Dear colleagues!

The modern trends of biological threats growing, emergent diseases (Lumpy skin disease, Foot-and-mouth disease, African swine fever, Avian influenza and other in Europe and Asia) determine the necessarily to pay the extremely high attention to the biosafety issues and biological hazards control.

The National Scientific Center 'Institute of the Experimental and Clinical Veterinary Medicine' is the leading specialized research institution in Ukraine created for solving scientific and practical tasks of veterinary animal. NSC IECVM's basic research are focused on: immunogenesis and disease pathogenesis, indications, authentications, isolations and studies of biological features of their causative agents, developments of facilities and systems of monitoring, diagnostics, prophylaxis and prognostication of infectious diseases of animals, monitoring of quality and unconcern of agricultural produce and development of the normative basis for animal diseases control and biosafety. NSC IECVM coordinates implementation of scientific researches on questions veterinary medicine, that conduct scientific establishments of NAAS, State Service of Ukraine for Food Safety and Consumer Protection, and Higher educational establishments of Ukraine of agrarian profile.

New journal 'Journal for Veterinary Medicine, Biotechnology and Biosafety", discovered in 2015, aimed to consolidate and share the new developments and achievements in the area of biological science. This was recognized as the profile edition for veterinary medicine doctors and biologists in Ukraine. Our journal promotes the research of Ukrainian institutions, publishing their achievements in English, and sharing it among the scientific community. It includes cooperative veterinary and medical aspects, fitting to One Health Approach declared by WHO, OIE, and FAO. It was included in Index Copernicus and eLibrary scientific databases.

The Editorial board hopes, that our issue will be interesting for wide auditorium of scientists and practical specialists in veterinary medicine, biology, biotechnology and biosafety. We invite new authors for fruitful collaboration and joint development.

Sincerely yours,
Editors-in-Chief

Prof. Borys STEGNIY

Prof. Anton GERILOVYCH

GUIDELINES FOR THE PREPARATION OF THE PAPERS SUBMITTED FOR PUBLICATION AT THE 'JOURNAL FOR VETERINARY MEDICINE, BIOTECHNOLOGY AND BIOSAFETY'

1. Papers must be submitted in an electronic variant and should be sent directly to the editorial board at nsc.iecvm.kharkov@gmail.com or inform@vet.kharkov.ua with subject 'Article in JVMBBS'

2. Papers must be written in English

3. Authors make sure there are no typographical errors in the manuscript

4. Papers must be presented in Word format, in an A4 layout, using Times New Roman 14 point font, which should be single-spaced with 25 mm margins

5. Tables and illustrations must be must be submitted as separate files and inserted in the text

6. Papers must be assembled in the following order:

(a) UDC code

(b) Title of the article

(c) Surname and initials of the author(s)

(d) Name of organization, city, country, and e-mail address of corresponding author

(e) Summary in English (between 200 to 300 words), which should be included: the aim of the work, materials and methods, the results of the work, conclusions

(f) Keywords (up to 8)

(g) Text of the article in the following order: introduction (include brief literature review, actuality, and aim of the work), materials and methods, the results of the work, conclusions, acknowledgements, references

7. References and citation must be formatted according to the 'Harvard – Cite Them Right 9th ed.' style only (use: examples at www.citavi.com/csecodes/csedocs/Cite_them_right_9th_Edition.docx; or one of online reference generators as www.refme.com; or one of reference management software as Zotero) with completed list of authors, the full name of the journal, and DOI or direct link to the publication (if available)

8. References and citation on papers published in non-Latin alphabet languages should be translated into English (or taken from the English summary of the articles) and transliterated into the Latin alphabet from original languages (for Ukrainian use KMU 2010 system at ukrlit.org/transliteratsiia and for Russian use BGN system at ru.translit.net/?account=bgn). Transliterated text must be placed in square brackets. For example: Gerilovich, A., Bolotin, V., Rudova, N., Sapko, S. and Solodyankin, A. (2011) 'Etiological structure of circovirus-associated diseases of pigs in the Eastern region of Ukraine' [Etiolohichna struktura tsyrkovirus-asotsiiovanykh khvorob svynei v hospodarstvakh Skhidnoho rehionu Ukrainy], *News of Agrarian Sciences [Visnyk ahrarnoi nauky]*, 1, pp. 34–36. [in Ukrainian]

