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The efficacy of the method for producing micro-preparations from *Ctenocephalides felis* fleas, which parasitize on cats

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Fleas of the genus *Ctenocephalides* attack mammals such as carnivores, lagomorphs, marsupials, primates, rodents, and ungulates. Among arthropods, the fleas of *Ctenocephalides felis* species are the most important ectoparasites of cats worldwide, which negatively affect the animal's body and are carriers of dangerous diseases. The parasites cause allergic dermatitis, anemia, especially in young animals. Therefore, timely detection and identification of ctenocephalidosis pathogens is relevant, the effectiveness of which depend on the high-quality production of preparations of micro-preparations from parasitic insects. The aim of the work was to improve and test the method for making micro-preparations from *C. felis* fleas, which parasitize on cats. The study was conducted in the conditions "Yashma" private veterinary clinic (the city of Kremenchuk) and the laboratory of the Department of Parasitology and Veterinary and Sanitary Expert Examination of Poltava State Agrarian University (the city of Poltava). The proposed method is related to veterinary medicine, namely veterinary parasitology, and can be used for making anatomical and morphological preparations from *C. felis* fleas with the aim of studying their morphological and metric features in the structure. It was found that the use of the proposed method for making total micro-preparations from fleas turned out to be more effective concerning the general assessment of the intensity of coloring of the fleas' body morphological structures (by 72.51 %, $P<0.001$) and the time required to make one micro-preparation (by 80.39 %, $P<0.001$) compared to similar indicators in the method of preparing pathogens of Mallophaga order *in toto*. The use of the proposed method allowed to obtain good, uniform coloring of all the fleas' antennal segments, as evidenced by the obtained high average score (4.91). At the same time, the use of the method of preparing the pathogens of Mallophaga order *in toto* led to oversaturation of the above-mentioned morphological structures with dye, as indicated by the obtained low score (1.35). The obtained results allow us to recommend the proposed method of producing micro-preparations from *Ctenocephalides felis* fleas to increase the efficacy of microscopic studies for feline ctenocephalidosis.

Keywords: parasitology, *Ctenocephalides felis*, cats, diagnostics, micro-preparations, efficacy.

Ефективність способу виготовлення мікропрепаратів з бліх *Ctenocephalides felis*, що паразитують у котів

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Блохи роду *Ctenocephalides* нападають на таких ссавців, як хижі, зайцеподібні, сумчасті, примати, гризуни, копитні. Серед членистоногих вид бліх *Ctenocephalides felis* є найважливішим ектопаразитом котів у всьому світі, які негативно впливають на організм тварини, та є переносниками небезпечних хвороб. Паразити викликають алергічний дерматит, анемію, особливо у молодих тварин. Тому, актуальним є своєчасне виявлення та ідентифікація збудників ктеноцефальозу, ефективність яких залежить від якісного виготовлення мікропрепаратів з паразитичних комах. Метою роботи було удосконалення та випробування способу виготовлення мікропрепаратів з бліх *C. felis*, що паразитують у котів. Дослідження проводили в умовах приватної ветеринарної клініки «Яшма» (м. Кременчук) та лабораторії кафедри паразитології та ветеринарно-санітарної експертизи Полтавського державного аграрного університету (м. Полтава). Запропонований спосіб відноситься до ветеринарної медицини, а саме – ветеринарної паразитології, і може бути використаний для приготування анатомо-морфологічних препаратів бліх *C. felis* з метою вивчення їх морфологічних й метричних особливостей у будові. Встановлено, що використання запропонованого способу для виготовлення тотальних мікропрепаратів з бліх виявилося ефективнішим за загальною оцінкою інтенсивності забарвленості морфологічних структур тіла бліх (на 72,51 %, $P<0,001$) та часом, що йде на виготовлення одного мікропрепарату (на 80,39 %, $P<0,001$) порівняно з аналогічними показниками у способі приготування збудників ряду Mallophaga *in toto*. Застосування запропонованого способу дозволило отримати добре, рівномірне забарвлення всіх члеників антен бліх, про що свідчить отриманий високий середній бал (4,91). Водночас, застосування способу приготування збудників ряду Mallophaga *in toto* призводило до перенасичення фарбою вищенаведених морфологічних структур, на що вказує отриманий низький бал (1,35). Отримані результати дозволяють рекомендувати запропонований спосіб виготовлення мікропрепаратів з бліх *Ctenocephalides felis* для підвищення ефективності проведення мікроскопічних досліджень за ктеноцефальозу котів.

