Comparative efficiency of laboratory diagnostic methods of dog toxocarosis

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Article info

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The causative agents of toxocarosis are cosmopolitan nematode parasites that infect a wide range of domestic and wild animals through several routes of transmission, producing tissue-localized larvae and persistent eggs that can survive in the external environment. Despite the growing awareness of their zoonotic potential and importance for health care, the issue of using highly effective methods of lifelong diagnostics that are easy to use, inexpensive and have high diagnostic efficiency remains relevant. The aim of the work was to compare the effectiveness of the well-known and the proposed method in the diagnosis of toxocarosis in dogs. The proposed method relates to the field of veterinary medicine, namely veterinary parasitology, to methods of coproscopy, in particular, methods of detecting eggs of Toxocara canis nematodes parasitizing dogs. In laboratory conditions, well-known methods of coprooviscopy (Fulleborn's, Kotelnikov-Khrenov's, method of coprooviscopy for trichurosis of sheep) and the proposed method for laboratory diagnosis of toxocarosis in dogs were compared. When using the Fulleborn's method, 80 % of positive samples were detected, and when using the Kotelnikov-Khrenov's method, the method of coprooviscopy for sheep trichurosis, and the proposed method, 100 % of positive samples were detected. Indicators of the intensity of toxocarous infestation in dogs when applying the Fulleborn's method averaged 61.7 eggs/g, Kotelnikov-Khrenov's – 88.3 eggs/g, the method of coprooviscopy for sheep trichurosis – 88.8 eggs/g, the proposed method – 102.1 eggs/g. It was proved that the proposed method of laboratory diagnosis of toxocarosis in dogs was more effective compared to the method of Fulleborn by 39.6 %, Kotelnikov-Khrenov – by 13.5 %, and the method of coprooviscopy for trichurosis of sheep – by 13.0 %. When applying the proposed method, high coagulation properties of the improved floatant were established relative to undigested feed residues. The obtained results of experimental studies allow us to recommend the proposed method for effective laboratory diagnosis of toxocarosis in dogs.

Keywords: parasitology, toxocarosis, dogs, laboratory diagnostics, flotation, coprooviscopy, efficiency.

Порівняльна ефективність методів лабораторної діагностики токсокарозу собак

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Збудники токсокарозу є космополітичними нематодами-паразитами, які заражають широкий спектр домашніх і диких тварин за допомогою кількох шляхів передачі, продукуючи личинок, що локалізуються у тканинах, і стійкі яйця, які можуть виживати у зовнішньому середовищі. Незважаючи на зростання обізнаності про їх зоонозний потенціал і значення для охорони здоров'я, залишається актуальним питання застосування високоефективних методів зажиттєвої діагностики, які є простими у використанні, недорогими та мають високу діагностичну ефективність. Метою роботи було провести порівняння ефективності загальновідомих та запропонованого способу при діагностиці токсокарозу собак. Запропонований спосіб відноситься до галузі ветеринарної медицини, а саме – ветеринарної паразитології, до способів копроскопії, зокрема, способів виявлення яєць нематод Toxocara canis, що паразитують у собак. У лабораторних умовах порівнювали загальновідомі способи копрооовоскопії (Фюллеборна, Котельникова-Хренова, способу копрооовоскопії за трихурозу овець) та запропонованого способу при лабораторній діагностиці токсокарозу собак. При використанні методу Фюллеборна виявлено 80 % позитивних проб, а при використанні методу Котельникова-Хренова, способу копрооовоскопії за трихурозу овець та запропонованого способу виявлено 100 % позитивних проб. Показники інтенсивності токсокарозної інвазії собак при застосуванні методу Фюллеборна у середньому становили 61,7 яєць/г, Котельникова-Хренова – 88,3 яєць/г, способу копрооовоскопії за трихурозу овець – 88,8 яєць/г, запропонованого способу – 102,1 яєць/г. Доведено, що запропонований спосіб лабораторної діагностики токсокарозу собак виявився ефективнішим порівняно зі способом Фюллеборна на 39,6 %, Котельникова-Хренова – на 13,5 %, способу копрооовоскопії за трихурозу овець – на 13,0 %. При застосуванні запропонованого способу встановлено високі коагуляційні властивості удосконаленого флотанту відносно неперетравлених решток корму. Отримані результати експериментальних досліджень дозволяють рекомендувати до застосування запропонований спосіб для ефективної лабораторної діагностики токсокарозу собак.