ISSN 2411-0388

NATIONAL ACADEMY OF AGRARIAN SCIENCES OF UKRAINE

NATIONAL SCIENTIFIC CENTER 'INSTITUTE OF EXPERIMENTAL AND CLINICAL VETERINARY MEDICINE'

JOURNAL FOR VETERINARY MEDICINE, BIOTECHNOLOGY AND BIOSAFETY

Volume 6
Issue 2

KHARKIV
2020

The Journal for Veterinary Medicine, Biotechnology and Biosafety is included in the list of scientific special serial publications of Ukraine that can publish the results of Ph.D. and Dr.Habil. theses in veterinary sciences (order of the Ministry of Education and Science No. 241, March 9, 2016) and biological sciences (order of the Ministry of Education and Science No. 515, May 16, 2016)

Materials approved for publication and to spread via the Internet by the Scientific Council of the National Scientific Center 'Institute of Experimental and Clinical Veterinary Medicine' (protocol No. 4 of 27.02.2020)

The full text of articles available at jvmbbs.kharkov.ua. JVMBBS covered in the abstract and citation databases Google Scholar (scholar.google.com), RISC (elibrary.ru), Index Copernicus (indexcopernicus.com), and CrossRef (crossref.org)

Editorial Board Address:
NSC 'Institute of Experimental and Clinical Veterinary Medicine'
83 Pushkinska Str., Kharkiv, Ukraine, 61023
tel. +38 (057) 707-20-53, 704-10-90
E-mail: nsc.iecvm.kharkov@gmail.com, inform@vet.kharkov.ua

Certificate of state registration:
KB No. 21398-11198P of June 25, 2015

Part 1. Veterinary medicine

UDC 619:616.98:579.873.21:598.271 **DOI 10.36016/JVMBBS-2020-6-2-1**

DOMESTIC PARROTS AS A POTENTIAL SOURCE OF MYCOBACTERIOSIS

Zavgorodniy A. I., Pozmogova S. A., Kalashnyk M. V.

National Scientific Center 'Institute of Experimental and Clinical Veterinary Medicine', Kharkiv, Ukraine, e-mail: nick.v.kalashnik@gmail.com

Summary. The article presents the results of bacteriological examination of five samples of feces from grey parrots (*Psittacus*) (n = 3), cockatoo (*Cacatua*) (n = 1), yellow-crowned amazon (*Amazona*) (n = 1). Five cultures of mycobacteria were bacteriologically isolated from the five samples. According to biochemical and cultural-morphological characteristics, mycobacterial cultures are classified as *Mycobacterium scrofulaceum* (n = 1) and *Mycobacterium genavense* (n = 4). Isolated cultures of mycobacteria are important in human pathology. Infected exotic poultry pose a potential risk of mycobacterial infection in their owners, so it is necessary to conduct research on biological material

Keywords: identification, isolation, mycobacteriosis, *Mycobacterium scrofulaceum*, *Mycobacterium genavense*, parrots

Introduction. Avian tuberculosis is one of the most significant diseases of domestic, exotic birds, and poultry, and birds kept as pets are the most frequently infected. It was previously thought that in most cases the infectious agent of avian tuberculosis was *Mycobacterium avium* complex (MAC), namely *M. avium*, belonging to serotypes 1, 2, 3, and 6 (genotype *IS901*+ and *IS1245*+) (Dvorska et al., 2007; Fulton and Sanchez, 2013; OIE, 2018; Aranaz et al., 1997).

However, when using molecular-genetic methods, it was found that most mycobacterial infections (up to 80%) in captive birds, especially in species of birds Passeriformes (Sparrow) and Psittaciformes (Parrot), are caused by *M. genavense*, while MAC identified only in 5–10% of cases (Hoop, 1997). *M. genavense* has also been found in birds of Coraciiformes (Gray-breasted), Piciformes (Woodpeckers), Columbiformes (Doves), Ciconiiformes (Storks) and Galliformes (Chickens) (Tell, Woods and Cromie, 2001; Schmitz et al., 2018a, 2018b).