Ключові слова: паразитологія, *Ctenocephalides felis*, коти, діагностика, мікропрепарати, ефективність.

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Introduction

Ctenocephalides felis is one of the most important ectoparasites of dogs and cats worldwide due to its geographical spread, dual parasitological action as an invasive agent and transmitter of diseases, economic losses, and acquired resistance to common insecticides. *C. felis* is more adaptable than *C. canis*, infecting more host species and therefore establishing itself over larger areas. Besides, fleas of this species pose a serious threat to humans [1–6]. *C. felis* ectoparasites are known to have the ability to transmit bacteria and viruses such as *Bartonella*, hemotropic *Mycoplasma, Rickettsia,* and *Wolbachia* through salivary inoculation during feeding [7–10]. They are also intermediate hosts for helminthes such as *Dipylidium caninum* and *Acanthocheilonema reconditum* [11].

Fleas are very effective at infecting their hosts by jumping from the environment onto the animal or from animal to animal [12, 13]. Immediately after reaching the animals' bodies, fleas begin to feed on blood [14–16]. As a result, severe itching, dermatitis, and anemia may occur in kittens [17, 18].

Therefore, timely detection and identification of ctenocephalidosis pathogens is relevant, the effectiveness of which also depends on the high-quality production of micro-preparations from parasitic insects. In particular, the method is known of making permanent preparations of the fleas of *Ctenocephalides* genus in toto, according to which the fleas selected and fixed in 70 % ethyl alcohol solution are transferred for 12 hours to a glass with 3 % hydrogen peroxide solution, having previously pierced the chitinous covering in the middle third segment of the abdomen with a needle. Then the insects are removed from the hydrogen peroxide and thoroughly washed with water. In order to dehydrate the insects, they are gradually passed through alcohols of increasing concentration (70.0 %, 80.0 % and 96.0 %), in each of which the object is kept for 60 minutes. Then the fleas are carefully transferred to a cavity glass slide, into which a combined mixture of juniper and caryophyllus oils in a ratio of 1 : 1 is previously added and left alone for 4–5 hours. After that, the fleas are placed on the glass slide, where a small amount of Canadian balsam is applied and covered with a cover slip [19].

Also, the researchers propose to dip the fleas in 10 % potassium hydroxide for 2 hours at making micro-preparations, after that to dehydrate the fleas using a series of ethanol concentrations (50–100 %). In the future, the authors suggest treating the sample with aniline oil and washing it with xylene. Then the sample is placed on a glass slide, applied a small amount of Canada balsam and covered with a cover slip [20].

The aim of the study

The aim of the research was the improving and testing of the method for producing micro-preparations from *C. felis* fleas that parasitize on cats.

Materials and methods

The research was conducted during 2025 in the conditions of "Yashma" private veterinary clinic (Kremenchuk) and the laboratory of the Department of Parasitology and Veterinary and Sanitary Expert Examination of Poltava State Agrarian University (Poltava).

In order to find the optimal time for staining the morphological structures of *C. felis* fleas, 80 insect specimens were examined using the proposed method for making micro-preparations. The degree of visibility of the fleas' morphological body structures, which have a naturally weak (the frontal, second and third antennal segments and parietal bristles) and pronounced coloring (main ctenidium and maxillary palps), was determined by microscopy at magnification ×40 and ×100. The degree of visibility was conditionally divided into high, satisfactory, unsatisfactory, as a result of insufficient coloring; unsatisfactory, because of the oversaturation with dye.

To establish the effectiveness of the method of producing permanent preparations from fleas of *C. felis* genus it was compared with the method of preparing the pathogens of Mallophaga order *in toto* [21]. In all, 40 flea specimens (20 by each method) were examined, which was resumed on the bodies of cats that came to the veterinary clinic. The evaluation was conducted according to the intensity of staining indicators of the fleas' morphological body structures that have naturally weak coloring in accordance with the 5-point scale (where: 1 is oversaturation with dye, 2 – barely noticeable coloring, 3 – insignificant coloring, 4 – satisfactory uneven coloring, 5 – good uniform coloring) and according to the time spent on making one total micro-preparation.

The mathematical analysis of the obtained data was carried out using the Microsoft EXCEL applied program package by determining the arithmetic mean (M), standard deviation (SD) and probability level (P) applying the one-way analysis of variance technique using Fisher's criterion.

