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CONTENTS

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FOLIA VETERINARIA, 66, 1: 15—25, 2022

DEWORMING SCHEMES' EFFICACY FOR ADULT DOGS WITH MIXED GASTRO-INTESTINAL HELMINTHOSES

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ABSTRACT

Mixed gastrointestinal helminthoses, which combines *Toxocara canis, Trichuris (T.) vulpis, Toxascaris leonina, Uncinaria stenocephala, Ancylostoma caninum*, and *Dipylidium caninum* in various combinations, are very common pets' problems worldwide. It is unlikely necessary to choose between 100 % efficiency and for the patient's body to heal the infected animals safely. The present work aims to develop an affordable scheme for adult dogs' deworming, which will create a minimum load on the body due to the low drugs' toxicity. Mixed breed dogs, 1—5 years old, representing both sexes, spontaneously infected with *T. vulpis* (100 % prevalence) in combination with other gastrointestinal helminths (from 12.7 to 45.1 %) were selected for study. Regimens combining Caniquantel® Plus (fenbendazole + praziquantel) and fenbendazole with a 24-hour interval were tested. After a single treatment of experimental animals with Caniquantel® Plus, no helminth eggs were detected in their faeces after three days, except for *T. vulpis.* Seven days after the start of the experiment, the intensity of infection of this nematode decreased by only 22.0 % ($P < 0.001$). Bodies and fragments of dead helminths were found in faeces 1—4 days after deworming, with *T. vulpis* isolated only in 2 days in small quantities (4.54 ± 0.21 specimens per 100 g of faeces). Two-stage deworming with Caniquantel® Plus and fenbendazole after 24 hours resulted in 100 % efficiency against eggs of all parasites after five days. Helminths' bodies stopped excreting after four days, and *T. vulpis* was detected within three days in substantive quantities (from 10.03 ± 0.45 to 36.8 ± 1.2 specimens per 100 g of faeces).

Key words: canine; co-infection; fenbendazole; parasites; praziquantel; prevalence; *Trichuris vulpis*; *Toxocara canis*

INTRODUCTION

Mixed gastrointestinal helminthoses are one of the most common problems in pet veterinary medicine worldwide. However, such helminthoses are rare in the form of mono-infections. The main component of co-infections is usually *Toxocara (T.) canis* (Werner, 1782) in puppies or young dogs and *Trichuris (T.) vulpis* (Froelich, 1789) in adult dogs [10, 14, 31, 48]. Other members of helminthic parasitocenoses in dogs are usually *Toxascaris (T.) leonina* (Linstow, 1902), representatives of Ancylostomatidae family—*Uncinaria (U.) stenocephala* (Railliet, 1884) and *Ancylostoma (A.) caninum* (Ercolani, 1859), *Dipylidium (D.) caninum* (Linnaeus, 1758). According to the analysis, the worldwide prevalence of *Toxocara* infection in dogs is 11.1% (10.6—11.7%). The regional distribution of this nematode varies from 6.4% to 19.2%: Eastern Mediterranean (19.2%, 13.7—25.5%), Africa (18.5%, 13.7—23.9%), South-East Asia (11.9%, 6.8—18.2%), North America (11.1%, 10.6—11.7%), South America (10.9%, 7.6—14.6%), Europe (10.8%, 8.9—12.9%), and Western Pacific (6.4%, 3.3—10.2%).

Young animals under 12 months, stray, rural and male animals have a significantly ($P < 0.001$) higher prevalence of *T. canis* than older, pet, urban or female dogs [43]. According to a similar monitoring of *T. leonina* spread among dogs, the pooled prevalence is 7.2% (3.5—12.0%) in the Eastern Mediterranean region, 5.7% (1.4—12.2%) in South-East Asia, 3.6% (1.2—6.9%) in Africa, 2.6% (1.6—3.8%) in Europe, 2.0% (1.1—3.2%) in North America), 1.0% (0.1—3.4%) in the Western Pacific and 0.6% (0.1—2.1%) in South America [44].