Ключові слова: паразитологія, токсокароз, собаки, лабораторна діагностика, флотація, копрооовоскопія, ефективність.

Introduction

Scientists note that 26 species have been recognized among parasites of the genus *Toxocara*, where only species *T. canis* and *T. cati* are zoonoses. These nematodes are very common parasites in dogs, which are definitive hosts, and release eggs into the environment, resulting in significant environmental pollution. The danger of the causative agent *T. canis* is explained by the possibility of human infection, where the main way of transmission of the parasite is through ingestion of eggs from the soil or soil-contaminated hands, food, etc. [1–5].

Analysis of the available literature shows that the seroprevalence of *Toxocara* species among humans is 19 %. Moreover, seroprevalence is the highest in the African region (35 %), and the lowest in the Eastern Mediterranean region – 8.2 % [6].

Recent complex environmental changes, including destruction of natural habitats and increased urbanization, have contributed to increased contact between wildlife, domestic animals, and humans, which may increase the risk of *T. canis* nematode spread [7–10].

The final diagnosis of toxocarosis in dogs can be made only when using direct research methods in case of detection of helminth eggs, using lifelong coproscopic diagnostics [11–13]. To detect nematode eggs parasitizing in the gastrointestinal tract of animals and humans, it is recommended to use coproovoscopic flotation, sedimentation and combined (sedimentation-flotation) methods. It should be noted that the majority of existing methods and techniques are based on the use of various flotation liquids with a high specific gravity. For the manufacture of flotation fluids, veterinarians and scientists use a variety of chemical compounds and their combinations in various proportions and combinations. Many improved methods have also been proposed, including for the diagnosis of nematodoses of the alimentary canal of sheep, using combined flotation solutions [14–16].

Also, scientists recommend using quantitative methods using special counting cameras: McMaster's, Galat-Yevstafieva's, et al [17–19]. Quantitative counting of helminth eggs is also carried out without the use of special equipment. For this purpose, the volume of the test material, the area of the test surface and the parasitological loop are taken into account. Such methods include the method of Trach, Mazanny, et al, Lyashenko, et al [20–22]. Therefore, testing and improvement of modern, effective methods of coproovoscopy for toxocarosis in dogs is urgent.

The aim of the study

The purpose of the research was to compare the effectiveness of the well-known and the proposed method in the diagnosis of toxocarosis in dogs.

Materials and methods

The work was carried out during 2024 on the basis of the Laboratory of the Department of Parasitology and Veterinary-Sanitary Examination of the Poltava State Agrarian University and in the conditions of a private veterinary clinic in Poltava.

In order to establish the effectiveness of the proposed method of coproscopic examination of dogs for the presence of toxocares eggs, a comparison was made between the proposed method and the well-known Fulleborn's, Kotelnikov-Khrenov's [23], method of coproovoscopy for trichurosis in sheep [24].

Feces from dogs spontaneously infected with the causative agent of toxocarosis were used for the experiment. With each flotation solution, 15 samples of feces were examined using the technique proposed in the proposed method. Samples were settled in each of the flotation solutions for 10 minutes. The number of detected toxocares eggs was counted in 1 g of feces.

Evaluation of the methods was carried out according to the indicators of: actual specific gravity of the flotation solution; flotation capacity (number of positive samples and average number of detected toxocares eggs); coagulation ability (• – a small number of small foreign remains; •• – simultaneous detection of a large number of small and a small number of large-sized remains; ••• – a large number of both small and significant foreign remains).

Statistical processing of the results of experimental studies was carried out by determining the arithmetic mean (M), standard deviation (SD) and probability level (p) using the technique of univariate analysis of variance, using Fisher's test.

