

## POTENTIATION OF ACARICIDAL DRUGS WITH THE HELP OF A PHYTOCOMPLEX THAT UNDERGOES CRYODESTRUCTION

Kiptenko A. V., Dunaiev Yu. K., Paliy A. P., Bogach M. V., Keleberda M. I.

National Scientific Center 'Institute of Experimental and Clinical Veterinary Medicine', Kharkiv, Ukraine, e-mail: [bogach\\_nv@ukr.net](mailto:bogach_nv@ukr.net)

**Summary.** The objective of this study was to develop a novel natural veterinary pharmaceutical agent for the treatment of tick and mite infections, with the aim of experimentally confirming its efficacy *in vivo*. The dogs selected for the experiment were divided into three groups (n = 27) according to the type of tick infection, with each group divided into three subgroups (n = 9). The first experimental group of dogs was affected by ixodid ticks, the second by sarcoptic mites, and the third by thrombidiform mites. The groups were then subdivided into three smaller groups. Group I received treatment with 'AnimAll VetLine' antiparasitic tablets for dogs and cats, Group II received treatment with 'Acaro Spectra' antiparasitic tablets for dogs, and Group III served as the control group, whose condition was monitored without treatment. The antiparasitic tablets, designated 'Acaro Spectra', demonstrated efficacy within 24 hours. The test results indicated that the ticks were removed within the same timeframe, and no new ticks attached. Additionally, the administration of 'Acaro Spectra' did not elicit any allergic reactions or signs of distress in the animals. In the treatment of dogs affected by ixodid ticks, sarcoptic, and thrombidiform mites, the drug 'Acaro Spectra' antiparasitic tablets for dogs showed 100% effectiveness. The results obtained allow us to recommend the drug 'Acaro Spectra' antiparasitic tablets for dogs for the treatment and prevention of ixodid ticks (*Ixodes ricinus*, *Rhipicephalus sanguineus*, *Dermacentor reticulatus*), sarcoptic mites (*Otodectes cynotis*, *Notoedres cati*, *Sarcoptes canis*), thrombidiform mites (*Demodex* spp., *Cheyletiella* spp.)

**Keywords:** ixodid ticks, sarcoptic mites, thrombidiform mites, treatment

**Introduction.** External and internal parasites can cause serious diseases in domestic animals, which is a concern for their owners. Antiparasitic treatment is challenging due to the large number of available products and the limited understanding of parasitocides among dog owners (Bebrysz et al., 2021; Paliy et al., 2021). Some owners do not protect their dogs against all parasites, while others use effective doses less frequently than recommended (Boost et al., 2017; Lavan et al., 2018). The most common types of ticks and mites that parasitize dogs and cats are ixodid ticks (*Ixodes ricinus*, *Rhipicephalus sanguineus*, *Dermacentor reticulatus*), sarcoptic mites (*Otodectes cynotis*, *Notoedres cati*, *Sarcoptes canis*), thrombidiform mites (*Demodex* spp., *Cheyletiella* spp.) (Kruchynenko, 2020).

Ticks and mites can transmit various viruses, bacteria or parasites that can cause serious infections or diseases in humans and animals. Tick-borne diseases are becoming a growing and serious problem in Europe and worldwide (Beck et al., 2006; Heyman et al., 2010).

*Rhipicephalus sanguineus*, the brown dog tick, is adapted to colonize both human and dog habitats and can be found in areas near human habitation (Aguilar-Meraz et al., 2024).

Several species of fleas and ticks are established in different bioclimatic zones (Estrada-Pena et al., 2017). Protecting domestic animals from the adverse effects of ectoparasites, including ectoparasite-induced blood loss, skin diseases, and vector-borne pathogens, may require long-term protection against ticks and fleas (Lavan et al., 2018). Ectoparasites present a persistent challenge to veterinarians and pet owners throughout the year (Paliy et al., 2023; Tishyn, Yuskiv and Yuskiv, 2024).

The successful control of ectoparasites in dogs and cats is possible with the availability of highly effective antiparasitic drugs (Matos et al., 2015; Paliy et al., 2024; Giannelli et al., 2024).