Infestation of dogs with the nematode *T. vulpis*, according to various authors, is 15.1% in Bulgaria [19], 9.9% in Italy [49], 9.6% in Serbia [18], 3% in Palestine [34] from 2.43% [52] to 2.74% in the USA [33], 1.31% in Pakistan [22]. Thus, this indicator has the highest values in Europe.

Strongylatoses incidence in dogs is 31.6% in India [34], 31.4% in Australia [5], 30.23% in Kenya [32], 18.33% in Indonesia [39], 15—16.5% in Serbia [18], 8.3% in Brazil [2], 8% in Palestine [34], from 5.63% [52] to 8.23% in USA [33], and 3.94% in Pakistan [22]. Infestation with the tapeworm *D. caninum* is characteristic of 23% of dogs in Palestine [34] 21% in Ethiopia [15], 11.8% in Pakistan [22] 5.4% in Serbia [18], 1.67% in Indonesia [39].

The stability of geohelminths' eggs in the environment contributes to their spread among dogs. According to studies conducted in the Kharkiv region (Ukraine), the soil contamination level in urban areas of cities by exogenous stages of helminths is 5—55%, and in residential areas 20.0—23.3%. In general, in soil samples, the authors identified eggs of Strongylata, Ascaridata, *Trichocephalus*, and Cestoda. The researchers also point out that dogs and cats infected with helminths in the environment pollute from faeces with eggs of *Toxocara* spp. (75 ± 4 eggs.g^{-1}) and *D. caninum* (6 ± 1 eggs.g^{-1}) [36]. According to a survey in the Poltava region, in 31.18% of the dogs, fleas of the genus *Ctenocephalides* mostly parasitize in the form of associations with Nematoda (*T. canis, T. vulpis, U. stenocephala*), Cestoda (*D. caninum*), protozoa and other ectoparasites [56]. The prevalence of *T. vulpis* in the Kyiv region, according to previous studies, was high enough at 27.1% [47]. The trematode *Alaria (A.) alata* (Goeze, 1792) deserves special attention. Researchers say this helminth is widespread among domestic and wild carnivores: in Germany (25% of red foxes) [55], Serbia (28% of dogs) [29], Denmark (32.9% of raccoon dogs) [23] and many others in Europe. But, there is not any data about its prevalence in Ukraine.

Effective deworming is a necessary condition for maintaining the health of not only pets but also their owners [45]. Control of gastrointestinal tract helminths in dogs requires the use of anthelmintics containing various active substances. Thus, benzimidazole compounds [27, 52] or macrocyclic lactones [16, 20, 40] are usually used to release nematodes. Praziquantel is the most widely used substance to control trematodes and cestodes in dogs [24, 28]. Niclosamide is much less commonly used today [8]. Parasites' morphological and biological features require not only the correct choice of anthelmintics' active substance. The mode of its use has to be chosen correctly. Thus, *T. vulpis* feed periodically, and they are firmly fixed on the mucous membrane of the large intestine. Therefore, a single application of anthelmintics (benzimidazoles) does not give sufficient effectiveness [3, 41]. The use of long-acting drugs (macrocyclic lactones) allows holding a sufficient concentration in patients for the *T. vulpis* death. But, it's ineffective against trematodes and cestodes in mixed infections [1, 16, 25]. Excess doses or multiplicity of antiparasitic drugs can be justified, strictly dictated by the need [42]. Thus, it is grave to find a maximum effective treatment regimen that will not harm the patient [9, 51]. Fenbendazole is a low-toxic substance with a safety factor of 200 [7]. It is highly effective against nematodes. Although, it has low solubility and reaches low concentrations in the blood, which leads to low bioavailability [21].

The combination of fenbendazole and praziquantel is optimal for combating mixed-infection (trematodes, cestodes and most nematodes) in pets. It was weighty to research the fenbendazole+praziquantel (as Caniquantel® Plus) combination followed by fenbendazole (with 24-hour interval), taking into account the need to destroy *T. vulpis* in dogs.

Given the above, the present work aimed to develop an affordable deworming scheme for adult dogs. It was grave for us to create a minimum load on patients' bodies due to the low toxicity of the selected drugs. Such data will allow us to safely free adult dogs from all members of the combined helminth infection.

