Received: 19.10.2024 | *Accepted: 28.10.2024*

Veterinary Sciences

doi: 10.31210/spi2024.27.04.30
UDC 636.7:616.99:595.132:330.341.1
ORIGINAL ARTICLE

Scientific Progress & Innovations
https://journals.pdaa.edu.ua/visnyk

27 (4)
2024

Efficiency of the improved method of laboratory diagnostics of canine cystoisosporosis

R. Suvorov[1] ✉ | V. Melnychuk[1, 2]

Article info

Correspondence Author
R. Suvorov
E-mail:
romeovinnie88@gmail.com

[1] Poltava State Agrarian University,
Skovorody Str., 1/3,
Poltava, 36003, Ukraine

[2] Institute of Veterinary Medicine of the National Academy of Agrarian Sciences of Ukraine,
Donetska Str., 30, Kyiv,
03151, Ukraine

Citation: Suvorov, R., & Melnychuk, V. (2024). Efficiency of the improved method of laboratory diagnostics of canine cystoisosporosis. *Scientific Progress & Innovations*, 27 (4), 176–180. doi: 10.31210/spi2024.27.04.30

It is known that the study of the species composition of protozooses in dogs parasitizing the digestive tract, their distribution, extensiveness and intensity of invasion, as well as age, seasonal dynamics and breed susceptibility to parasite infection is necessary in the study of epizootology and epidemiology of invasions of domestic carnivores. This is possible only with the use of highly effective, sensitive, easy-to-use laboratory methods of coproovoscopy. The aim of the work was to improve, test and determine the effectiveness of the method of laboratory diagnosis of canine cystoisosporosis. The proposed method refers to the field of veterinary medicine, namely, veterinary parasitology, to methods of coproscopy, in particular to methods of detecting cystoisosporosis oocysts in dogs. We conducted tests of flotation methods of coproovoscopy, such as: Fulleborn's, Kotelnikov-Khrenov's, the method of coproovoscopy for trichurosis in sheep and the proposed method with an improved composition of the flotant for the laboratory diagnosis of canine cystoisosporosis. It was found that when using the above methods, oocysts of cystoisospores were detected 100 % of the time. At the same time, indicators of the intensity of cystoisosporous invasion when using different methods of laboratory diagnostics differed significantly. When using the Fulleborn method, the indicators of the intensity of cystoisosporous invasion were on average 162.1 oocysts/g, Kotelnikov-Khrenov's – 215.2 oocysts/g, the coproovoscopy method for sheep trichurosis – 244.8 oocysts/g, the proposed method – 277.3 oocysts/g. The proposed method of laboratory diagnosis of cystoisosporosis in dogs turned out to be more effective compared to Fulleborn's method by 41.5 %, Kotelnikov-Khrenov's method by 22.4 %, and the method of coproovoscopy for trichurosis in sheep by 11.7 %. The proposed method and the method of coproovoscopy for trichurosis in sheep showed the highest coagulation properties relative to undigested feed residues. At the same time, a small amount of small remains of undigested feed floated to the surface of the floating solutions. The obtained results make it possible to recommend the proposed method for more effective lifelong laboratory diagnosis of cystoisosporosis in dogs.

Keywords: parasitology, cystoisosporosis, dogs, coproovoscopy, effectiveness.

Ефективність удосконаленого способу лабораторної діагностики цистоізоспорозу собак

Р. С. Суворов[1] | В. В. Мельничук[1, 2]

[1] Полтавський державний аграрний університет,
м. Полтава, Україна

[2] Інститут ветеринарної медицини Національної академії аграрних наук України,
м. Київ, Україна