The advent of new technologies has facilitated the development of a superior antiparasitic drug that is more natural and efficacious. There are ongoing endeavors to create environmentally benign and cost-effective products. It is imperative to control the damage caused to the skin by infection and to minimize the re-infection by pathogens, such as ticks and fleas.

**The objective** of this study is to develop a novel natural veterinary drug for the treatment of tick and mite infections. The aim is to establish and subsequently confirm *in vivo* acaricidal efficacy of the drug.

**Materials and methods.** To develop a new antiparasitic drug with the addition of a phytocomplex, we studied the composition of 'AnimAll VetLine' and 'Quadro Tab' antiparasitic tablets for dogs and cats.

'AnimAll VetLine' was selected for potentiation based on its superior efficacy indicators. The drug's composition is as follows: 100 mg of the drug contains the active substances imidacloprid (2.4 mg), lufenuron (20.0 mg), and milbemycin oxime (0.6 mg). The excipients are lactose, starch, artificial flavor, aerosil, and calcium stearate.

To create our drug 'Acaro Spectra' antiparasitic tablets for dogs with the addition of a phytocomplex, we used the same composition of chemicals and excipients as in 'AnimAll VetLine'. After studying the different types of plant action, we chose the following composition of the phytocomplex for cryodestruction and subsequent addition to the drug (wt. %): laminaria (*Laminaria*) —

1.5–2.4%; neem leaves (*Azadirachta indica*) — 2.8–3.2%; garlic (*Allium sativum*) — 0.9–1.8%; lemon balm (*Melissa officinalis*) — 1.4–2.6%, which work together to repel common parasites, helping to protect the animal from ticks and fleas (Schmahl et al., 2010; Bharadwaj, Hayes and Stafford, 2015).

The selected phytocomplex has anti-allergic properties, provides soothing relief from itching, and is rapidly absorbed by the animal body.

The equipment utilized in the cryodestruction of plant raw materials plays a pivotal role in the process. In this instance, a cryogenic vibratory chopper (CVC-3) is employed, which provides continuous cooling with liquid nitrogen through a built-in cooling system both prior to and during grinding (<https://www.hd-grinder.com/info/advantages-of-cryogenic-grinders-in-processing-95091427.html>).

One of the active substances of ‘Acaro Spectra’, imidacloprid, is subjected to cryo-grinding in conjunction with the phytocomplex.

The phytocomplex, along with imidacloprid, a component of the veterinary drug, is manufactured using cryo-grinding technology (cryodestruction). This process offers a significant advantage in that the biologically active compounds in plants are not subjected to harsh processing and are preserved in their natural forms and proportions. This technology enables the concentration of the primary attributes of plant materials in a limited volume, and it is currently regarded as the most effective of all existing technologies.

The use of cryotechnology on plant raw materials in conjunction with the active ingredient imidacloprid results in the production of fine and ultrafine powders with a considerable specific surface area. This has an impact on the rate of biochemical reactions within the animal’s body. Additionally, the drug comprises a micro-structured combination of plant fibers and an insecticide belonging to the neonicotinoid class. This unique formulation confers the drug with efficacious ectoparasitocidal, insecticidal, and repellent properties, which collectively repel common parasites and protect the animal from ticks, particularly the ixodid ticks (*Dermacentor* spp., *Rhipicephalus* spp., *Ixodes* spp.), sarcoptic mites (*Otodectes cynotis*, *Notoedres cati*, *Sarcoptes canis*), thrombidiform mites (*Demodex* spp., *Cheyletiella* spp.).

Thus, we have developed a tick control tablet for dogs that contains the following active ingredients imidacloprid (a neonicotinoid belonging to the group of chloronicotinyl compounds), lufenuron (a compound of the benzoylphenylurea group), milbemycin oxime (a second-generation macrocyclic lactone of milbemycins, a compound of biosynthetic products of the species *Streptomyces hygroscopicus* subsp. *aureolacrimosus*), and a phytocomplex, which is included as an excipient. The phytocomplex contains a mixture of fully active bioavailable ingredients, also as excipients: lactose, starch, artificial flavor, aerosil, calcium stearate, etc.