MATERIALS AND METHODS

Animal materials and study design

The research took place in 2020—2021 in private yards of villages of the Bila Tserkva district of the Kyiv region. Laboratory studies were conducted in the Laboratory of the Department of Parasitology and Pharmacology, Bila Tserkva National Agrarian University, Ukraine. To set up the experiment, two groups of adult dogs were formed (n = 35 and n = 36). Animals of both groups were 1—5 years old, 10—25 kg weight, mixed breeds and sexes. Animals of both groups were spontaneously infected with *T. vulpis* nematode or its combination with other helminths (*T. canis*, *T. leonina*, Ancylostomatidae family, *D. caninum*) in different variations (Fig. 1).

Parasitological study

The faeces examination method using the "Counting Chamber for Ovoscopic Researches" was used to make the initial diagnosis [48]. During the 7-day experiment, both ovoscopic (with a determination of the content of parasites' eggs or packets in 1 g of faeces) and helminthoscopic examinations (with a determination of the number of parasites' eggs or proglottids in 100 g of faeces) were performed daily. Faeces helminthoscopy was performed by sequential lavage [30, 37]. According to the results of helminthoscopy after deworming, infection of 9 from 71 experimental dogs with *A. alata* Trematoda was also established. A sorting of experimental animals between two experimental groups was carried out on the principle of analogues.

Caniquantel® Plus (Euracon Pharma GmbH, Germany) and Fenbendazole ultra 5% (O.L.KAR Animal Health, Ukraine) were used in the deworming scheme (Fig. 2). According to results of further parasitological studies of faeces' samples, the efficiency of animal treatment was established. Results indicate the frequency, which assumes that one day is 24 hours after the start of the previous stage, not the current day.