Results and discussion

The conducted studies to determine the optimal modes for implementing the proposed method have established that a high degree of the fleas' morphological body structures visibility with naturally weak and pronounced body coloring is ensured by the insects' being kept in the proposed environment for 5–6 minutes (***Table 1***).

During this period, the morphological structures of the body with naturally weak coloring, namely: the frontal, second, and third antennal segments (***Fig. 1***) and parietal bristles (***Fig. 2***) have time to be colored. At the same time, there is no oversaturation with dye of the fleas' body morphological structures with naturally pronounced coloring, namely: main ctenidium and maxillary palps (***Fig. 3***).

Table 1

The degree of the morphological body structures visibility of *Ctenocephalides felis* genus fleas using the proposed method (N=80)

Morphological structures of the body	Exposure, minutes			
	1–2	3–4	5–6	7–8
Morphological structures with naturally weak coloring				
Frontal antennal segment	*	**	***	***
Second antennal segment	*	***	***	***
Third antennal segment	**	***	***	***
Parietal bristles	**	***	***	●
Morphological structures with naturally pronounced coloring				
Main ctenidium	***	***	***	●
Maxillary palps	***	***	***	●

Note: degree of visibility: *** – high; ** – satisfactory; * – unsatisfactory, as a result of insufficient coloring; ● – unsatisfactory, as a result of dye oversaturation.

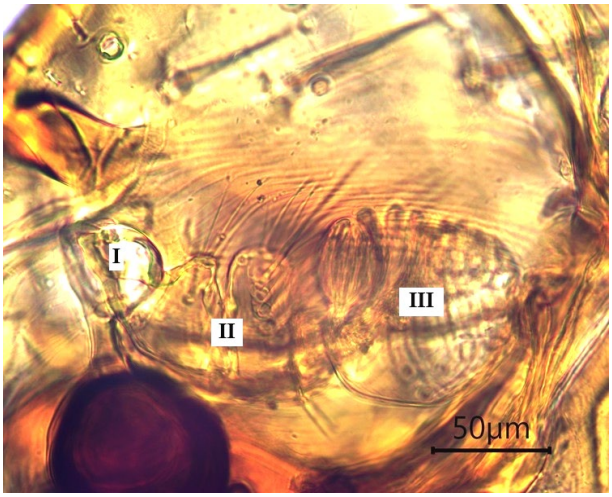

Fig. 1. The degree of *Ctenocephalides felis* fleas' antennal segments visibility at using the proposed method: I –the frontal antennal segment, II – the second antennal segment, III – the third antennal segment (club)

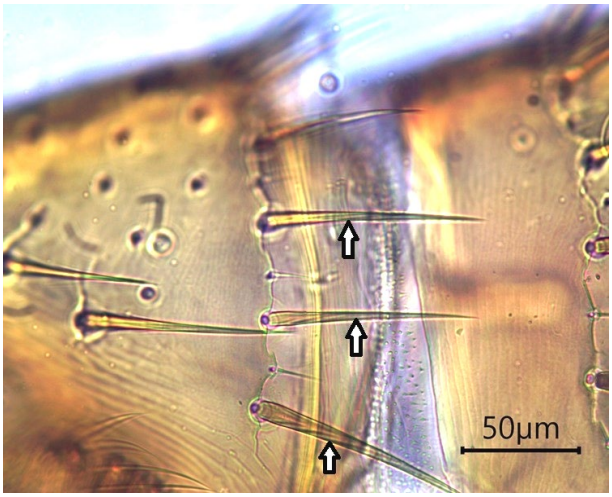

Fig. 2. The degree of *Ctenocephalides felis* fleas' parietal bristles visibility at using the proposed method

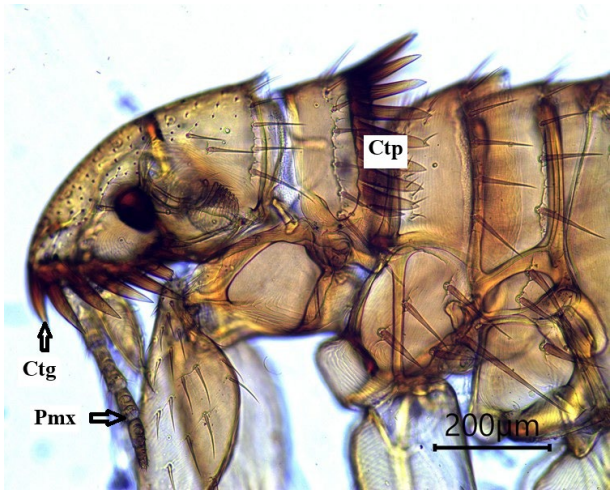

Fig. 3. The degree of *Ctenocephalides felis* fleas' body visibility at using the proposed method: Ctp – pronotum ctenidium, Ctg – main ctenidium, Pmx – maxillary palps

