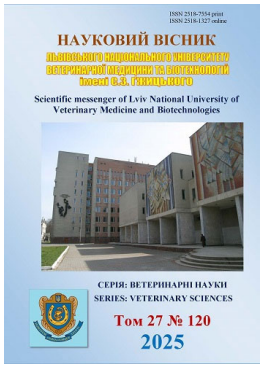

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Efficacy of laboratory diagnostic methods for associative trichurosis–toxocarosis infection in dogs

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Gastrointestinal nematodoses are widespread and dangerous parasitic diseases of dogs, and it is known that some of them have zoonotic potential and pose a threat to human health. For rapid intravital diagnosis of gastrointestinal nematodoses in dogs, a wide range of salts and other substances is available for use in flotation examinations. Since polyparasitism is a very common phenomenon, there is a need for sensitive and accurate diagnostic tools that are easy to apply and allow simultaneous detection of several pathogens during integrated control of parasitic mixed infections. The aim of this study was to determine the sensitivity of known coproovoscopic methods in dogs with associative trichurosis–toxocarosis infection. The research was carried out at the private veterinary clinic "Dovira" (Kharkiv) and at the Parasitology Laboratory of Poltava State Agrarian University (Poltava). The following flotation methods were tested: Kotelnikov–Khrenov (using an ammonium nitrate solution), Sorokova (using a combined solution of calcium and ammonium nitrate), Petrenko (using a combined solution of calcium nitrate, sugar, and sodium chloride), and Melnychuk (using a combined solution of calcium nitrate and sodium chloride). The conducted studies established that in dogs with trichurosis–toxocarosis infection, all tested methods, regardless of the exposure time of fecal samples, allowed simultaneous detection of Toxocara and Trichuris eggs, although the intensity of infection varied. The highest intensity values of toxocarosis and trichurosis infection were detected when applying the Melnychuk method with a fecal sample exposure of 15 min, reaching 69.3 and 84.4 eggs/g, respectively. This method demonstrated higher sensitivity in dogs with trichurosis–toxocarosis infection compared with the Kotelnikov–Khrenov method by 21.9–73.1 %, the Sorokova method by 21.2–30.9 %, and the Petrenko method by 27.6 %. The obtained results allow recommending the Melnychuk method, which uses a combined solution of calcium nitrate and sodium chloride as the flotation medium, for effective intravital laboratory diagnosis of the associative course of trichurosis and toxocarosis in dogs.

Key words: *parasitology, dogs, associative infections, nematodoses, coproovoscopy, efficacy.*

Ефективність методів лабораторної діагностики асоціативної трихурозно-токсокарозної інвазії у собак

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Шлунково-кишкові нематодози є значно поширеними і небезпечними паразитичними агентами собак, і відомо, що окремі з них мають зоонозний потенціал і несуть загрозу для здоров'я людини. Для швидкої зажиттєвої діагностики нематодозів травного тракту у собак існує велика кількість солей та інших речовин, які можна використовувати у флотаційних дослідженнях. Оскільки поліпаразитизм є дуже поширеним явищем, тому під час інтегрованого контролю паразитарних мікстінвазій існує потреба в чутливих та точних діагностичних інструментах, які легко застосовувати і які дозволяють виявляти одночасно декількох збуд-

ників. Мета досліджень полягала у визначенні чутливості відомих методів копроовоскопії за асоціативної трихурозно-токсокарозної інвазії у собак. Дослідження проводили в умовах приватної ветеринарної клініки «Довіра» (м. Харків) та лабораторії паразитології Полтавського державного аграрного університету (м. Полтава). Випробувано способи флотації Котельникова-Хренова (із розчином аміачної селітри), Сорокової (із комбінованим розчином кальцієвої та аміачної селітри), Петренка (із комбінованим розчином кальцієвої селітри, цукру та натрію хлориду), Мельничука (із комбінованим розчином кальцієвої селітри та натрію хлориду). Проведеними дослідженнями встановлено, що за трихурозно-токсокарозної інвазії собак всі випробувані способи незалежно від експозиції копропроб дозволяли одночасно виявити яйця токсокар і трихурисів, де показники інтенсивності інвазій різнилися. Найвищі значення інтенсивності токсокарозної та трихурозної інвазії було виявлено під час застосування способу Мельничука за експозиції копроб 15 хв – 69,3 та 84,4 яєць/г. Цей спосіб проявив вищу чутливість за трихурозно-токсокарозної інвазії собак порівняно зі способами Котельникова-Хренова – на 21,9–73,1 %, Сорокової – на 21,2–30,9 %, Петренка – на 27,6 %. Отримані результати досліджень дозволяють впроваджувати спосіб Мельничка, де в якості флотанту використано комбінований розчин кальцієвої селітри та натрію хлориду, для ефективної зажиттєвої лабораторної діагностики асоціативного перебігу трихурозу і токсокарозу в собак.