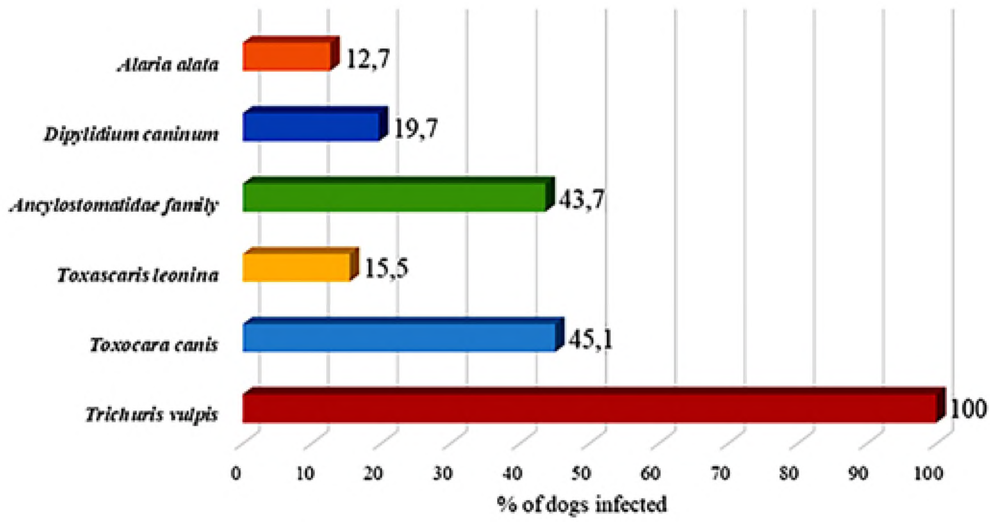

Fig. 1. Prevalence of helminths in dogs of experimental groups, n = 71

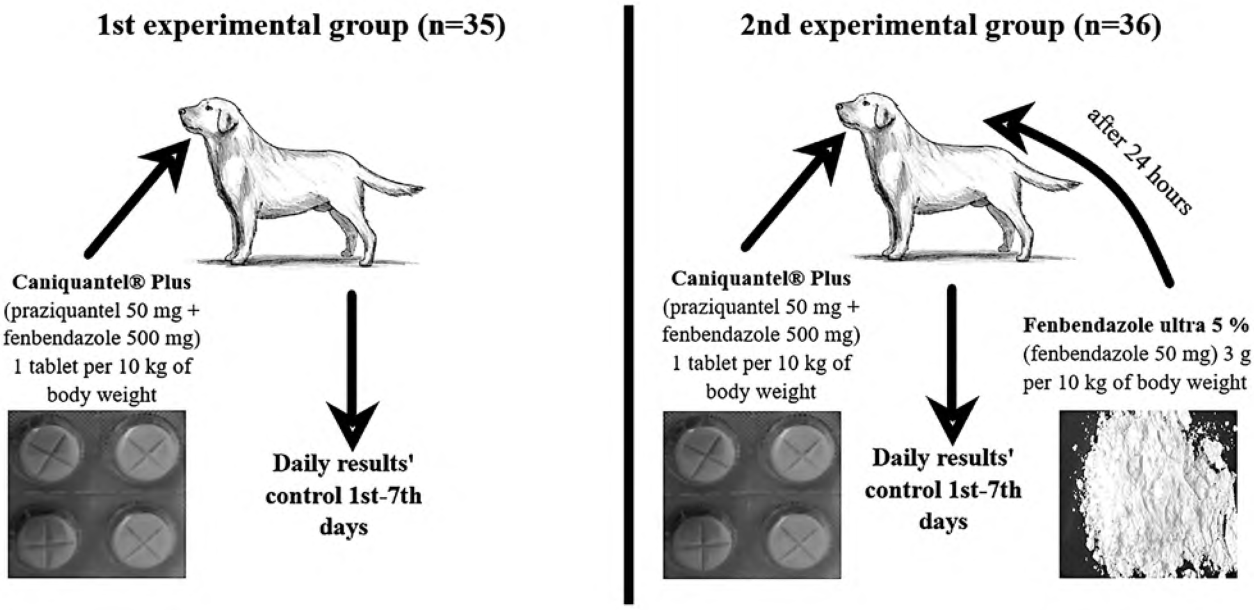

Fig. 2. Experimental deworming scheme

Statistical analysis

Datasets of helminths eggs' or packets' content were expressed as mean (x) ± standard error of the mean (SE). Mathematical analysis of study results was conducted by means of Statistica 13.3 IT Application (StatSoft Inc., USA). Differences between average values were considered statistically significant at $P < 0.05$ (ANOVA).

Ethical considerations

The research protocol of the present study was approved by the Ethic Committee of Bila Tserkva National Agrarian University in Ukraine (Approval №7, conclusion 3/1, 21.05.2020).

RESULTS

The dynamics of helminth eggs' content in faeces of the 1st experimental group's dogs (treated with Caniquantel® Plus once) shows an increase in this indicator (from 11.8 % for *T. vulpis* to 3.2 times for Ancylostomatidae family) one day after the study start (Fig. 3). After two days, last portions of *T. canis* eggs (73.7 ± 2.6 specimens.g^{-1} of faeces) and *D. caninum* packets (12.64 ± 0.51) were detected. Eggs of *T. leonina* (2.08 ± 0.06 specimens.g^{-1} of faeces) and Ancylostomatidae family (11.13 ± 0.58 specimens.g^{-1} of faeces) were last recorded three days after an anthelmintic administration. Regarding the excretion of *T. vulpis* eggs, after two days, their content in the faeces significantly decreased by 19.4 % compared to the previous indicator ($P < 0.01$), no longer having a significant difference from the data before the experiment. Subsequently, by the end of the study, a concentration of eggs of this pathogen fluctuates slightly, reaching after seven days a decrease relative to the initial value of 22.0 % ($P < 0.001$).

