

## Effectiveness of the improved method of lifelong diagnostics trichurosis of sheep

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### Abstract

Today, the most accurate way to diagnose animal helminthiasis is laboratory research. Among them, lifelong coproovoscopy methods play a leading role in the study of the spread of gastrointestinal helminthiasis, particularly trichurosis, and in establishing the effectiveness of anthelmintics. Therefore, a promising research direction includes improving and testing modern methods of coproovoscopy for trichurosis in sheep. The work aimed to determine the diagnostic efficiency of the improved flotation method of coproovoscopy for trichurosis in sheep. In laboratory conditions, the effectiveness of well-known flotation methods and the proposed method of coproovoscopy in diagnosing sheep trichurosis were determined. The leading indicators of the effectiveness of laboratory methods were the indicator of the intensity of trichurous invasion, the coagulation ability of the flotation solution, and the crystallization time of a drop of flotation liquid on a glass slide. It was established that all methods of coproovoscopy used in the experiment have flotation properties relative to *Trichuris* eggs. However, the improved method of coproovoscopy showed a higher flotation ability concerning *Trichuris ovis* nematode eggs, where the proposed flotation liquid has pronounced coagulation properties concerning undigested feed residues, and also has a slow crystallization time of droplets on a slide, which is more than 50 min. The improved method uses a combined flotation liquid, whose main components were  $\text{Ca}(\text{NO}_3)_2$ ,  $\text{C}_{12}\text{H}_{22}\text{O}_{11}$ , and  $\text{NaCl}$ . The indicator of the intensity of trichuriasis invasion when using the improved method was  $67.0 \pm 17.5$  eggs in 1 g of feces and exceeded the number of trichuriasis eggs detected by flotation methods using  $\text{NaCl}$  – by 2.3 times ( $P < 0.001$ ),  $\text{C}_{12}\text{H}_{22}\text{O}_{11}$  – by 2.1 times ( $P < 0.001$ ),  $\text{NH}_4\text{NO}_3$  – 1.3 times ( $P < 0.01$ ),  $\text{C}_{12}\text{H}_{22}\text{O}_{11} + \text{NaCl}$  – 1.1 times. The obtained data on the effectiveness of the improved method of coproovoscopy allows us to recommend it for introduction into production for effective and accurate laboratory lifelong diagnosis of trichurosis in sheep.

**Keywords:** parasitology; trichurosis; sheep; coproovoscopy; diagnostic efficiency.

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### 1. Introduction

Scientific literature shows that the primary method of lifelong laboratory diagnosis of gastrointestinal helminthiasis in animals is coproscopic research (Cringoli, 2004; Glinz et al., 2010; Alvarado-Villalobos et al., 2017; Kruchynenko, 2021). For nematodes, flotation occupies an essential place among the known methods of coproovoscopy. These methods differ in the composition of the flotation liquid, the research technique, exposure, etc., and the diagnostic efficiency. In general, flotation methods are based on the use of a solution that has a higher density than parasite eggs, the morphological structure of which is used to identify the pathogen and establish the diagnosis of invasion (Quinn et al., 1980; Cringoli et al., 2004; Bowman & Lynn, 2009; Dakhno & Dakhno, 2010).

Many flotation and combined coproscopy methods have been proposed for diagnosing animal helminthiasis. It

should be noted that some authors obtain different results of efficiency when using one or another technique. Some scientists believe that solutions of individual salts with increased density increase helminth eggs' flotation ability. Others, on the contrary, contribute to the delay of egg flotation. In addition, according to the authors, the proposed methods of coproovoscopy should be convenient, inexpensive, and ergonomic (Yevstafieva, 2007a, 2007b; Cebra & Stang, 2008; Danko & Stybel, 2012; Halat et al., 2015).

