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ORIGINAL ARTICLE

Efficacy of flea and tick collars against the ectoparasites of domestic animals

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One of the most common parasitic diseases of domestic animals is their lesions by bloodsuckers, including fleas, lice, ticks. According to the study's results, the ectoparasite infestation of stray dogs (n=28) and cats (n=22) is mono - and a mix of invasions. The highest intensity of flea invasions in dogs and cats was from 2.5±2.0 to 8.5±2.0 and from 1.5±1.5 to 5.5±2.0 individuals per 10 cm² of animal skin area, respectively. Regardless of the year's season, it is necessary to pay special attention to the control and prevention of ectoparasitosis in animals. The purpose of our research was to determine the effectiveness of the use of flea and tick collars for pets with the active ingredient fipronil. Collars containing fipronil are well tolerated by pets and do not cause any side effects or changes in their clinical condition. Clinical studies have established a high activity of the investigated tick and flea collars against fleas, lice, lice, and parasitomorphic ticks. The effectiveness of flea and tick collars in production trials for dogs and cats' defeat with ectoparasites is 100%.

Keywords: fleas, lice, ticks, dogs, cats, collar, fipronil, invasion intensity.

Introduction

Despite the achieved success in the prevention and control of animal parasitic diseases, the issue of their eradication through the use of effective antiparasitic agents of various origins remains relevant today (Geary & Thompson, 2003; Shkromada et al., 2019; Paliy et al., 2020b). Climate change and the uncontrolled use of chemical compounds significantly affected parasitic species' biodiversity and natural habitat (Winter et al., 2018; Paliy et al., 2019; Bogach et al., 2020).

The use of innovative means to combat animal ectoparasites is of particular and important importance (Swiger & Payne, 2017; Paliy et al., 2018b; Pérez de Leónet al., 2020). To date, pharmacists have proposed many antiparasitic agents, which differ in both the content of active substances and the method of application (Liu & Weller, 1996; Woods et al., 2011; Paliy et al., 2020d). It is proposed to use their natural enemies to fight against parasitic Diptera (El-Husseini et al., 2018; Paliy et al., 2018d; 2020a). Ectoparasites such as fleas, lice, and ticks often parasitize domestic animals, and their prevalence is influenced by many factors, particularly climate and geography (Abarca et al., 2016). Ectoparasite infestation in dogs was 42.4%, and in cats, it was 31.3% (Colella et al., 2020).

The most common ectoparasites of dogs are fleas (Siphonapteridae, Ctenocephalus canis) (Cadiergues et al., 2000). They are ubiquitous and can be mechanical and biological carriers of pathogens of many dangerous diseases (Bitam et al., 2010; Abdullah et al., 2019; Ng-Nguyen et al., 2020).

Along with this, they are intermediate hosts of the cestodes *Dipylidium caninum* and the filariae of dogs *Dipetalonema reconditum*. Flea bites are painful, cause itching, inflammation of the skin, which determines animals' emaciation (Abdulkareem et al., 2019). In young animals, progressive exhaustion and anemia are observed. Puppies, in case of high intensity of infestation, can die. Dogs affected by fleas gnaw back, belly, tail. The skin is covered with ulcers; the hair falls out. Simultaneously, the animals are exhausted, an unpleasant odor spreads from the skin (Scaramozzino et al., 2018). Effective flea control requires an understanding of their biology, which affects which strategies will and will not be effective against them (Bitam et al., 2010; Bourne et al., 2018).

Pediculosis is a contagious disease caused by the species-specific blood lice *Linognathus setosus* (dogs) and *Felicola subrostratus* (cats). They are most often found in young, poorly fed, unkempt dogs and cats. Symptoms usually include anxiety and itching, with secondary seborrhea, baldness, excoriation. Tousled hair, small papules, and crusts may be present, and in severe disease, anemia, and general weakness (Kumsa & Mekonnen, 2011). The dog hair beetle (*Trichodectes canis*) is a permanent ectoparasite of dogs; it parasitizes on animals' heads and necks. It is also an intermediate host of the cucurbitaceous tapeworm (*Taenia cucumerina*) and often transmits these worms to dogs and even humans, especially children (Martins et al., 2013). Dangerous ectoparasites of animals are ticks that feed exclusively on host animals' blood and can spread several pathogenic microorganisms (Walker, 2017; Paliy et al., 2018b; Saleh et al., 2019).