The use of a combination of drugs in dogs of the 2nd experimental group (Fig. 4) allowed to record the last presence *T. canis* eggs (91.1 ± 3.9 specimens.g^{-1} of faeces) and packets of *D. caninum* (7.26 ± 0.42 specimens.g^{-1} of faeces) 2 days after the start of deworming. Eggs of *T. leonina* (5.26 ± 0.22 specimens.g^{-1} of faeces) and Ancylostomatidae family (3.25 ± 0.17 specimens.g^{-1} of faeces) last appeared in faeces of dogs after three days. The concentration of *T. vulpis* eggs gradually increased, almost doubling after three days relative to baseline ($P < 0.001$). After four days, their content in faeces decreased significantly. The last *T. vulpis* eggs appeared five days after the start of the experiment (12.32 ± 0.45 specimens.g^{-1} of faeces).

Regarding the dynamics of helminth bodies' excretion with faeces of experimental dogs from the 1st group, *T. vulpis* appeared in insignificant quantity two days after the beginning of the experiment (4.54 ± 0.21 specimens

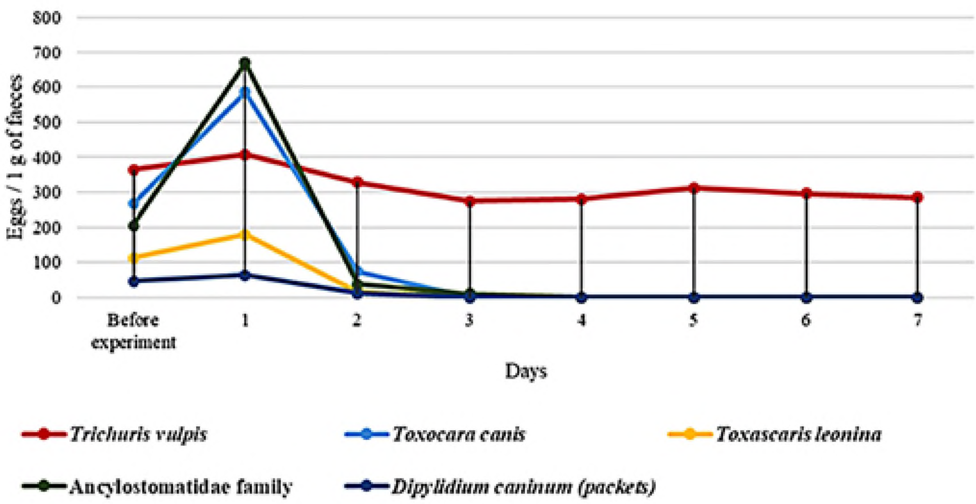

Fig. 3. Helminths eggs' excretion with canine faeces (n = 35) after deworming with Caniquantel® Plus

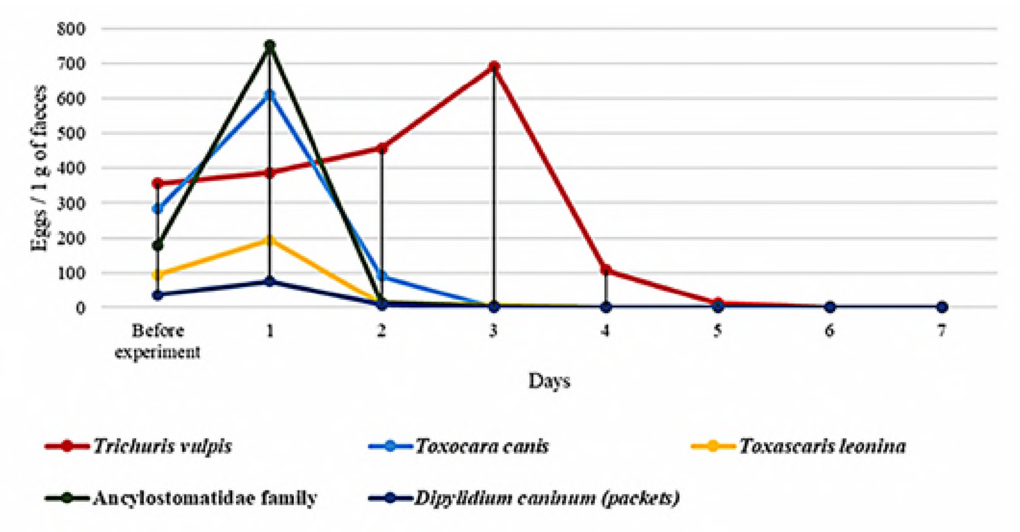

Fig. 4. Helminths eggs' excretion with canine faeces (n = 36) after deworming with Caniquantel® Plus and fenbendazole (24 h interval)