Відомо, що вивчення видового складу протозоозів у собак, що паразитують у травному тракті, їх поширення, екстенсивності та інтенсивності інвазії, а також вікової, сезонної динаміки та породної сприйнятливості до зараженості паразитами необхідно у дослідженні епізоотології та епідеміології інвазій домашніх м'ясоїдних тварин. Це можливе лише за використання високоефективних, чутливих, зручних у використанні лабораторних методів копроовоскопії. Метою роботи було удосконалити, випробувати та визначити ефективність способу лабораторної діагностики цистоізоспорозу собак. Запропонований спосіб відноситься до галузі ветеринарної медицини, а саме – ветеринарної паразитології, до способів копроскопії, зокрема способів виявлення ооцист цистоізоспорозу собак. Проводили випробування флотаційних методів копроовоскопії таких як: Фюллеборна, Котельникова-Хренова, способу копроовоскопії за трихурозу овець та запропонованого способу з удосконаленим складом флотанту при лабораторній діагностиці цистоізоспорозу собак. Виявлено, що при використанні вищезазначених методів 100 %-во виявляли ооцисти цистоізоспор. Водночас, показники інтенсивності цистоізоспорозної інвазії при використанні різних методів лабораторної діагностики значно різнилися. При використанні методу Фюллеборна показники інтенсивності цистоізоспорозної інвазії в середньому становили 162,1 ооцист/г, Котельникова-Хренова – 215,2 ооцист/г, способу копроовоскопії за трихурозу овець – 244,8 ооцист/г, запропонованого способу – 277,3 ооцист/г. Запропонований спосіб лабораторної діагностики цистоізоспорозу у собак виявився ефективнішим порівняно зі способом Фюллеборна на 41,5 %, Котельникова-Хренова – на 22,4 %, способу копроовоскопії за трихурозу овець – на 11,7 %. Запропонований спосіб та спосіб копроовоскопії за трихурозу овець проявили найвищі коагуляційні властивості відносно неперетравлених решток корму. При цьому на поверхню флотантів розчинів спливала незначна кількість дрібних решток неперетравленого корму. Отримані результати дають можливість рекомендувати запропонований спосіб для більш ефективної зажиттєвої лабораторної діагностики цистоізоспорозу собак.

Ключові слова: паразитологія, цистоізоспороз, собаки, копроовоскопія, ефективність.

Бібліографічний опис для цитування: *Суворов Р. С., Мельничук В. В.* Ефективність удосконаленого способу лабораторної діагностики цистоізоспорозу собак. *Scientific Progress & Innovations*. 2024. № 27 (4). С. 176–180.

Introduction

To ensure the health and well-being of domestic carnivores, coproscopic examination for parasite eggs and oocysts is an important part of lifelong diagnosis. Many different procedures and techniques are used, each with its own advantages and limitations. Examination of faecal smears is useful for detecting motile protozoa, examination of feces using sedimentation methods reveals high specific gravity helminth eggs that do not float well in a flotation solution [1–6].

The methods most commonly used to detect parasite eggs and oocysts are flotation methods, which are based on the difference in the specific gravity of the eggs or oocysts and the flotation solution. The specific gravity of most parasite eggs or oocysts is known to range from 1.05 to 1.23. It has been proven that for eggs or oocysts of parasites to float well, the specific gravity of the flotation solution should be greater than that of the eggs. Ideally, all helminth eggs and protozoan oocysts should float and retain their morphological structure, while faecal remains should settle to the bottom in the selected flotation solution. The scientists note that flotation solutions are made by adding a measured amount of salt or sugar to a certain amount of water to produce a solution with a desired specific gravity. There are ordinary, well-known, most common flotation solutions, which include saturated sodium chloride (NaCl; 1.18), sugar (1.27–1.33), sodium nitrate (NaNO3; 1.18–1.20), magnesium sulfate (MgSO4; 1.20), zinc sulfate (ZnSO4; 1.20) etc. These solutions are reasonably effective, easy to manufacture or commercially available, and relatively inexpensive. Flotation procedures range from simple to complex. The simplest procedure involves mixing a small amount of feces with a flotation solution in a beaker or centrifuge tube, then adding the solution until the beaker is nearly full. Then it is left alone until the eggs float to the surface of the sample and are collected using a parasitological loop [7–11].

Researchers believe that when choosing methods of flotation coproovoscopy, it is necessary to take into account some factors, namely: specific gravity of the flotation solution, viscosity, volume of the flotation solution used, the use of an additional centrifugation stage, the duration and speed of centrifugation, exposure to sedimentation, preservation of the morphological structure of the eggs or oocysts of parasites [12–15]. In this regard, it is important to test modern and new methods of coproovoscopy, to establish their effectiveness for a certain type of pathogen.