A successful fight against domestic animals' invasive diseases is possible only with the availability of highly effective veterinary drugs (Pollmeier et al., 2002). Providing pet owners with the necessary assortment of effective and inexpensive ectoparasite control agents in easy-to-use forms is the path to well-being regarding these diseases. This can be achieved only through the development and production of highly effective competitive domestic drugs or the improvement of already known veterinary drugs (Kovalenko et al., 2020; Paliy et al., 2021). Drugs to combat ectoparasites belong to different classes of compounds and, as a rule, are effective against a narrow range of pathogens, which prompts animal owners to use a variety of drugs for treatment and prevention, which are far from impeccable in their effect on the animal's body and are environmentally hazardous. Therefore, specialists have always been interested in the possibility of creating and using drugs with a broad spectrum of action (Pink et al., 2005), as well as complex disinfectants (Stegniy et al., 2019; Paliy et al., 2020c; 2020e).

In recent years, manufacturers have proposed several agents that are used for therapeutic and prophylactic purposes in case of infections with ectoparasites in dogs and cats in the form of collars (Stanneck et al., 2012; Otranto et al., 2017; Rust, 2020). Its spectrum of action can judge the effectiveness of a drug. Besides, the drug's effectiveness is characterized by an improvement in the clinical state of sick animals, the speed of their recovery, the animal's tolerance to the drug, and the manifestation of side effects (Beugnet & Franc, 2010).

Materials and methods

In the course of experimental studies, the effectiveness of the action of flea and tick collars for pets was determined:

No. 1 – polyvinyl chloride (PVC) tape (1.0 g contains 40.0 mg of fipronil) 35±5 cm long (for cats) and 70±5 cm (for dogs);

No. 2 – polymer tape 65±5 cm long, contains 1.0 g of fipronil (for dogs);

No. 3 – polymeric tape 35±5 cm long, contains 0.5 g of fipronil (for cats).

Fipronil is widely used in a general antiparasitic complex, the effectiveness of which has been proven over ten years of use (Beugnet & Franc, 2010). The collars' flea and tick action under study was determined on dogs and cats affected by ectoparasites, which were kept in animal shelters: dogs (n=22), cats (n=14) of different breeds, and ages with different body weights. The study also involved dogs (n=6) and cats (n=8), which move freely in the city; the animals are sterilized and chipped. Clinical studies of the therapeutic efficacy of collars were carried out in the Laboratory of Veterinary Sanitation and Parasitology of the National Scientific Center "Institute of Experimental and Clinical Veterinary Medicine" (Kharkiv) and also based on an animal shelter (Kharkiv region).

Experimental studies were carried out according to the scheme:

1) Clinical examination of animals in the shelter, establishing the initial diagnosis, sampling of ectoparasites from the skin of animals and bedding for laboratory research, and constant clinical observation of experimental animals' physiological states.

2) Microscopic examination of samples to determine the type of pathogens of ectoparasitic diseases in biological material, their identification, the establishment of the extent of invasion in dogs and cats. 3) The use of collars individually in a group of animals in a shelter, sampling for laboratory studies after 5, 10, 30 days during the use of collars, determines the extensibility of its action. 4) Daily clinical examination of the health of experimental animals throughout the experiment.

During the research, laboratory utensils, a microscope, a refrigerator, Petri dishes, subject and cover glasses, reagents for microscopic studies (glycerin), arthropods from the animals studied (dogs, cats) were used. Following the tasks, the studies were carried out by visual and microscopic methods (Yuskiv, 1998; Galat et al., 2009; Fourie et al., 2013). For the experiment, adult dogs and cats of both sexes and different breeds were selected, which had not been treated with insecticidal acaricides at least three months before the start of the experiment. When setting up the experiment, all animals were examined for the presence of pathogens of ectoparasitic diseases. The microscopic method identified the causative agents of animal diseases (Mathison & Pritt, 2014). The intensity of invasion was determined by counting ectoparasites on an area of 10 cm^2 of an animal's skin. For each series of experiments, an appropriate number of animals (dogs, cats) were selected from which research groups were formed following general rules on the principle of analogs. The animals were numbered and housed in the enclosure. Before and during the use of the collars, a clinical examination of the animals was carried out. The collars were used individually for the test and control animals. After 5, 10, 20, and 30 days of using the collars, the research results were recorded based on the condition of the treated animals, the count of live ectoparasites on them, as well as the degree of invasion after treatment, and the extensibility of the collars was determined. To collect ectoparasites from the skin, the animals were fixed in a supine position. The review of the skin of the animals began with the head. The neck, back, flanks, abdomen, and limbs were then examined. The hair was parted and combed during the examination. The survey was first carried out with the naked eye and then with a magnifying glass. The identified ectoparasites were removed from the skin of animals using tweezers. The removed ectoparasites were placed in glassware filled with Barbagallo liquid (3% formalin aqueous solution in physiological saline) or 70% ethanol. Some of the ectoparasites were delivered alive to the laboratory in test tubes with wet filter paper inside. The strips of filter paper were moistened with boiled water. The tubes and jars were covered with a layer of cloth and tied. Each tube and jar was labeled.