per 100 g of faeces), Fig. 5. Subsequently, parasites of this species were absent in faeces samples. Adult bodies of *T. canis* appeared in faeces samples for the first time after one day from the beginning of the experiment (19.50 ± 0.72 specimens per 100 g of faeces), and last—after two days (3.32 ± 0.14 specimens per 100 g of faeces). Excretion of *T. leonina* nematodes occurred only one day after deworming. Ancylostomatidae family helminth bodies appeared two days later (179.5 ± 9.1 specimens per 100 g of faeces) and last time after four days from the start of the experiment (5.39 ± 0.25 specimens per 100 g of faeces). A similar gradual decrease in faeces content was characteristic of *D. caninum* proglottids, but they were isolated in the period from 1 to 3 days. Adults of *A. alata* trematodes also appeared after one (18.76 ± 0.77 specimens per 100 g of faeces) and two days (28.4 ± 0.93 specimens per 100 g of faeces) from the study start.

A similar pattern to results of group 1 was observed for all helminths except *T. vulpis* (Fig. 6). Thus, *T. canis* bodies appeared in samples one (20.4 ± 0.85 specimens per 100 g

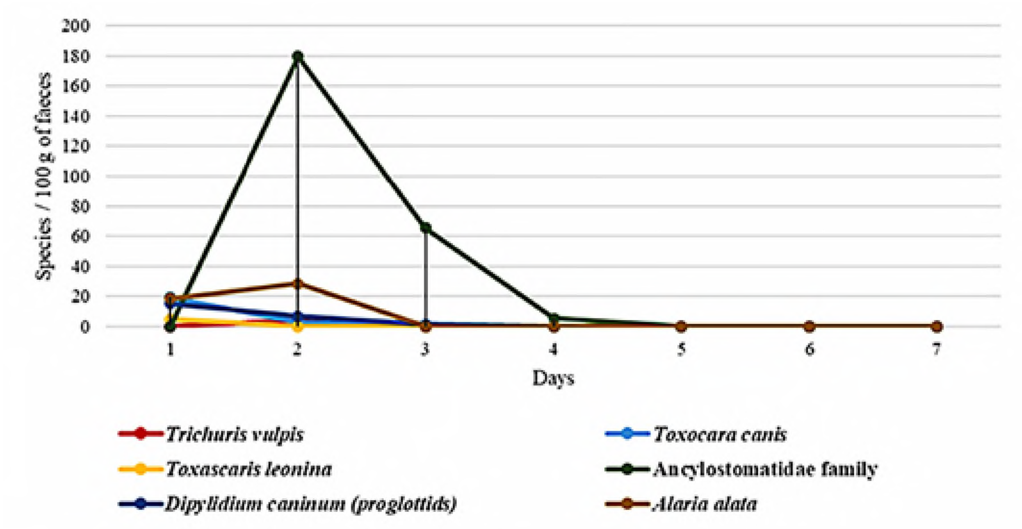

Fig. 5. Helminths' excretion with canine faeces (n = 35) after deworming with Caniquantel® Plus

