composition with the active compounds fipronil, ivermectin, and pyriproxyfen of the tested drug was safe and highly effective against *Otodectes cynotis*. Therapeutic efficacy of the tested drug against *Otodectes cynotis* mites was 81.4% on the 7th day and 100.0% on days 14 and 21. Ivermectin in the combination drug PerFect TRIO expands its properties against ecto- and endoparasites. Studies demonstrated effectiveness of ivermectin after local application against otocariosis

(96%) and toxocarosis and cheyletiellosis of cats (100%) (Pagé et al., 2000). Inferring from the study results, using 0.01% ivermectin (Acarexx) locally into the ear canal, 0.5 mL into each ear, as the main therapy, was efficient (100%) against the *Otodectes cynotis* mites in kittens below the age of 4 weeks 72 h after treatment (Nunn-Brooks et al., 2011).

The study focusing on effects of ivermectin and fipronil-containing ectoparasiticide drugs alone and in combination on ectoparasites in populations of cats found that fipronil and its combination with ivermectin had an advantage above ivermectin alone against ectoparasites (Shehzad et al., 2019). Ivermectin was the most commonly used acaricide and the most efficient one in treating sarcopitosis in both wild animals and animals in captivity (Rowe et al., 2019).

Ivermectin was effective against the nematodes *Strongyloides stercoralis* of dogs in shelters. Following the regular deworming protocols for dogs decreased the number of invasive larvae in the environment, which is important to protect dogs, as well as workers of the shelter, from the risk of infestations (Paradies et al., 2019; Unterköfler et al., 2022).

According to clinical studies, the tested drug PerFect TRIO was a highly effective anthelmintic against endoparasites – the nematode *Uncinaria stenocephala* (percentage efficacy > 98%), displaying 100% effect against hookworm disease of cats on days 7 and 14.

The new drug on the pharmaceutical market of Ukraine, PerFect TRIO antiparasitic drops for dogs and cats with the active compounds fipronil, ivermectin, and pyriproxyfen, provided high and stable efficacy against ectoparasite insects, ectoparasite mites, and nematodes of the digestive canal after spot-on usage with month-long interval.

Conclusions

Our parasitological studies revealed that the tested drug, PerFect TRIO antiparasitic drops for dogs and cats, was a highly effective insecticide against the ectoparasite fleas *Ctenocephalides canis*, *C. felis*, *Pulex irritans*, the chewing lice *Trichodectes canis*, *Felicola subrostratus*; acaricidal drug against the Sarcopitiformes mite *Otodectes cynotis*, the Trombidiformes mite *Cheyletiella yasguri*; and nematocidal drug against the *Strongyloides* nematode of the digestive-canal *Uncinaria stenocephala* in dogs and cats. The field trials demonstrated that the drug is easy-to-use, and the doses recommended by manufacturer were well received by the animals, independent of breed, age, sex, body mass, and caused no toxic effect on the animals, and provided a broad range of treatment against ectoparasitic and endoparasitic invasions of dogs and cats.

The authors declare that there is no conflict of interest.

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