The study of dust and debris from the enclosures with the treated animals was carried out by the flotation method. A sample weighing 30 g was placed in a cylinder or conical flask, filled with water, and thoroughly mixed. The particles that floated to the surface were removed, and the water was carefully drained, leaving sediment. The precipitate was mixed with 20 parts of a saturated sodium chloride solution and settled for 20 minutes. A sample (three drops) was then removed from the flotation film's surface with a bacteriological loop, placed on a glass slide, covered with a cover glass, and examined under a microscope. The extent of invasion (EI) was determined as the number of infected animals' ratio to the number of examined animals, expressed as a percentage. The extent of invasion was determined by the formula:

$$EI = (X/Y) \times 100$$

where: X – the number of animals in which ectoparasites were found;

Y – the total number of animals.

The intensity of invasion (II) was determined by the number of ectoparasites per 10 cm^2 of animal skin. The extensibility (EE) of the collar's action was calculated by the number of treated animals as an utterly free percentage of parasites. Experiments on animals were carried out according to current bioethical requirements (Festing & Wilkinson, 2007; Kabene & Baadel, 2019).

Results and discussion

As a result of a clinical examination of sick dogs admitted to the shelter, redness, inflammation of the skin were found, a well-visible itching reflex on the skin was noted for the presence of papules and scales. Bald spots were noticeable; the skin was rough and cracked. General emaciation of the animals was noted. Ectoparasites were collected from animal skins (Table 1).

Table 1. Determination of the extensiveness and intensity of parasite invasions (qualitative and quantitative composition) in dogs (n=22)

Arthropod	Extensiveness, %	Intensity on 10 cm^2
Ctenocephalus canis	63.6	8.5±2.0
Linognathus setosus	18.2	1.5±1.5
Trichodectes canis	36.4	2.5±1.5

By visual examination, 63.6% of the animals showed persistent infestation by fleas, eight dogs were infested with lice, and four dogs were diagnosed with mixed infestations with lice and chewing lice. Simultaneously, the intensity of infestation in dogs by fleas was 8.5±2.0 individuals per 10 cm^2 of animal skin area, by lice – 1.5±1.5 individuals per 10 cm^2 of animal skin area, chewing lice infestation was 2.5±1.5 individuals per 10 cm^2 of animal skin area.

Flea and tick collars were stuck to old groups of twins (Fig 1, Table 2).

a b

Fig. 1. Use of flea and tick collars in dogs. **a**) collar No. 1; **b**) collar No. 2.

Table 2. Determination of the effectiveness of the insecticidal action of collars on dogs

Number of animals	Initially		After processing							
	EI, %	II, average	Five days		Ten days		15 days		30 days	
			EI, %	II	EI, %	II	EI, %	II	EI, %	II
investigated collar No. 1										
n=11	100	5.8	18.2	2.5	0	0	0	0	0	0
investigated collar No. 2										
n=11	100	5.8	27.3	2.8	0	0	0	0	0	0

From 2 to 7 days after collars, dead fleas and lice were detected on the treated animals. On day 10, fleas and lice were absent on the body of the animals. When examining the 30th day after collars application, fleas and lice were not found on the animals' bodies. The investigated flea and tick collars' extensibility during production tests on dogs infected with fleas, lice, and chewing lice was 100%. During the treatment and clinical observation of experimental and control animals, no complications or changes in the clinical state were observed after the use of collars.

As a result of a clinical examination of sick cats placed in a shelter, it was found redness, inflammation of the skin, a visible itching reflex on the skin, there are papules, scales, noticeable bald spots. The general depletion of the animals was noted. Ectoparasites were collected from animal skin (Table 3).

Table 3. Determination of the extent and intensity of parasite infestation (qualitative and quantitative composition) in cats (n = 14)

Arthropod	Extensiveness, %	Intensity on 10 cm^2
Ctenocephalides felis	85.7	5.5±2.0
Felicola subrostratus	28,5	2.5±1.5

The visual study found a stable invasive cat with fleas (85.7%), two animals with mixed invasion fleas and chewing lice, and two cats with chewing lice. The invasion rate in flea cats was 5.5±2.0 individuals per 10 cm^2 of the animal's skin area; chewing lice was 2.5±1.5 individuals per 10 cm^2 of the animal's skin area.