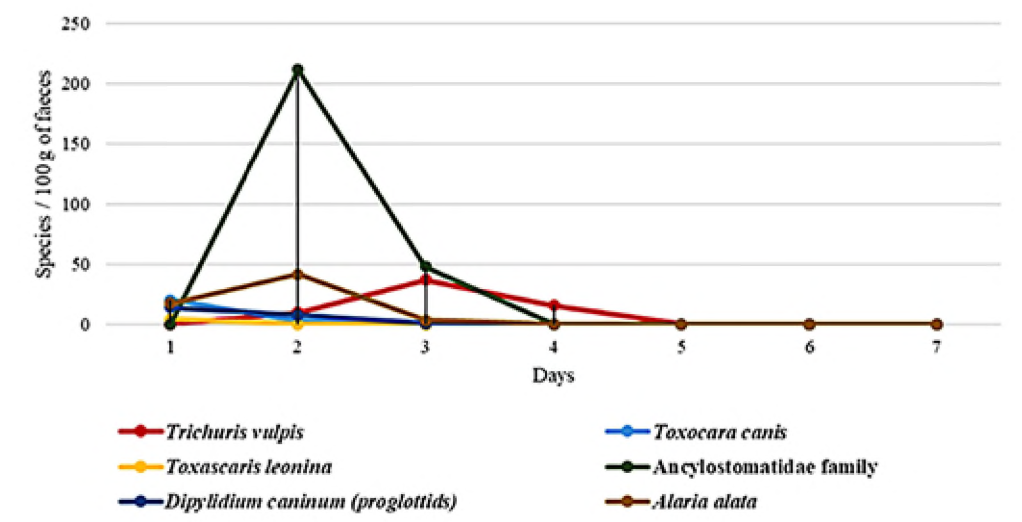

Fig. 6. Helminths' excretion with canine faeces (n = 36) after deworming with Caniquantel® Plus and fenbendazole (24 h interval)

of faeces) and two days (2.92 ± 0.11 specimens per 100 g of faeces) after the study started, *T. leonina* after one day (4.30 ± 0.18 specimens per 100 g of faeces), Ancylostomatidae family appeared after two (211.6 ± 12.4 specimens per 100 g of faeces) and three days (47.9 ± 2,3 specimens per 100 g of faeces), *A. alata* from one (17.30 ± 0.76 specimens per 100 g of faeces) to three days (3.37 ± 0.19 specimens per 100 g of faeces), and proglottids of *D. caninum* from one (14.14 ± 0.55 specimens per 100 g of faeces) to three days (1.05 ± 0.04 specimens per 100 g of faeces). Nematodes *T. vulpis* began excreted in faeces of dogs two days after the experiment started (10.03 ± 0.45 specimens per 100 g of faeces). Their number increased sharply 3.67 times after three days ($P < 0.001$). The last time these parasites were isolated after four days in the amount of 15.71 ± 0.80 specimens per 100 g of faeces.

Helminth bodies were not excreted with faeces of dogs in both groups after 5—7 days from the beginning of the experiment.

DISCUSSION

In previous decades, manuscripts have been published stating that Caniquantel® Plus can be used three times with an interval of 24 hours to successfully deworm dogs with mixed helminthoses [53]. Fenbendazole is needed to control parasitic nematodes in dogs (*T. vulpis, T. canis, T. leonina*, Ancylostomatidae family), and praziquantel—with cestodes (*D. caninum*, Taeniidae). Both of these substances are considered as low-toxic. However, more recent studies have shown that a single dose of praziquantel-containing drugs is sufficient to kill mature cestodes in dogs [24, 28] and there is no urgent need to re-feed this substance. But fighting some parasitic nematodes (*T. vulpis*, and in some cases *T. canis*), a single treatment with benzimidazoles may not be enough and should be duplicated with a daily interval [12, 57]. This evidence prompted us to investigate the effectiveness of a deworming regimen involving a single application of praziquantel and a double application of fenbendazole. This regimen has to be the most effective against mixed helminthiasis in adult dogs and the least toxic to patients, i.e. the golden mean of canine deworming.

The result of the present study was the identification of differences in the excretory of eggs and bodies of helminths with faeces of two experimental groups' dogs. Significantly, these differences were manifested in the indicators related to the nematode *T. vulpis*. Thus, a single application of the anthelmintic drug Caniquantel® Plus led to the effective release of the dogs' body at the 1st experimental group from helminths *T. canis, T. leonina*, Ancylostomatidae family, *D. caninum, A. alata*. It manifested itself in the cessation of their eggs' excretion (or packets) and/or the bodies of adults with dogs' faeces. However, such a deworming scheme proved to be ineffective against *T. vulpis*. The number of eggs of this nematode in canine faeces only partially decreased at the end of the experiment by 22.0 % ($P < 0.001$) compared to the initial data. The bodies of adult parasites were excreted in faeces only after two days and in small quantities (4.54 ± 0.21 specimens per 100 g of faeces). Such results give the right to speak only about the partial release of the host from parasites because the continued excretion of eggs indicates the presence of alive adult *T. vulpis* in the intestine.