To ascertain the efficacy of the pharmaceutical agent, a series of tests were conducted to substantiate or refute the purported beneficial impact of the pharmaceutical preparation designated as ‘Acaro Spectra’. It is our contention that the phytocomplex amplifies the efficacy of the drug. To substantiate this assertion, we conducted a series of tests and comparisons between ‘AnimAll VetLine’ tablets for dogs and cats and ‘Acaro Spectra’ antiparasitic tablets for dogs.

The study involved 81 canines aged between two months and seven years with a live weight from 2 kg to 32 kg. The animals were housed in standardised cages at an air temperature of  $24 \pm 1.5^\circ\text{C}$ , 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 Municipal Enterprise ‘Animal Treatment Center’.

The experimental animals were selected based on a clinical evaluation of their natural infection status and the presence of dermatological lesions, including dermatitis, alopecia, and the overall condition of the skin. A total of 81 canines presenting with dermatological abnormalities were selected. The study population consisted of 76 adult dogs and five puppies. The animals were crossbreeds (Labrador, German Shepherd, Australian Shepherd, Pomeranian Spitz, etc.) and outbreeds (OB). The objective was to test the effectiveness of the developed drug, ‘Acaro Spectra’ antiparasitic tablets for dogs, and to compare its effect with that of the drug ‘AnimAll VetLine’ antiparasitic tablets for dogs and cats.

The clinical examination of the affected animals revealed the presence of ixodid ticks (*Ixodes* spp., *Dermacentor* spp., *Rhipicephalus* spp.), sarcoptic mites (*Otodectes cynotis*, *Notoedres cati*, *Sarcoptes canis*), and thrombidiform mites (*Demodex* spp., *Cheyletiella* spp.). The presence of flea allergic dermatitis was documented, and the examination revealed the presence of *Ctenocephalides* sp. fleas. Among the naturally affected experimental animals, a mixed course of infection was observed.

The canines selected for the experiment were divided into three groups ( $n = 27$ ) according to the type of tick infection, with each group further divided into three subgroups ( $n = 9$ ). The experimental canines were maintained under identical conditions. All data and animal condition were recorded in the observation log.

The first experimental group of dogs was infected with ixodid ticks, the second — with sarcoptic mites, and the third — with thrombidiform mites. Each group was divided into three subgroups: I — treatment with ‘AnimAll VetLine’ antiparasitic tablets for dogs and cats; II — treatment with ‘Acaro Spectra’ antiparasitic tablets for dogs; III — control group, to monitor the condition of animals without treatment.

The experimental animals were administered the antiparasitic tablets ‘AnimAll VetLine’ and ‘Acaro Spectra’ in oral tablet (*per os*) form. The tablets are palatable, as they also contain a flavoring, and were readily consumed by most animals. In some cases, the

animal did not take the tablet voluntarily; in these instances, it was administered with food or directly into the oral cavity. The study was conducted for 21 days.

**Results and discussion.** In accordance with the established protocols, parasitological examinations of canines were conducted through visual inspection and sampling for subsequent laboratory analysis. This was done in order to detect the presence of ectoparasites, in accordance with the standards set forth by the Good Clinical Practice (GCP) guidelines, as well as in alignment with the guidelines set forth by the European Union (EU) and the World Association for the Advancement of Veterinary Parasitology (WAAVP) for the evaluation of the efficacy of antiparasitic substances in the treatment and prevention of tick infection. The identification of ectoparasite pathogens was conducted through microscopic examination, in accordance with

the established practical guidelines (Jacobs et al., 1994; Geurden et al., 2022).

In the first group of dogs affected by ixodid ticks, a visual examination of the animals, brushing and collection of ticks were performed. Five ticks were left on each animal to observe the effect of the drugs.

On the first day, the dogs treated with ‘AnimAll VetLine’ antiparasitic tablets for dogs and cats and ‘Acaro Spectra’ antiparasitic tablets for dogs were given the drugs in the form of tablets according to the dosage by weight of the experimental animals. The control group did not receive the drugs.

On the second day, the dogs were subjected to a visual examination and brushing. Subsequently, the aforementioned procedures were repeated on days 7, 14, and 21. The results of the examinations are presented in Table 1.