Results and discussion

It was determined that the diagnostic sensitivity of the tested methods was different, and although *T. canis* eggs (*Fig. 1*) were detected by all methods, at the same time, the sensitivity was 80 % when using the Fulleborn's method, when using Kotelnikov-Khrenov's, the method of coproovoscopy for sheep trichurosis, and the proposed method – 100 % (*Fig. 2*).

The same difference was found when determining the intensity of toxocarosis infestation. In particular, when using the Fulleborn's method, the indicators of the intensity of invasion were on average 61.7±21.3 eggs/g, Kotelnikov-Khrenov's – 88.3±17.4 eggs/g, the method of coproovoscopy for sheep trichurosis – 88.8±18, 4 eggs/g, the proposed method – 102.1±16.7 eggs/g. It was also established that the highest diagnostic efficiency in terms of indicators of the intensity of toxocarosis invasion was found when using the proposed method, where it was more effective compared to the Fulleborn's method by 39.6 %, P<0.001 (*Fig. 3*), Kotelnikova-Khrenova – by 13.5 % , P<0.01 (*Fig. 4*), the method of coproovoscopy for sheep trichurosis – by 13.0 %, P<0.05 (*Fig. 5*).

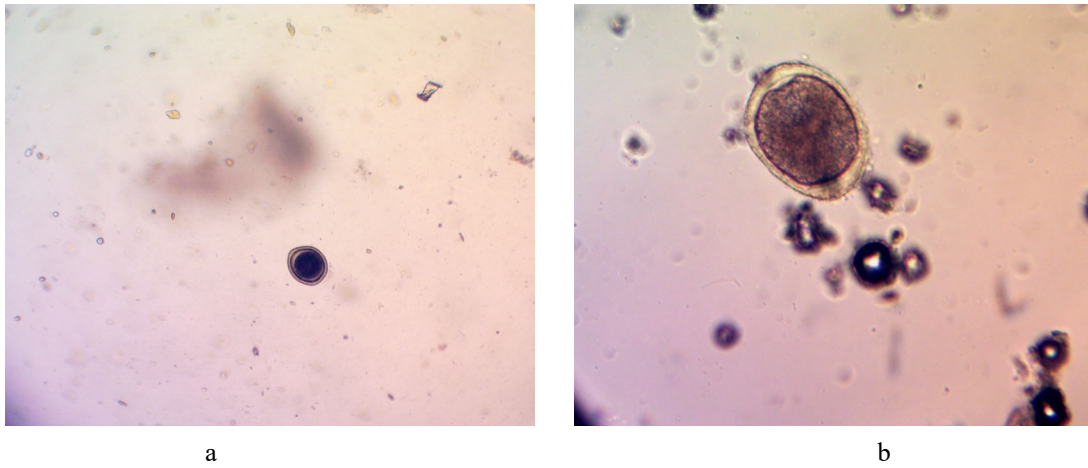**Fig. 1.** Eggs of the nematodes *Toxocara canis* detected when using the investigated methods of coproovoscopy:
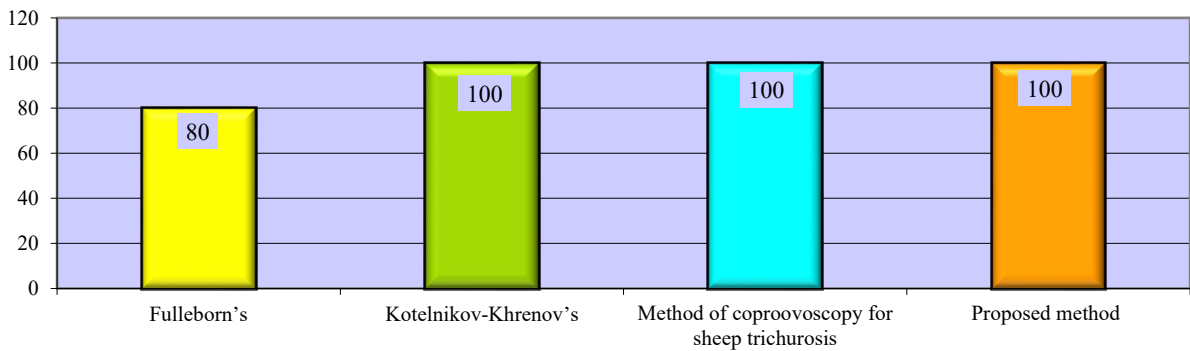a – × 120, b – × 400