Results of the presented study about the insufficient effectiveness of standard regimens for anthelmintics' use against *T. vulpis* echo the scientific reports from different European countries. Thus, Hinaidy from Austria describes that the usual deworming carried out by owners for bitches and their puppies were only partially effective [17]. The presence of *T. vulpis* eggs in faeces of dogs from Finland, which are dewormed at least once a year were indicated by Pullola [38]. Data published in Switzerland emphasize that even in the case of canine deworming every 3 months (with a combination of pyrantel embonate, praziquantel and febantel), *T. vulpis*, *Capillaria* spp. and Taeniidae eggs were detected in faeces with a prevalence 11—22 % [46] The authors also emphasize that the frequency and mode of deworming directly depend on the characteristics of the environment, nutrition and lifestyle of pets [11].

Significantly better results in the present study were obtained in a comprehensive parasitological study of canine faeces at the 2nd experimental group. Thus, the excretion of dead specimens of *T. vulpis* lasts three days and stops four days after the experiment start, and their eggs after five days. The excretion of eggs (packets) of helminths *T. canis, T. leonina*, Ancylostomatidae family, *D. caninum* stopped in 2—3 days from the beginning of deworming, and bodies (proglottids) of *T. canis, T. leonina, Strongylata* sp., *D. caninum*, and *A. alata*—after 1—3 days. It means that the gastrointestinal tract is free of helminths.

A significant increase of helminth eggs' concentration in faeces of experimental dogs in the first days after the use of anthelminthics is due to the lysis of the bodies of female parasites after death. The decay of bodies leads to the mass release of helminth eggs (as well as somatic toxins) into the environment, which is the intestine's content [4]. The same applies to the situation with *T. vulpis*, when four days after the beginning of deworming of animals of the 2nd experimental group, these parasites were no longer excreted with faeces, and their eggs—yes. Similarly, in *T. leonina*, the release of eggs stopped after three days, and bodies of these parasites stopped after one day.

A surprise result of the present study was the isolation of mature individuals of *A. alata* with faeces of dogs in both experimental groups, as there are no reliable data on the spread of this parasite in Ukraine. In addition, as a result of ovoscopic examination of canine faeces, previous researchers didn't find eggs of this trematode. Although in Ukraine's neighbouring countries, this parasite is quite common among domestic and wild Canids, as well as among wild boars in Belarus [50], Poland [6, 54], and Latvia [35]. This phenomenon is easy enough to explain by the method most often used to prepare samples for ovoscopic research. Thus, Ukrainian parasitologists classically use flotation or combined methods of laboratory examination for canine faeces [56]. Just for samples from cattle, it is considered appropriate to use sedimentation techniques to diagnose the fasciolosis [13]. However, eggs of *A. alata*, as in many other trematodes, are quite large, 0.115—0.130 × 0.068—0.093 mm [26]. Therefore, the detection of these pathogens by flotation methods is unlikely. This situation may lead to further study of this trematode's prevalence among domestic and wild animals in Ukraine.

CONCLUSIONS

Deworming of adult dogs with Caniquantel® Plus allows fighting successfully the helminths *T. canis, T. leonina*, Ancylostomatidae family, *D. caninum*, and *A. alata*. However, such treatment is ineffective in controlling the nematode *T. vulpis*. Alternate use of Caniquantel® Plus and fenbendazole (with 24-hour interval) allows to completely rid the body of adult dogs against the causative agents of mixed gastrointestinal tract helminthoses. It is manifested by the complete cessation of the release of eggs (packets) and bodies (proglottids) of mature helminths of different species with animal faeces five days after the deworming start. Therefore, such a scheme has the maximum efficiency and safety due to the combination of low-toxic substances.

Further study could usefully investigate *A. alata* prevalence among domestic and wild Canids' populations in the Kyiv region particularly, and Ukraine generally.

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