**Table 1** — Effectiveness of treatment of dogs affected by ixodid ticks

Group	Number of ticks				
	1 <sup>st</sup> day	2 <sup>nd</sup> day	7 <sup>th</sup> day	14 <sup>th</sup> day	21 <sup>st</sup> day
‘AnimAll VetLine’ antiparasitic tablets for dogs and cats	45	1	0	0	0
‘Acaro Spectra’ antiparasitic tablets for dogs	45	0	0	0	0
Control group (no treatment)	45	45	42	55	48

The control group of dogs exhibited a range of symptoms, including weakness, increased sleeping, redness of the skin, allergic reactions, a poor appetite, and fever. The dogs in the experimental group, which were treated with ‘AnimAll VetLine’ antiparasitic tablets for dogs and cats and ‘Acaro Spectra’ antiparasitic tablets for dogs, exhibited a notable improvement in their condition. The administration of the pharmaceutical agents resulted in the crawling of ticks on the dogs’ fur, yet none of the ticks proceeded to bite.

Thus, the antiparasitic tablets ‘Acaro Spectra’ for dogs resulted in the death of all ticks on the second day, which were subsequently captured. The antiparasitic tablets ‘AnimAll VetLine’ for dogs and cats led to the death of 44 out of 45 ticks on the second day, with no fixed ticks observed on the seventh day. Notably, the ticks that were initially planted on the dogs did not become fixed, and there was no evidence of re-infection.

In the control group, we noticed that only those ticks that had already drunk blood were sucked out, and in a few days, they would bite the dogs again. The majority of

the ticks that had been implanted remained attached to the dogs and proceeded to bite them.

In the second group of dogs affected by sarcoptic mites, a visual examination of the animals was conducted on control days. Additionally, smears and scrapings were taken from the ears and skin, examined under a microscope, and the hair follicles were examined.

The animals exhibited a range of symptoms, including lethargy, an unwillingness to eat, and a constant scratching behavior. Additionally, they displayed partial hair loss, reddened skin, self-inflicted wounds, and a tendency to shake their heads.

To enhance the effect of the drug and to speed up healing and soothe the skin, remove itching and redness, the damaged areas were treated twice a day with an antibacterial spray with chlorhexidine and ketonazole (Vitomax, ‘Pet Skin Spray’). The auricles were treated and cleaned every day with ‘Auricap’ to remove mite vital secretions in the animal’s auricle as quickly as possible. The results of the examinations are presented in Table 2.

**Table 2** — Effectiveness of treatment of dogs affected by sarcoptic mites

Group	Intensity of skin infection in percentage, %				
	1 <sup>st</sup> day	2 <sup>nd</sup> day	7 <sup>th</sup> day	14 <sup>th</sup> day	21 <sup>st</sup> day
‘AnimAll VetLine’ antiparasitic tablets for dogs and cats	30	30	25	18	12
‘Acaro Spectra’ antiparasitic tablets for dogs	30	30	26	15	10
Control group (no treatment)	30	30	40	45	52

On the first day, when examining the scrapings under a microscope, a considerable number of mites was identified in all animals.

On the second day, a notable reduction in the number of mites was evident in the groups of animals treated with ‘AnimAll VetLine’ antiparasitic tablets for

dogs and cats and ‘Acaro Spectra’ antiparasitic tablets for dogs. By the seventh day, no mites were observed under the microscope.

The animals treated with ‘AnimAll VetLine’ antiparasitic tablets for dogs and cats and ‘Acaro Spectra’ antiparasitic tablets for dogs exhibited notable improvements in health. The animals displayed enhanced physical condition, with wool growth on exposed skin, absence of skin redness and itching, and favorable changes in appetite and mood. Additionally, the dogs exhibited increased activity levels and a cessation of ear shaking.

In contrast, the control group exhibited a decline in the animals’ condition, accompanied by a notable intensification of the aforementioned symptoms, and three animals displayed a complete refusal to eat.

In the third group of dogs affected by sarcoptic mites on the control days, a comprehensive examination was conducted, including a visual assessment and skin scrapings, which were subsequently examined under a microscope. The clinical manifestations of sarcoptic mite infection are highly analogous to those observed in thrombidiform mite infection. Consequently, the examination and treatment are nearly identical.

Dogs display a consistent tendency to scratch, resulting in partial hair loss, cutaneous redness, and self-inflicted wounds caused by itching.