***Ключові слова**: паразитологія, собаки, асоціативні інвазії, нематодози, копроовоскопія, ефективність.*

Introduction

It is known that domestic dogs are carriers of a significant number of gastrointestinal helminth species, some of which can be transmitted to humans and therefore have zoonotic potential. Among the most widespread gastrointestinal nematodoses in dogs, researchers in many countries identify *Toxocara canis* and *Trichuris vulpis* (Fang et al., 2015; Silva et al., 2020; Zhang et al., 2022; Mubarak et al., 2023). Therefore, intravital laboratory diagnosis is an important component of modern veterinary parasitology, and its effectiveness depends on the correct choice of diagnostic method.

For the diagnosis of gastrointestinal nematodoses in dogs, coproovoscopic examinations are most commonly used. These methods are sufficiently effective and are based on flotation or combined techniques (Quinn et al., 1980; Cringoli et al., 2004; Bowman & Lynn, 2009). In contrast to existing molecular-genetic and immunological methods, coproovoscopic flotation techniques, although they do not always provide precise identification in certain nematodoses, are characterized by simplicity of performance without the need for specialized equipment, as well as by the possibility of conducting morphometric analysis and isolating nematode eggs for in vitro studies (Taglioretti et al., 2014; Melnychuk & Yuskiv, 2019).

Scientists note that the efficacy and sensitivity of flotation coproovoscopic methods are influenced by factors such as the technique of fecal sample collection, the time and conditions of transportation, the composition of the flotation solution, and the examination procedure. At the same time, some authors demonstrate that for a given helminthosis, the same coproovoscopic method may exhibit different diagnostic efficacy (Stationery, 1986; Foreyt, 1989; Sobotyk et al., 2021; Segura et al., 2023).

During the diagnosis of gastrointestinal parasitoses in dogs, researchers continuously monitor the effectiveness of various coproovoscopic methods and refine existing techniques that show higher efficacy than classical ones. In particular, the effectiveness of flotation methods using sodium nitrate and sugar solutions has been studied. It was established that flotation with sodium nitrate solution allowed detection of *T. vulpis* in 68.4 % and *T. canis* in 54.5 % of cases. At the same time, flotation using a sugar solution enabled detection of *T. vulpis* in 83.3 % and *T. canis* in 77.8 % of cases (Adolph et al., 2017).

Researchers from Brazil evaluated the effectiveness of four methods for diagnosing gastrointestinal parasitoses in dogs: two classical methods (Willis and Hoffman) and two modern methods (FLOTAC and Mini-FLOTAC). Eggs and oocysts of gastrointestinal parasites were detected in 93.3 % of samples. Specifically, 20 % were detected using the Hoffman method, 53.3 % using the Willis method, and 90 % and 63.3 % using the FLOTAC and Mini-FLOTAC methods, respectively. The most frequently detected parasites were nematodes of the family *Ancylostomatidae* and the species *T. vulpis* and *T. canis*. The FLOTAC and Mini-FLOTAC methods proved to be the most effective for detecting eggs and/or oocysts of gastrointestinal parasites in dogs (Lima et al., 2015).

Research objective

The aim of this study was to determine the sensitivity of known coproovoscopic methods in dogs with associative trichurosis–toxocarosis infection.

Materials and Methods

The study was conducted in 2025 at the private veterinary clinic “Dovira” (Kharkiv) and at the Parasitology Laboratory of Poltava State Agrarian University (Poltava).

The following flotation methods were tested: Kotelnikov–Khrenov (using an ammonium nitrate solution, specific gravity 1.30) (Kotelnikov, 1974); Sorokova (using a combined solution of calcium and ammonium nitrate, specific gravity 1.32–1.34) (Sorokova, 2019); Petrenko (using a combined solution of calcium nitrate, sugar, and sodium chloride, specific gravity 1.32) (Petrenko et al., 2024); and Melnychuk (using a combined solution of calcium nitrate and sodium chloride, specific gravity 1.34) (Melnychuk et al., 2025).

Fecal samples from dogs spontaneously infected with an association of trichurosis and toxocarosis pathogens were used for the study. In total, 180 coproovoscopic examinations were performed (15 per each method). Sedimentation of samples in each flotation solution was carried out for 5, 10, and 15 minutes. The evaluation criteria included the number of positive samples and the mean number of detected nematode eggs (intensity of infection, II (eggs/g)).