Animal research groups have used individual flea and ticky collars (Fig 2, Table 4).

a b

Fig. 2. Use of flea and tick collars in cats. **a**) collar No. 1; **b**) collar No. 3.

Table 4. Determination of the effectiveness of the insecticidal effect of collars on cats

Number of animals	Initially		After processing							
	EI, %	II, average	5 day		10 day		20 day		30 day	
			EI, %	II	EI, %	II	EI, %	II	EI, %	II
investigated collar No. 1										
n=7	100	4.5	14.3	1.5	0	0	0	0	0	0
investigated collar No. 3										
n=7	100	4.75	28.6	2.0	0	0	0	0	0	0

From 2 to 5 days after applying the collars, dead fleas, lice and lice were detected on the treated animals. On the 10th day, no fleas and lice were found on the animals' bodies. On the 30th day, fleas and lice were also not found on the body of the animals. Extensibility (EE) of research drugs in production trials for the defeat of cats by fleas and lice was 100%. During treatment in two Siamese animals after donning the collars, both the study and the control group noted short-term salivation. From the second day and during the experimental and control animals' clinical observation, no complications or changes in the clinical state were observed after the use of the drugs.

We examined the litter samples from aviaries with the treated animals for the presence of larvae and imago ectoparasites (Table 5).

Table 5. Results of the study of litter samples from enclosures for the presence of larvae and imago of animal ectoparasites

Research method	The number of larvae and imago of ectoparasites in the litter at the place of detention	
	Initially	After processing on a 10 day
Flotation method	dogs	
	14.5±1.5	14.5±1.5
	cats	
	12.0±1.5	12.0±1.5

As shown in Table 5, after the animals' treatment on the 10th day, no larvae and imago of animal ectoparasites were found in the litter. As a result of a clinical examination of dogs (6) and cats (8), which move freely in the city, a well-noticeable itching reflex was revealed and the presence of scales on the skin. The animals were moderately well-fed. Ectoparasites were collected from animal skin (Table 6).

Table 6. Determination of the extensiveness and intensity of parasite infestation (qualitative and quantitative composition) in dogs (n=6) and cats (n=8)

Animal species	Arthropod	Extensiveness infestation, %	Intensity infestation on 10 cm^2
Dogs	*Ctenocephalus canis*	100.0	2.5±2.0
	Ixodes ricinus	33.3	0.7±0.3
	Dermacentor reticulatus	16.7	1.7±0.3
	Rhipicephalus sanguineus	16.7	1.7±0.3
Cats	*Ctenocephalides felis*	100.0	1.5±1.5
	Ixodes ricinus	25.0	1.5±1.5
	Haemaphysalis eliptica	25.0	1.5±1.5

Visual examination revealed persistent infestation of dogs and cats with fleas (100%). Three dogs and four cats were found to have parasitomorphic ticks. The intensity of flea infestation in dogs was 2.5±2.0 individuals per 10 cm^2 of animal skin area, and in cats – 1.5±1.5 individuals per 10 cm^2 of animal skin area. The experimental and control groups of animals have been put on individual collars (Table 7). The animals were observed for 30 days during feeding. No changes in the behavior of the animals were observed.

Table 7. Determination of the effectiveness of antiparasitic collars on dogs and cats

Animal species, number	Initially		After processing							
	EI, %	II, average	5 day		10 day		20 day		30 day	
			EI, %	II	EI, %	II	EI, %	II	EI, %	II
			investigated collar No. 1							
Dogs (n=3)	100	4.5	33.3	1.5	0	0	0	0	0	0
			investigated collar No. 2							
Dogs (n=3)	100	4.75	33.3	2.0	0	0	0	0	0	0
			investigated collar No. 1							
Cats (n=4)	100	4.6	25.0	1.5	0	0	0	0	0	0
			investigated collar No. 3							
Cats (n=4)	100	4.8	25.0	2.0	0	0	0	0	0	0