The aim of the study

The purpose of the research was to improve, test and determine the effectiveness of the method of laboratory diagnosis of canine cystoisosporosis.

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 the conditions of the private veterinary clinic "Dovira" (Kharkiv).

In order to establish the effectiveness of the proposed method of coproscopic examination of dogs for the presence of cystoisospores oocysts, a comparison was made between the proposed method and the well-known Fülleborn, Kotelnikova-Khrenova [16], method of coproovoscopy for sheep trichurosis [17].

Feces from dogs spontaneously infected with cystoisosporosis were used for the experiment. With each flotation solution, 15 samples of feces were examined using the technique proposed in the method of coproscopic examination of dogs for the presence of cystoisospores oocysts. Samples were settled in each of the flotation solutions for 10 minutes. Counting of the number of detected oocysts of cystoisospores was carried out 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 (the number of positive samples and the average number of detected cystoisospes oocysts); 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;

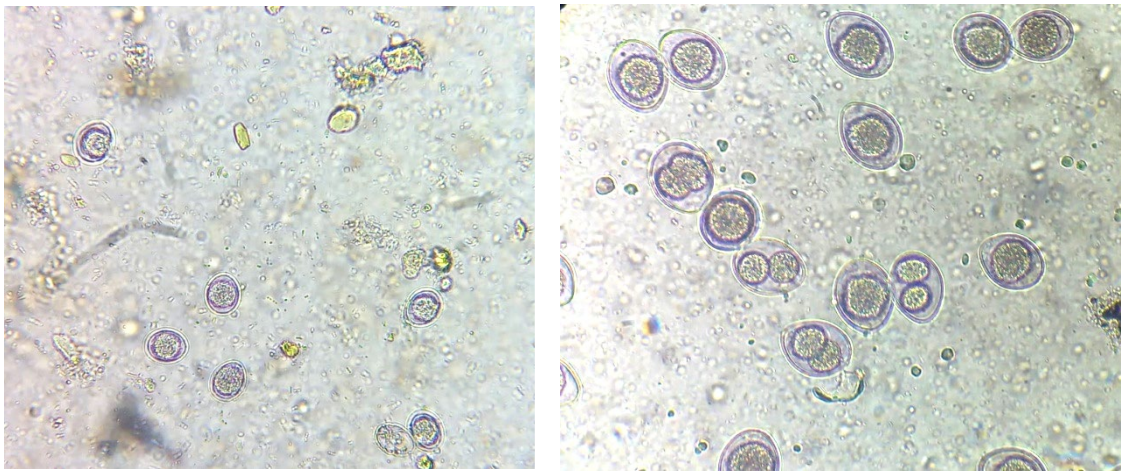
••• – a large number of both small and significant foreign remains.

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 all methods of coproovoscopy used in the experiment allowed the detection of cystoisospores oocysts in dog feces (***Fig. 1***) in 100 % of cases, where cystoisospores oocysts were detected in 15 cases out of 15 examined copro samples (***Fig. 2***).

At the same time, indicators of the intensity of cystoisosporous invasion when using different methods of laboratory diagnostics differed significantly. When using the Fulleborn method, the indicators of the intensity of cystoisosporous invasion were on average 162.1±25.6 oocysts/g, Kotelnikov-Khrenov – 215.2±34.3 oocysts/g, the method of coproovoscopy for sheep trichurosis – 244.8±28.3 oocysts/g, of the proposed method – 277.3±48.7 oocyst/g. Moreover, the proposed method turned out to be the most effective in terms of indicators of the intensity of cystisosporous invasion, where it was more effective compared to the Fulleborn method by 41.5 %, $P<0.001$ (***Fig. 3***), Kotelnikov-Khrenov – by 22.4 %, $P<0.001$ (***Fig. 4***), the method of coproovoscopy for sheep trichurosis – by 11.7 %, $P<0.05$ (***Fig. 5***).