Mathematical analysis of the obtained data was performed using the Microsoft Excel software package (Microsoft Corporation, Redmond, Washington, USA) by calculating the arithmetic mean (M), standard deviation

(SD), and significance level (P) using one-way analysis of variance with Fisher's criterion.

Results and Discussion

The conducted studies demonstrated that in dogs with trichurosis–toxocarosis infection, all tested methods, regardless of fecal sample exposure time, allowed simultaneous detection of *Toxocara* and *Trichuris* eggs, although the intensity of infection varied.

Comparison of the Kotelnikov–Khrenov and Melnychuk methods showed that the latter was more effective in terms of the intensity of toxocarosis and trichurosis infection (Table 1).

Yes, the effectiveness of the Melnychuk method was higher: at an exposure time of 5 min, it detected 73.1 % more *Trichuris* eggs ($P < 0.01$) and 21.9 % more *Toxocara* eggs ($P < 0.05$). At an exposure time of 10 min, 31.7 % more *Trichuris* eggs ($P < 0.01$) and 11.9 % more *Toxocara* eggs were detected, whereas at an exposure time of 15 min, 21.7 % more *Trichuris* eggs ($P < 0.01$) and 18.4 % more *Toxocara* eggs ($P < 0.05$) were recorded.

When comparing the Sorokova and Melnychuk methods, it was established that the intensity indicators of toxocarosis and trichurosis infection were higher when using the Melnychuk method (Table 2).

Table 1

Comparative efficacy of the Kotelnikov–Khrenov and Melnychuk methods in dogs with associative trichurosis–toxocarosis infection

Method	Flotation exposure, min	Nematode eggs	Positive samples	%	M ± SD, eggs/g
Kotelnikov–Khrenov (ammonium nitrate solution)	5	*Trichuris*	15	100.0	31.1 ± 19.3**
		Toxocara	15	100.0	39.6 ± 18.3*
	10	*Trichuris*	15	100.0	45.8 ± 19.0**
		Toxocara	15	100.0	65.3 ± 31.1
	15	*Trichuris*	15	100.0	47.6 ± 17.3**
		Toxocara	15	100.0	68.9 ± 39.6*
Melnychuk (combined calcium nitrate and sodium chloride solution)	5	*Trichuris*	15	100.0	54.7 ± 21.0
		Toxocara	15	100.0	50.7 ± 17.8
	10	*Trichuris*	15	100.0	67.1 ± 16.6
		Toxocara	15	100.0	74.2 ± 28.8
	15	*Trichuris*	15	100.0	69.3 ± 20.9
		Toxocara	15	100.0	84.4 ± 43.6

Note: * $P < 0.05$; ** $P < 0.01$ compared with the Melnychuk method at the corresponding exposure time

Table 2

Comparative efficacy of the Sorokova and Melnychuk methods in dogs with associative trichurosis–toxocarosis infection

Method	Flotation exposure, min	Nematode eggs	Positive samples	%	M ± SD, eggs/g
Sorokova (combined calcium and ammonium nitrate solution)	5	*Trichuris*	15	100.0	37.8 ± 20.7*
		Toxocara	15	100.0	47.1 ± 18.9
	10	*Trichuris*	15	100.0	52.9 ± 17.5*
		Toxocara	15	100.0	69.8 ± 27.4
	15	*Trichuris*	15	100.0	53.8 ± 21.2*
		Toxocara	15	100.0	72.9 ± 37.8
Melnychuk (combined calcium nitrate and sodium chloride solution)	5	*Trichuris*	15	100.0	54.7 ± 21.0
		Toxocara	15	100.0	50.7 ± 17.8
	10	*Trichuris*	15	100.0	67.1 ± 16.6
		Toxocara	15	100.0	74.2 ± 28.8
	15	*Trichuris*	15	100.0	69.3 ± 20.9
		Toxocara	15	100.0	84.4 ± 43.6

Note: * $P < 0.05$ compared with the Melnychuk method at the corresponding exposure time

At an exposure time of 5 min, the Melnychuk method detected 30.9 % more *Trichuris* eggs ($P < 0.05$) and 7.1 % more *Toxocara* eggs. At an exposure time of 10 min, the intensity of trichurosis infection was higher by 21.2 % ($P < 0.05$), and the intensity of toxocarosis infection by 5.9 %. At an exposure time of 15 min, the intensity of trichurosis infection was higher by 22.4 % ($P < 0.05$), and the intensity of toxocarosis infection by 13.6 %.