From 2 to 5 days after putting on the collars, dead fleas were found on the treated animals. From 10 to 30 days, no fleas were found on the body of the animals. Along with this, ticks were not detected on animals for 30 days. The extensibility (EE) of the action of antiparasitic collars in production tests when dogs and cats are affected by fleas and ixodid ticks was 100%. The active fight against animal ectoparasites is because they can be infectious carriers (Narladkar, 2018; Hartman et al., 2019) and parasitic (Paliy et al., 2018c; Ng-Nguyen et al., 2020) animal diseases and people and are common in many biotopes. The defeat of dogs and cats by ectoparasites is an urgent problem worldwide, especially stray ones (Thamer & Faraj, 2019). It has been proven that dogs and cats in 99.5% and 91.5% of cases, respectively, are infected with one or more species of ticks, fleas, or lice. Moreover, the overall prevalence of ectoparasites is higher among dogs than among cats. *Ctenocephalides feli*s (67.0-82.9%), *Ctenocephalides canis* (18.0-73.8%), *Heterodoxus spiniger* (4.0%), nymphs *Amblyomma* spp. were isolated from the affected animals. (3.5%), *Pulex irritans* (2.5-6.0%) and *Haemaphysalis leachi* (0.5%). Fleas and lice were observed more often among females than males. The study found that fleas' prevalence, ticks, and lice on dogs were not significantly different between male and female animals or between young and adult dogs. However, these ectoparasites' prevalence was significantly higher in females than in males and adults than in young cats (Kumsa & Mekonnen, 2011).

In a survey of 921 dogs, other researchers identified four flea species (*Ctenocephalides canis, Ctenocephalides felis, Pulex irritans, Echidnophaga gallinacea*) and three species of ticks (*Rhipicephalus sanguineus, Amblyomma tigrinum, Amblyomma triste*). In general, a high prevalence of fleas in dogs was observed in rural areas (Abarca et al., 2016). The study showed that the prevalence of fleas, ticks, and lice in Nigeria, when 333 dogs were examined for the presence of ectoparasites, two hundred and seventy-one animals (81.4%) were infected with one type of ectoparasites, and multiple infestations were recorded mainly among female dogs. Six species of ectoparasites of three taxa were identified: ticks (*Rhipicephalus sanguineus, Haemaphysalis leachii, Amblyomma variegatum*), fleas (*Ctenocephalides canis, C. felis*), lice (*Heterodoxus spiniger*). The intensity of invasion was significantly higher in female and younger dogs ($p < 0.001$) (Abdulkareem et al., 2019). The most common parasitism (59%) is *C. felis* and *P. simulans* (Troyo et al., 2012). The most common clinical symptoms of ectoparasitosis are scratches (13.8%), allergic dermatitis (13.2%), and alopecia (7.9%). Most often, ectoparasites are found on the necks of animals – 31.6% and animals with dark hair have a higher intensity of damage than individuals with light hair (Bahrami et al., 2012). In animals under one year of age, the lesion's intensity is more potent than in other age groups (66.7%) (Chee et al., 2008). There is now a wide range of drugs that can be used to control fleas both in the environment and directly on animals, including sprays and point depots, collars, oral pills, and injections. When used correctly, they are generally safe and highly effective (Bourne et al., 2018). Tick control is mainly based on the use of common acaricides and macrocyclic lactones. However, intensive use of such compounds has led to mites populations that show resistance to all major chemical classes of acaricides. So, there is a need to develop alternative approaches that include innovative livestock practices, rotation of acaricides, and improved composition (Abbas et al., 2014; Rodriguez-Vivas et al., 2018). Along with disinfectants (Paliy et al., 2018a), it is necessary to rationally use chemical acaricide agents, taking into account the specific epizootic situation (Henrioud, 2011). Fipronil is a common agent in the fight against animal ectoparasites. It has been proven to be effective against fleas and lice in cats (Gracia et al., 2017) and dogs (Pollmeier et al., 2002). The combination of fipronil and (S) -methoprene has a prolonged flea and tick action for four weeks (Young et al., 2004; Dryden et al., 2013; Nambi et al., 2016). Combining fipronil with other chemical derivatives is an effective agent (Horak et al., 2012; Navarro et al., 2015). A single topical application of a combination of fipronil and permethrin to the skin provides acaricidal efficacy against *I. ricinus* and *R. sanguineus* for four weeks (Dumont et al., 2015). Thus, the use of fipronil as an active ingredient in flea and tick agents has been confirmed by our studies and other experiments.

Conclusions

We have found a persistent infection of stray dogs and cats with ectoparasites. In this case, both mono and mixed invasion of animals by fleas, lice, and Mallophaga are diagnosed. The intensity of invasion in dogs per 10 cm² of the animal's skin area by fleas was from 2.5±2.0 to 8.5±2.0, by lice – 1.5±1.5, by chewing lice (were formerly classified as Mallophaga) – 2.5±1.5 individuals. Based on the studies carried out on flea and tick collars with the active ingredient fipronil, it was established that they are well tolerated by dogs and cats and do not give any side effects and changes in the clinical state of animals. Clinical studies have established flea and tick collars' high activity against fleas, lice, chewing lice, and parasitomorphic ticks.

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