a b

Fig. 1. Isospores oocysts detected during coproscopic examination of dogs using tested methods: a – × 400; b – × 150

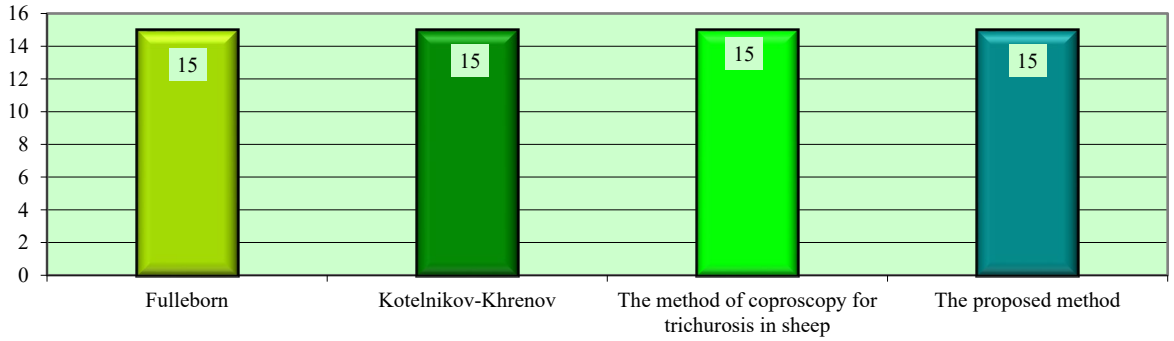


Fig. 2. The number of detected positive samples during laboratory diagnostics canine cystoisosporosis

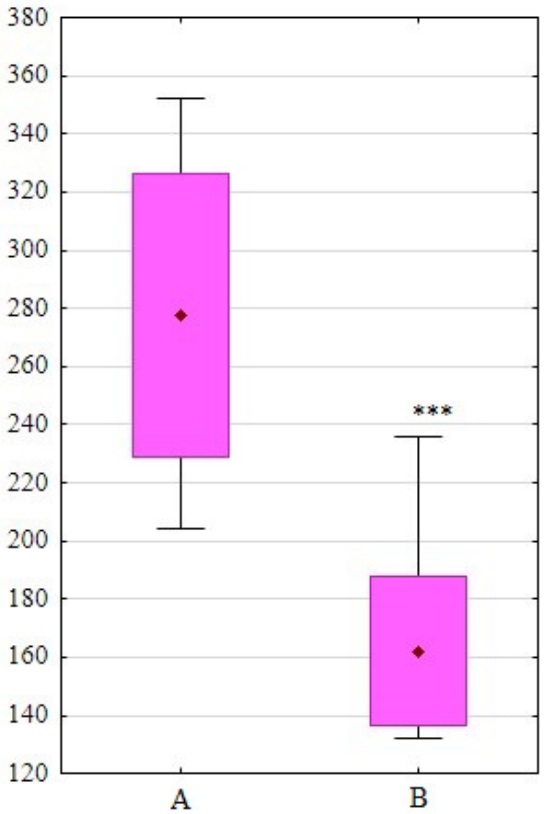


Fig. 3. Comparative efficiency of coproscopy methods for cystoisosporosis in dogs (n=15): A – proposed method, B – Fulleborn's method; P<0.001 – relative to A