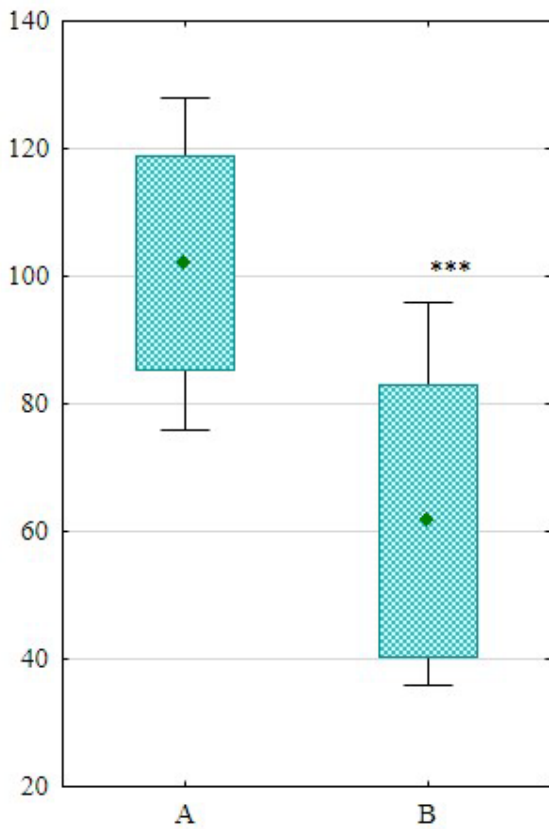**Fig. 2.** The number of detected positive samples during coproovoscopic diagnosis of toxocarosis in dogs

Fig. 3. Comparative effectiveness of coproscopy methods for toxocarosis in dogs (n=15): A – proposed method, B – Fulleborn's method; P<0.001 – relative to A
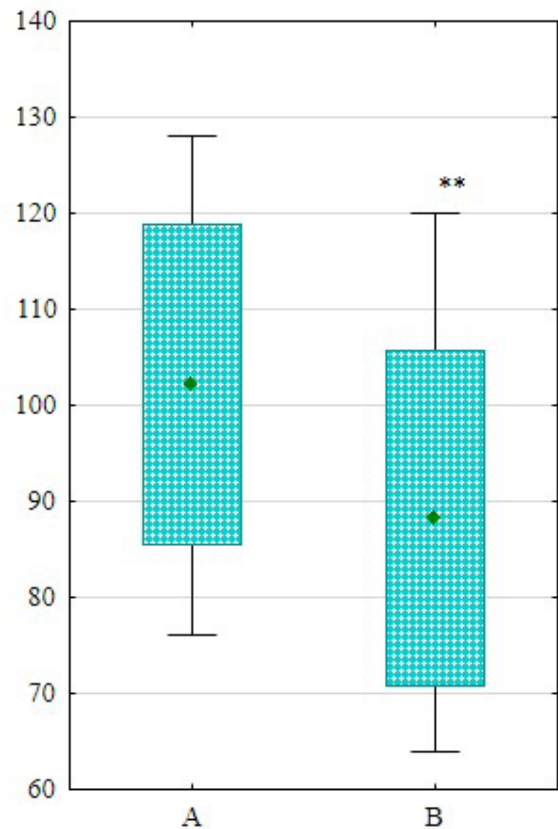