To optimize the efficacy of the medication and facilitate the healing process, the affected areas were treated twice daily with an antibacterial spray containing chlorhexidine and ketonazole (Vitomax, ‘Pet Skin Spray’). The findings of the examinations are presented in Table 3.

**Table 3** — Effectiveness of treatment of dogs affected by thrombidiform mites

Group	Intensity of skin infection in percentage, %				
	1 <sup>st</sup> day	2 <sup>nd</sup> day	7 <sup>th</sup> day	14 <sup>th</sup> day	21 <sup>st</sup> day
‘AnimAll VetLine’ antiparasitic tablets for dogs and cats	45	45	40	30	10
‘Acaro Spectra’ antiparasitic tablets for dogs	45	45	41	28	8
Control group (no treatment)	44	44	56	60	68

On the initial examination of the scrapings under a microscope, a considerable number of mites were recorded. On the second day, the number of mites exhibited a notable decline in dogs treated with ‘AnimAll VetLine’ antiparasitic tablets for dogs and cats and ‘Acaro Spectra’ antiparasitic tablets for dogs. On the seventh day, no mites were observed under the microscope. Following treatment, the dogs exhibited improved physical condition, with improved skin appearance, hair growth on exposed skin, absence of redness, itching, and enhanced appetite.

The healing of the skin was observed to occur at a rate 2% faster when the dogs were administered the antiparasitic tablets ‘Acaro Spectra’ than when they were administered the antiparasitic tablets ‘AnimAll VetLine’ for dogs and cats.

In the control group, the condition of the dogs worsened. This was evidenced by an increase in the number of areas of skin devoid of hair, an increase in the frequency of scratching, a change in the color of the skin to a reddish hue, and the presence of wounds that were actively licked by animals.

A comparison was conducted between ‘AnimAll VetLine’ antiparasitic tablets for dogs and cats, which are similar in effect and chemical composition, and ‘Acaro Spectra’ antiparasitic tablets for dogs, which were developed by our team. The results demonstrated that both products exhibited 100% effectiveness against ixodid ticks starting from the second day. No reinfection with ticks was observed until the 21<sup>st</sup> day of observation. The weekly attachment rates are an important parameter for understanding the role of the drug in controlling

reinfection. All animals were successfully cured of the tick infection.

In the fight against ixodid ticks, thrombidiform mites and sarcoptic mites, both drugs proved to be effective, and the number of ticks decreased significantly on the second day. The results of skin healing were 2% faster after administration of ‘Acaro Spectra’ antiparasitic tablets for dogs.

‘AnimAll VetLine’ antiparasitic tablets for dogs and cats showed its effectiveness within 21 days against thrombidiform and sarcoptic mites.

The efficacy of ‘Acaro Spectra’ antiparasitic tablets for dogs was demonstrated within 21 days against thrombidiform and sarcoptic mites, and ixodid ticks. The healing of skin lesions and the occurrence of allergic reactions in animals were observed to be slightly faster in comparison to the antiparasitic tablets ‘AnimAll VetLine’ for dogs and cats. It is postulated that the drug ‘Acaro Spectra’ is straightforward to use, highly efficacious, and more effective than other pharmaceutical agents.

**Conclusions.** 1. The developed drug ‘Acaro Spectra’ antiparasitic tablets for dogs showed that the acaricidal effect is manifested after 24 hours. The test results proved that ticks fall off after 24 hours, and new ones do not attach.

2. The administration of ‘Acaro Spectra’ antiparasitic tablets for dogs has been observed to elicit no allergic reactions or signs of anxiety in the animal.

3. The drug ‘Acaro Spectra’ antiparasitic tablets for dogs has been demonstrated to be 100% effective in the treatment of dogs affected by ixodid ticks, sarcoptic mites, and thrombidiform mites.

4. The results obtained allow us to recommend the drug 'Acaro Spectra' antiparasitic tablets for dogs for the treatment and prevention of infection with ixodid ticks (*Ixodes ricinus*, *Rhipicephalus sanguineus*, *Dermacentor*

*reticulatus*), sarcoptic mites (*Otodectes cynotis*, *Notoedres cati*, *Sarcoptes canis*), thrombidiform mites (*Demodex* spp., *Cheyletiella* spp.).

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