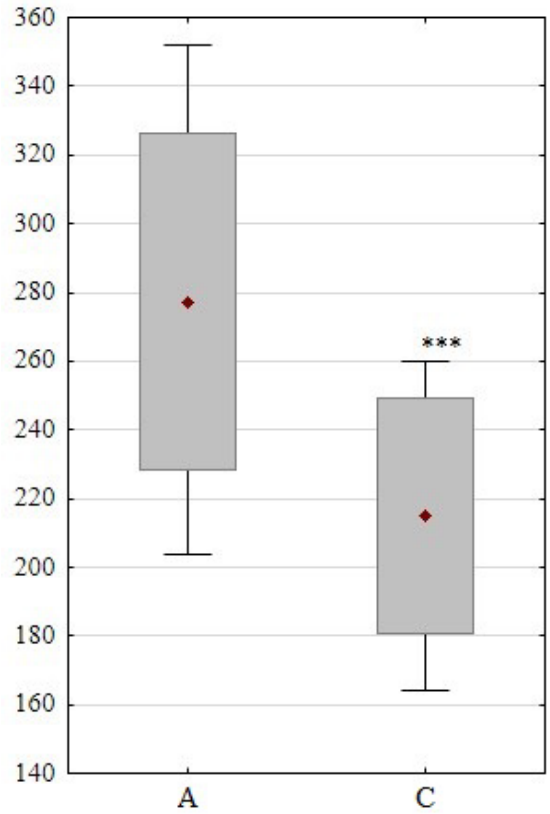


Fig. 4. Comparative efficiency of coproscopy methods for cystoisosporosis in dogs (n=15): A – proposed method, C – Kotelnikov-Khrenov's method; P<0.001 – relative to A

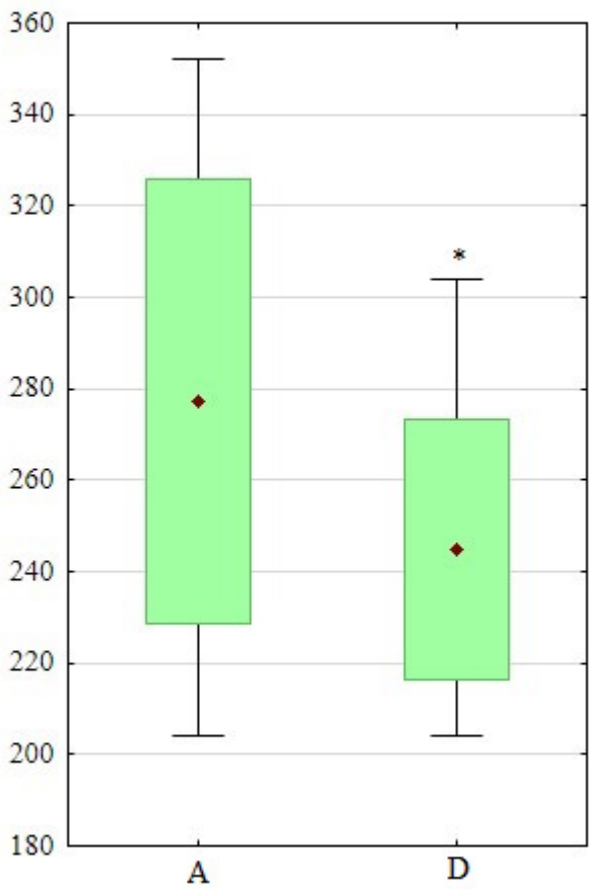


Fig. 5. Comparative effectiveness of methods of coprooscopy for cystoisosporosis in dogs (n=15): A – proposed method, D – method of coproovoscopy for trichurosis of sheep; P<0.05 – relative to A

The proposed method and the method of coproovoscopy for trichurosis in sheep showed the highest coagulation properties relative to undigested feed residues. At the same time, a small amount of small remains of undigested feed floated to the surface of the floating solutions.

Scientists testify that in order to ensure the health and well-being of domestic carnivores, it is necessary to carry out lifelong diagnostic coproscopic studies of animals, where the effectiveness of one or another method ensures the timeliness and accuracy of diagnosis [2–5, 18–20]. Therefore, it is urgent to improve, test and determine the effectiveness of the method of laboratory diagnosis of canine cystoisosporosis.

The proposed method refers to the field of veterinary medicine, namely, veterinary parasitology, to methods of coproscopy, in particular to methods of detecting canine cystoisosporosis oocysts. It was found that when using the above methods, oocysts of cystoisospores were detected 100 % of the time. At the same time, the proposed method (277.3±48.7 oocysts/g) turned out to be the most effective in relation to indicators of the intensity of cystisosporous invasion, where it was more effective compared to the Fulleborn method by 41.5 % (162.1±25.6 oocysts/g, P<0.001), Kotelnikov-Khrenov's – by 22.4 % (215.2±34.3 oocyst/g, P<0.001), the method of coproovoscopy for trichurosis in sheep – by 11.7 % (244.8±28.3 oocysts/g, P<0.05). The proposed method and the method of coproovoscopy for sheep trichurosis showed the highest coagulation properties relative to undigested feed residues.