In particular, there is a well-known method of coproovoscopy, according to Fülleborn, using a saturated solution of table salt as a flotation liquid. The method involves examining feces by dissolving them in a liquid, followed by filtration and microscopy of droplets from the surface film. Flotant consists of a saline solution, where table salt is used as a salt; its specific gravity is 1.18–1.2. At the same time, this method has disadvantages, namely: the low specific gravity of the flotation solution, the flotation

process takes a long time to perform – 45–60 min, the solution is ineffective against *Trichuris* eggs, the specific gravity of which ranges from 1.16 to 1.22, as well as low coagulation ability of such a solution, which leads to the floating to the surface of not only helminth eggs but also particles of undigested feed (Halat et al., 2004).

There is a modern method of lifelong coproovoscopic diagnosis of chicken capillariosis, which uses a flotation solution based on a combined solution of sugar and sodium chloride. The proposed method has a higher diagnostic efficiency concerning the causative agent of capillariosis compared to known methods: Fülleborn (by 21.5–47.4 %,  $P < 0.001$ ), Kotelnikov-Khrenov (14.7–15.5 %,  $P < 0.05$ ,  $P < 0.001$ ), Mallory (5.4–9.9 %,  $P < 0.05$ ) and the method using urea (3.0–6.3 %,  $P < 0.01$ ). At the same time, the method proposed by the authors is economically feasible, convenient to use, and ergonomic (Natiahla et al., 2017).

In addition, domestic scientists proposed the author's development of quantitative coproovoscopic diagnosis of nematodes of the alimentary canal of ruminants, where changes were made to the centrifuge-flotation technique. Thus, after centrifugation of the samples, the supernatant liquid is drained, and 8–12 ml of calcium nitrate flotation solution is added to the sediment in each test tube. The authors determined the diagnostic effectiveness of well-known and improved methods based on the number of detected nematode eggs in sheep. It was established that the author's development was more effective than Trach's methods – by 25.3–80.9 %, Lyashenko et al. – by 50–90.5 %, and Stoll – 17.6 %. Furthermore, the proposed method was more effective in terms of the average number of nematode eggs in the sample compared to the methods: Lyashenko et al. – by 86.9 % ( $P < 0.001$ ), Tracha – by 37.9 % ( $P < 0.01$ ), Stoll – by 27.7 % ( $P < 0.05$ ) (Melnychuk & Yuskiv, 2019; Melnychuk & Yuskiv, 2020).

Therefore, lifelong laboratory diagnosis of trichurosis in sheep is based on flotation methods of coproovoscopy and the detection of parasite eggs in feces. At the same time, a large number of methods have been proposed, which have different diagnostic effectiveness depending on many factors, such as the type of equipment, the composition of the flotation liquid, the methods of conducting, the causative agent of parasitosis, which must be isolated from the material. Therefore, it is crucial to establish the diagnostic effectiveness of well-known, modern methods of coproovoscopy for trichurosis in sheep and improve the existing ones, which will increase the effectiveness of lifelong laboratory diagnosis of trichurosis in sheep.

The work aimed to determine the diagnostic efficiency of the improved flotation method of coproovoscopy for trichurosis in sheep.

## 2. Materials and methods

The research was conducted throughout 2023 at the laboratory of the Department of Parasitology and Veterinary and Sanitary Examination of the Poltava State Agrarian University.

An experimental test was conducted to determine the diagnostic effectiveness of the proposed method of coproovoscopy for trichurosis in sheep. The proposed method was compared with well-known methods, namely Fülleborn, Mallory, Kotelnikova-Khrenova, and with the method of lifelong coproovoscopic diagnosis of chicken capillar-

iosis (Natiahla et al., 2017).

Feces from sheep free of parasite eggs were used for the experiment. One hundred specimens were added to a sample of feces weighing 1 g. eggs of trichuris, previously isolated from the terminal parts of the gonads of female nematodes of the species *Trichuris ovis*. With each flotation solution, 20 samples of feces with pre-introduced *Trichuris* eggs were examined. Samples were settled in each of the flotation solutions for 10 minutes. The number of detected *Trichuris* eggs was counted in 1 g of feces.