Fig. 4. Comparative efficiency of coproscopy methods for toxocarosis in dogs (n=15): A – the proposed method, B – the Kotelnikov-Khrenov's method; P<0.01 – relative to A
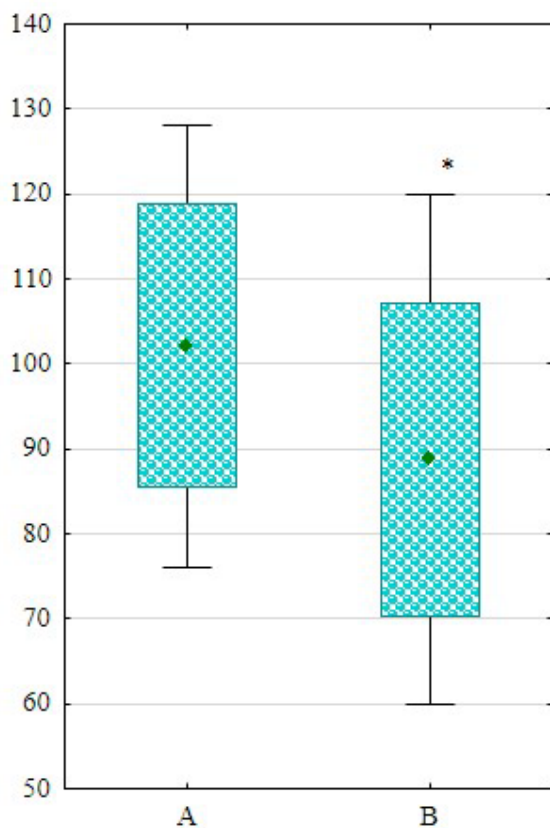
Fig. 5. Comparative effectiveness of methods of coprooscopy for toxocarosis in dogs (n=15): A – proposed method, B – method of coproovoscopy for trichurosis of sheep; P<0.05 – relative to A

When using the proposed method of laboratory diagnosis of toxocarosis in dogs, a small amount of small foreign remains was detected, which indicates a high coagulation property of the proposed floatant. The same high coagulation ability was found when using the coproovoscopy method for trichurosis in sheep. When using the Kotelnikov-Khrenov's method, a large number of both small and large foreign remains were detected. When using the Fulleborn's method, a large number of small and a small number of large remains were detected at the same time.

The scientific literature notes the relevance of the study of canine toxocarosis caused by the nematode *T. canis* in connection with its zooanthroponous potential [1–5]. Therefore, the application of effective, accurate and ergonomic methods of lifelong diagnosis of this infestation is relevant.

The conducted studies established that when using the Fulleborn's method, 80 % of positive samples were detected, the Kotelnikov-Khrenov's method, the method of coproovoscopy for sheep trichurosis, and the proposed method revealed 100 % of positive samples. It was proved that the proposed method of laboratory diagnosis of toxocarosis in dogs was more effective compared to the method of Fulleborn by 39.6 % (61.7±21.3 eggs/g, P<0.001), Kotelnikov-Khrenov – by 13.5 % (88.3±17.4 eggs/g, P<0.01), the method of coproovoscopy for sheep trichurosis – by 13.0 % (88.8±18.4 eggs/g, P<0.05).

So, in order to detect *T. canis* eggs in dog feces, scientists compared the effectiveness of flotation methods using $MgSO_4$ (density 1.20), $ZnSO_4$ (1.18–1.20), $NaNO_3$ (1.18–1.20), NaCl (1.18–1.20) and the proposed method (sugar + water + formaldehyde, density 1.27). The results indicate that the technique proposed by the authors and the use of centrifugation were more effective in terms of the intensity of invasion than other methods [25].

The obtained results of experimental studies allow recommending the use of the proposed method for effective laboratory diagnosis of toxocarosis in dogs.

Conclusions

The proposed method of coproscopic examination of dogs for the presence of eggs of pathogens of toxocarosis shows a high index of flotation ability relative to *T. canis* – up to 102.1±16.7 eggs/g during 10 minutes of fecal suspension settling compared to Fulleborn's method (by 39.6 %, P<0.001), Kotelnikov-Khrenov's (by 13.5 %, P<0.01) and the method of coproovoscopy for sheep trichurosis (by 13.0 %, P<0.05). In the proposed method of coproscopic examination of dogs for the presence of eggs of pathogens of toxocarosis, a flotation solution is used, which exhibits high coagulation properties relative to undigested food residues.

Conflict of interest

The author state that there is no conflict of interest.

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