In the scientific literature, there is a report on the high efficiency of the improved method of coproovoscopy proposed by the authors for trichurosis in sheep. It exceeds the effectiveness of Fulleborn's methods in lifelong diagnosis of infestation by 2.3 times (P<0.001), Mallory's methods by 2.1 times (P<0.001), Kotelnikov-Khrenov's – 1.3 times (p<0.01), Galat and Melnychuk's – in 1.5 times (P<0.01), Manoilo's – 1.3 times (P<0.05), Dakhno's – 1.4 times (P<0.01) [17].

The obtained results make it possible to recommend the proposed method for more effective lifelong laboratory diagnosis of cystoisosporosis in dogs.

Conclusions

The positive effect of the proposed method of coproovoscopy for cystoisosporosis in dogs 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 allows cystoisospores oocysts to rise to the surface. It was established that the proposed method exceeds the efficiency of the Fulleborn's methods – by 41.5 % (P<0.001), Kotelnikov-Khrenov's – by 22.4 % (P<0.001), the method of coproovoscopy for sheep trichurosis – by 11.7 % (P<0.05).

Conflict of interest

The authors state that there is no conflict of interest.

References

1. Sardella, N. H. (2012): Evaluación de la eficacia de la flotación con cloruro de zinc como método de enriqueci- miento para la recuperación de huevos de enteroparásitos. *Revista Argentina De Parasitologia*, 1, 164.
2. Alvarado-Villalobos, M. A., Cringoli, G., Maurelli, M. P., Cambou, A., Rinaldi, L., Barbachano-Guerrero, A., Guevara, R., Chapman, C. A., & Serio-Silva, J. C. (2017). Flotation techniques (FLOTAC and mini-FLOTAC) for detecting gastrointestinal parasites in howler monkeys. *Parasites & Vectors*, 10 (1). https://doi.org/10.1186/s13071-017-2532-7
3. Kruchynenko, O. V. (2021). Comparison of coproovoscopic diagnostic methods of V. N. Trach, McMaster and Mini-Flotac for hens' infestation with *Ascaridia galli* and *Trichostrongylus tenuis*. *Bulletin of Poltava State Agrarian Academy*, 2, 194–199. https://doi.org/10.31210/visnyk2021.02.24
4. Bowman, D. D., & Lynn, R. C. (2009). *Diagnostic parasitology. Georgi's parasitology for veterinarians. 9th ed.* St-Louis: Elsevier.
5. Mathison, B. A., Kohan, J. L., Walker, J. F., Smith, R. B., Ardon, O., & Couturier, M. R. (2020). Detection of intestinal protozoa in trichrome-stained stool specimens by use of a deep convolutional neural network. *Journal of Clinical Microbiology*, 58 (6). https://doi.org/10.1128/jcm.02053-19
6. Foreyt, W. J. (1989). Diagnostic parasitology. V*eterinary Clinics of North America: Small Animal Practice*, 19 (5), 979–1000. https://doi.org/10.1016/s0195-5616(89)50107-4
7. Dakhno, I. S., & Dakhno, Yu. I.(2010). *Ekolohichna helmintolohiia*. Sumy [in Ukrainian]