Evaluation of the methods was carried out according to the indicators of actual specific gravity, flotation capacity (number of positive samples and average number of detected *Trichuris* eggs), coagulation ability (• – a small amount of small extraneous remains; •• – simultaneous detection of a large number of small and a small number of considerable remains; ••• – a large number of both small and large foreign remains); crystallization time of a drop of flotation liquid on a glass slide at a temperature of 20 °C, min.

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.

## 3. Results and discussion

### 3.1. Results

The conducted studies revealed that all used methods of coproovoscopy have flotation properties relative to the eggs of nematodes of the *T. ovis* species. However, the proposed method of coproovoscopy for trichurosis in sheep showed a higher flotation capacity, where the indicator of the number of trichuriasis eggs detected reached  $67.0 \pm 17.5$  EPG (Table 1).

In comparison with well-known methods, the improved method turned out to be 2.3 times more effective ( $28.9 \pm 14.2$  EPG,  $P < 0.001$ ) than the Fülleborn method; by 2.1 times ( $31.8 \pm 10.1$  EPG,  $P < 0.001$ ) than the Mallory method; by 1.3 times ( $51.0 \pm 18.9$  EPG,  $P < 0.01$ ) than the Kotelnikov-Khrenov method. The lifelong coproovoscopic diagnosis of capillariosis in chickens also made it possible to detect *T. ovis* eggs; however, their number was 1.1 times less ( $56.0 \pm 21.1$  EPG) compared to the improved method (Fig. 1).

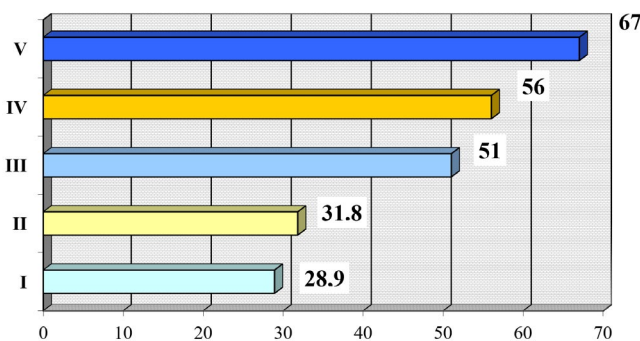


Fig. 1. Indicators of the intensity of trichurosis invasion using methods with the use: I – NaCl, II –  $C_{12}H_{22}O_{11}$ , III –  $NH_4NO_3$ , IV –  $C_{12}H_{22}O_{11} + NaCl$ , V –  $Ca(NO_3)_2 + C_{12}H_{22}O_{11} + NaCl$

**Table 1**

Effectiveness of the improved method of coproovoscopy for trichurosis in sheep (n = 20)

Method	Reagents used in the solution		Specific weight, g/l	II, EPG (M ± SD)	The presence of extraneous remains	Crystallization time, min
	chemical formula of salt	consumption rates, g/l				
Fülleborn	NaCl	420	1.19	28.9 ± 14.2***	••	≈ 5
Mallory	C <sub>12</sub> H <sub>22</sub> O <sub>11</sub>	1670	1.28	31.8 ± 10.1***	•	> 120
Kotelnikova-Khrenova	NH <sub>4</sub> NO <sub>3</sub>	1500	1.30	51.0 ± 18.9**	•••	≈ 4
The method of lifelong coproovoscopic diagnosis of capillariosis in chickens	C <sub>12</sub> H <sub>22</sub> O <sub>11</sub> + NaCl	1670 + 420	1.25	56.0 ± 21.1	••	> 50
Improved	Ca(NO <sub>3</sub> ) <sub>2</sub> + C <sub>12</sub> H <sub>22</sub> O <sub>11</sub> + NaCl	800 + 1670 + 420	1.32	67.0 ± 17.5	•	> 50

Note: P < 0.01; P < 0.001 – relative to the improved method

Analyzing the coagulation ability of flotation solutions used in the methods of coproovoscopy concerning undigested fodder remains, its highest manifestation was established when using the Mallory method and the improved method of coproovoscopy for trichurosis of sheep, in which a small number of small remains of undigested fodder floated to the surface. When applying the Fülleborn method and the method of lifelong coproovoscopic diagnosis of chicken capillariosis, a large number of small and a small number of significant remains were simultaneously detected. A low level of coagulation ability of the flotation solution during coproovoscopy was established when using the Kotelnikov-Khrenov method.