It was established that the Melnychuk method was more effective in detecting mixed infections in dogs with concurrent parasitism by *Toxocara* and *Trichuris* compared with the Petrenko method (Table 3).

Thus, using the Melnychuk method, the intensity of toxocarosis and trichurosis infection was higher, respectively: at an exposure time of 5 min – by 27.6 % ($P < 0.05$) and 4.5 %; at 10 min – by 15.2 % and 4.7 %; and at 15 min – by 12.8 % and 7.3 %.

Table 3
Comparative efficacy of the Petrenko and Melnychuk methods in dogs with associative trichurosis–toxocarosis infection

Method	Flotation exposure, min	Nematode eggs	Positive samples	%	M ± SD, eggs/g
Petrenko (combined solution of calcium nitrate, sugar, and sodium chloride)	5	*Trichuris*	15	100.0	39.6 ± 20.1*
		Toxocara	15	100.0	48.4 ± 17.2
	10	*Trichuris*	15	100.0	56.9 ± 17.4
		Toxocara	15	100.0	70.7 ± 27.2
	15	*Trichuris*	15	100.0	60.4 ± 23.4
		Toxocara	15	100.0	78.2 ± 44.8
Melnychuk (combined solution of calcium nitrate and sodium chloride)	5	*Trichuris*	15	100.0	54.7 ± 21.0
		Toxocara	15	100.0	50.7 ± 17.8
	10	*Trichuris*	15	100.0	67.1 ± 16.6
		Toxocara	15	100.0	74.2 ± 28.8
	15	*Trichuris*	15	100.0	69.3 ± 20.9
		Toxocara	15	100.0	84.4 ± 43.6

Note: * $P < 0.05$ compared with the Melnychuk method and others at the corresponding exposure time

It is known that associative gastrointestinal nematodoses are widespread among dogs, and some of them have zoonotic potential and pose a threat to human health (Silva et al., 2020; Zhang et al., 2022; Mubarak et al., 2023). Therefore, there is a need for sensitive and accurate diagnostic tools that are easy to apply and allow simultaneous detection of multiple pathogens.

Comparison of the flotation methods of Kotelnikov–Khrenov (ammonium nitrate solution), Sorokova (combined calcium and ammonium nitrate solution), Petrenko (combined calcium nitrate, sugar, and sodium chloride solution), and Melnychuk (combined calcium nitrate and sodium chloride solution) showed that, in dogs with trichurosis–toxocarosis infection, all methods, regardless of fecal sample exposure time, enabled simultaneous detection of *Toxocara* and *Trichuris* eggs. However, the intensity of infection differed substantially. The highest intensity values of toxocarosis and trichurosis infection were obtained using the Melnychuk method, which demonstrated higher sensitivity compared with the Kotelnikov–Khrenov method by 21.9–73.1 % ($P < 0.05$–$P < 0.01$), the Sorokova method by 21.2–30.9 % ($P < 0.05$), and the Petrenko method by 27.6 % ($P < 0.05$).

We obtained new data on the efficacy of known coproovoscopic methods in dogs with associative trichurosis–toxocarosis infection. At the same time, available literature reports high efficacy of the Melnychuk method in detecting *T. vulpis* and *T. canis* eggs in trichurosis and toxocarosis mono-infections. According to the author, its efficacy exceeds that of methods using sodium chloride solutions by 39.6–46.7 % ($P < 0.001$), ammonium nitrate solutions by 13.5–24.6 % ($P < 0.01$–$P < 0.001$), and calcium nitrate solutions by 11.7–18.4 % ($P < 0.05$) (Kitichenko, 2024).

The obtained results allow recommending the Melnychuk flotation method using a combined calcium nitrate and sodium chloride solution for effective intravital laboratory diagnosis of the associative course of trichurosis and toxocarosis in dogs.

Conclusions

High diagnostic efficacy of the Melnychuk method, which uses a combined calcium nitrate and sodium chloride solution as the flotation medium, was demonstrated in coproovoscopic diagnosis of trichurosis–toxocarosis mixed infection in dogs. The Melnychuk method showed higher performance, regardless of flotation exposure time, in simultaneous detection of *Trichuris* and *Toxocara* eggs compared with the Kotelnikov–Khrenov method (by 21.9–73.1 %, $P < 0.05$–$P < 0.01$), the Sorokova method (by 21.2–30.9 %, $P < 0.05$), and the Petrenko method (by 27.6 %, $P < 0.05$).

Conflict of interest
The authors declare no conflict of interest.

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