8. Taglioretti, V., Sardella, N., & Fugassa, M. (2014). Effectiveness of coproscopic concentration techniques. *Helminthologia*, 51 (3), 210–214. https://doi.org/10.2478/s11687-014-0231-x
9. Glinz, D., Silué, K. D., Knopp, S., Lohourignon, L. K., Yao, K. P., Steinmann, P., Rinaldi, L., Cringoli, G., N'Goran, E. K., & Utzinger, J. (2010). Comparing diagnostic accuracy of kato-katz, koga agar plate, ether-concentration, and FLOTAC for *Schistosoma mansoni* and soil-transmitted helminths. *PLoS Neglected Tropical Diseases*, 4 (7), e754. https://doi.org/10.1371/journal.pntd.0000754
10. Ghafar, A., Abbas, G., King, J., Jacobson, C., Hughes, K. J., El-Hage, C., Beasley, A., Bauquier, J., Wilkes, E. J. A., Hurley, J., Cudmore, L., Carrigan, P., Tennent-Brown, B., Nielsen, M. K., Gauci, C. G., Beveridge, I., & Jabbar, A. (2021). Comparative studies on faecal egg counting techniques used for the detection of gastrointestinal parasites of equines: A systematic review. *Current Research in Parasitology & Vector-Borne Diseases*, 1, 100046. https://doi.org/10.1016/j.crpvbd.2021.100046
11. Gates, M. C., & Nolan, T. J. (2009). Comparison of passive fecal flotation run by veterinary students to zinc-sulfate centrifugation flotation run in a diagnostic parasitology laboratory. *Journal of Parasitology*, 95 (5), 1213–1214. https://doi.org/10.1645/ge-2058.1
12. O'Grady, M. R., & Slocombe, J. O. (1980). An investigation of variables in a fecal flotation technique. *Canadian Journal of Comparative Medicine*, 44 (2), 148–157.
13. Cringoli, G., Rinaldi, L., Veneziano, V., Capelli, G., & Scala, A. (2004). The influence of flotation solution, sample dilution and the choice of McMaster slide area (volume) on the reliability of the McMaster technique in estimating the faecal egg counts of gastrointestinal strongyles and *Dicrocoelium dendriticum* in sheep. *Veterinary Parasitology*, 123 (1–2), 121–131. https://doi.org/10.1016/j.vetpar.2004.05.021
14. Cringoli, G., Rinaldi, L., Maurelli, M. P., & Utzinger, J. (2010). FLOTAC: new multivalent techniques for qualitative and quantitative copromicroscopic diagnosis of parasites in animals and humans. *Nature Protocols*, 5 (3), 503–515. https://doi.org/10.1038/nprot.2009.235
15. Quinn, R., Smith, H. V., Bruce, R. G., & Girdwood, R. W. A. (1980). Studies on the incidence of *Toxocara* and *Toxascaris* spp. ova in the environment. 1. A comparison of flotation procedures for recovering *Toxocara* spp. ova from soil. *Journal of Hygiene*, 84 (1), 83–89. https://doi.org/10.1017/s0022172400026553
16. Kotelnikov, G. A. (1974). *Diagnostics of animal helminthiasis*. Koloss, Moscow.
17. Petrenko, M. (2023). Effectiveness of the improved method of lifelong diagnostics trichurosis of sheep. *Ukrainian Journal of Veterinary and Agricultural Sciences*, 6 (3), 13–16. https://doi.org/10.32718/ujvas6-3.03
18. Segura, J., Alcala-Canto, Y., Figueroa, A., Del Rio, V., & Salgado-Maldonado, G. (2023). A Simple fecal flotation method for diagnosing zoonotic nematodes under field and laboratory conditions. *Journal of Visualized Experiments*, 202. https://doi.org/10.3791/66110
19. Liccioli, S., Catalano, S., Kutz, S. J., Lejeune, M., Verocai, G. G., Duignan, P. J., Fuentealba, C., Ruckstuhl, K. E., & Massolo, A. (2012). Sensitivity of double centrifugation sugar fecal flotation for detecting intestinal helminths in coyotes (*Canis latrans*). *Journal of Wildlife Diseases*, 48 (3), 717–723. https://doi.org/10.7589/0090-3558-48.3.717
20. Inpankaew, T., Schär, F., Khieu, V., Muth, S., Dalsgaard, A., Marti, H., Traub, R. J., & Odermatt, P. (2014). Simple fecal flotation is a superior alternative to guadruple Kato Katz smear examination for the detection of hookworm eggs in human stool. *PLoS Neglected Tropical Diseases*, 8 (12), e3313. https://doi.org/10.1371/journal.pntd.0003313

ORCID

R. Suvorov https://orcid.org/0009-0002-9391-9341
V. Melnychuk https://orcid.org/0000-0003-1927-1065