It was also found that the crystallization time of a drop of the flotation solution used in the improved method and the method of lifelong coproovoscopic diagnosis of chicken capillariosis at a temperature of 20 °C is more than 50 minutes. When using the Fülleborn and Kotelnikov-Khrenov methods, the crystallization time of a drop of flotation solution is 5 and 4 minutes, respectively. The crystallization time was more than 120 min when applying the Mallory method.

### 3.2. Discussion

The scientific community proves that the methods of helminthoscopy, which are based on the detection of helminth eggs, are used for lifelong diagnosis of gastrointestinal nematodes in animals and humans. Several methods are used in practical conditions, mainly based on egg-floating principles. These are flotation methods and their modifications, which are based on the principle of the difference in the specific gravity of eggs and hypertonic saline solutions (Knopp et al., 2009; Dakhno & Dakhno, 2010; Lobos-Ovalle et al., 2021). At the same time, a significant number of researchers propose improved, more modern, and effective methods of coproovoscopy for individual parasitosis (Yevstafieva, 2007a; Manoilo & Yevstafieva, 2016; Starodub, 2019). Therefore, the development and testing of an improved method of coproovoscopy for trichurosis in sheep is urgent. Analyzing the specific gravity of the flotation solutions used in the experiment, it can be noted that it was the highest in the proposed method – 1.32. This makes it possible to rise to the surface of *Trichuris* eggs; the specific gravity ranges from 1.16 to 1.22. The combination of its components provides such a high specific gravity of the flotation solution – Ca(NO<sub>3</sub>)<sub>2</sub>, C<sub>12</sub>H<sub>22</sub>O<sub>11</sub>, and NaCl. It was proved that the improved method of coproovoscopy for trichurosis of sheep shows a high indicator of flotation abil-

ity concerning *T. ovis* eggs – 67.0 ± 17.5 EPG for exposure of 10 min and exceeded (P < 0.01...P < 0.001) II indicators obtained by use of Fülleborn, Mallory, Kotelnikova-Khrenov methods and lifelong coproovoscopic diagnosis of chicken capillariosis. Also, the proposed flotation solution has pronounced coagulation properties. It has a slow crystallization time of droplets on a glass slide > 50 min, which is enough for conducting a perfect study of the sample or serial studies of many samples simultaneously.

The high diagnostic efficiency of the method of quantitative coproovoscopic diagnosis of nematodes of the alimentary canal of ruminants, where the use of calcium nitrate as a component of the flotation liquid is suggested, is evidenced by the works of domestic authors. This proves the relevance of studying the use of this salt in flotation liquids during coproovoscopic studies (Melnychuk & Yuskiv, 2019; Melnychuk & Yuskiv, 2020).

The obtained data on the effectiveness of the improved method of coproovoscopy allows us to recommend it for introduction into production for effective and accurate laboratory lifelong diagnosis of trichurosis in sheep.

### 4. Conclusions

The positive effect of the improved method of coproovoscopy for sheep trichurosis consists in the use of a flotation solution that has a sufficiently high specific gravity, has pronounced coagulation properties relative to undigested feed residues and has a slow crystallization time during research. It was established that the improved method exceeds the efficiency of methods using NaCl – by 2.3 times (P < 0.001), C<sub>12</sub>H<sub>22</sub>O<sub>11</sub> – by 2.1 times (P < 0.001), NH<sub>4</sub>NO<sub>3</sub> – by 1.3 times (P < 0.01), C<sub>12</sub>H<sub>22</sub>O<sub>11</sub> + NaCl – 1.1 times.

### Conflict of interest

The author claims no conflict of interest.

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