As a result of the conducted studies at comparing the proposed method and the method of preparing pathogens of the Mallophaga order *in toto*, it was found that the use of the proposed method for making total micro-preparations from fleas turned out to be more effective in terms of the general assessment of the coloring intensity of the fleas' body morphological structures (by 72.51 %, P<0.001) and the time required for making one micro-preparation (by 80.39 %, P<0.001) compared to the similar indicators in the method of preparing the pathogens of Mallophaga rder *in toto* (***Table 2***).

The use of the proposed method allowed to obtain good, uniform coloring of all the fleas' antennal segments, as evidenced by the obtained high average score (4.91). At the same time, the use of the method for preparing pathogens of the order of Mallophaga *in toto* led to oversaturation of the above-mentioned morphological structures with dye, as indicated by the low obtained score (1.35).

Table 2

The comparative effectiveness of the methods for producing total preparations of *Ctenocephalides felis* fleas (M±SD, n=20)

Morphological structures of the body	Research method, points	
	proposed	preparation of pathogens of the Mallophaga order *in toto*
Frontal antennal segment	4.95±0.22	1.60±1.47***
Second antennal segment	4.90±0.31	1.20±0.89***
Third antennal segment	4.95±0.22	1.20±0.89***
Parietal bristles	4.85±0.37	1.40±1.23***
General assessment of coloring	4.91±0.05	1.35±0.19***
Time spent on making the total micro-preparation, min	14.05±1.82	71.35±2.08***

Notes: *** – P<0.001 – compared to the similar indicators of the proposed method.

Scientific literature indicates a considerable spread of *C. felis* fleas among the domestic cats' population, as well as the formers' danger as invasive agent and transmitter of diseases [4–6, 8, 9]. Therefore, timely and accurate diagnosing of fleas' species is a relevant direction for the research. For this purpose, it is necessary to produce high-quality micro-preparations. The researchers note the presence of a small number of methods for making micro-preparations from different insects, which have different efficacy. Therefore, we proposed the method for producing micro-preparations from *C. felis* fleas isolated from domestic cats, tested it and established its effectiveness.

The conducted studies have revealed that the proposed method is more effective as far as the general assessment of coloring intensity of the fleas' body morphological structures (by 72.51 %, P<0.001) and the time required to make one micro-preparation (by 80.39 %, P<0.001) compared to the similar indicators in the method of preparing the pathogens of the order of Mallophaga *in toto*. The use of the proposed method allowed to obtain good, uniform coloring of all the fleas' antennal segments, as evidenced by the obtained high average score (4.91).

The scientists mention about the known method of making permanent preparations from the fleas of *Ctenocephalides* genus in toto [19]. At the same time, the disadvantage of the method is the long procedure of preparing insects for fixation, which takes about 21 hours. Besides, the double clearing procedure in 3 % hydrogen peroxide solution and the mixture of juniper and caryophyllus oils leads to significant discoloration of separate morphological structures of the insect body, which can interfere with the study of flea morphology.

Also, the known method of preparing pathogens of the order of Mallophaga in toto [21] is known, which was used in the experiment to compare the effectiveness of the proposed method. The application of the former method led to oversaturation of the fleas' body morphological structures with dye, as indicated by the low score we obtained (1.35). In our opinion, the disadvantage of this method is the use of a 1 % alcohol solution of brilliant green as the dye. The use of this dye leads to oversaturation of the insect body covering, which makes the produced micro-preparation unsuitable for research.

Thus, the obtained results allow us to recommend the proposed method of producing micro-preparations from *C. felis* fleas to increase the effectiveness of microscopic studies for feline ctenocephalidosis.

Conclusions

It was found that the proposed method of making micro-preparations from *C. felis* fleas, which parasitize on cats, allows to get good and uniform coloring of the fleas' body morphological structures with naturally weak coloring (the frontal, second and third antennal segments, parietal bristles), at the same time not to oversaturate with dye the morphological structures with pronounced natural coloring (main ctenidium, maxillary palps). The technique for conducting the proposed method is quite fast, with an average of 14.05 minutes spent on the production of one micro-preparation.

Conflict of interest

The authors state that there is no conflict of interest.

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