In studies conducted in Switzerland, *M. enavense* was isolated in 71%, and *M. vium* complex only in 17% of the samples. Other isolates included *M. fortuitum* (4%), *M. tuberculosis* (4%), *M. gordonae* (2%), and *M. nonchromogenicum* (2%) (Hoop, Böttger and Pfyffer, 1996). In a recent study of 170 birds from the Passeriformes and Cittaciformes orders, the infection rate was up to 91% (Schmitz et al., 2018a). In addition, isolated cases of infection have been reported in exotic captive birds caused by *M. scrofulaceum*, *M. kansasii*, and *M. fortuitum* (Dhama et al., 2011).

The geographical distribution of *M. genavense* (mainly in domestic birds, companion birds, birds in zoos and wildlife sanctuaries) includes countries in Europe, the United States and Australia. No cases of *M. genavense* have been detected in large commercial poultry farms. In birds, *M. genavense* causes disseminated disease with clinical and histopathological features that do not differ from infection caused by *M. avium* (Antinoff et al., 1996; Van der Heyden, 1997).

According to some authors, exotic birds and poultry have a species predisposition to mycobacterial infections (Painter, 1997; Quaranta et al., 1996; Morita et al., 1999; Hejlícek and Treml, 1995). Mycobacteriosis is much more common in parrots (Psittacinae) of the genera *Amazona*, *Pionus*, *Brotogeris*, *Melopsittacus undulatus*, as well as canaries (*Serinus canaria domestica*), snipe (*Carduelis spinus*) and toucans (*Ramphastidae*) than in other companion birds. Amazon (*Amazona* sp.) and gray-cheeked parrots (*Brotogeris pyrrophterus)* are most commonly affected among Psittacinae.

Grey parrots are the only birds in which cases of tuberculosis caused by *M. tuberculosis* and *M. bovis* are often detected (Schmidt et al., 2008; Washko et al., 1998; Fulton and Sanchez, 2013). The reason for the high prevalence of mycobacterial infections among parrots, especially caused by *M. genavense*, may be genetic factors, specific susceptibility of species, as well as exogenous causes (housing conditions, congestion, stress, etc.).

In addition, concomitant infections (*Macrorhabdus ornithogaster*, circovirus, polyomavirus, avian bornavirus, adenovirus, *Mycobacterium avium* ssp. *avium*, *Mycobacterium avium* ssp. *silvaticum*, *Mycoplasma* sp., Salmonella sp., *Escherichia coli, Aspergillus* sp., etc.), violating the immune status, promote the activation of *M. genavense* in the host (Schmitz et al., 2018a, 2018b; Manarolla et al., 2007).

Diagnosis of mycobacteriosis caused by *M. genavense* is based on the detection of acid-fast rods stained by the method of Ziehl–Neelsen in combination with the culture method on special media for 6–12 weeks (Realini et al., 1999). Detection of *M. genavense* by molecular methods, aimed at a fragment of the 16S rRNA gene with specific primers, can reveal a characteristic signature sequence.

Direct PCR determination of the amplified 16S rRNA sequence is used as an alternative method of bacterial identification. The danger of mycobacterial infections is that many infected birds appear clinically healthy because mycobacteriosis develops slowly and causes a chronic course of the disease, and in most cases in not young birds. Considering the high prevalence of mycobacteriosis among domestic parrots and their high susceptibility to mycobacterial infections, there is a high risk that a bird purchased from an unknown source (from a private owner) will be infected. Occurrence of mycobacteriosis is more probable in places with high population density and poor sanitary conditions.

Due to the fact that the above types of mycobacteria are of great importance in human pathology, domestic parrots can be a potential source of mycobacterial infection for owners, especially for people with weakened immune systems, children and the elderly.

The aim of the study was to conduct a bacteriological investigation of fecal samples for the presence of mycobacterial infection in parrots.

Materials and methods. Studies on the presence of mycobacteria in samples of fecal masses from five parrots, including three grey parrots (genus *Psittacus*, No. 1–3), one cockatoo (genus *Cacatua*, No. 4), and one yellow-crowned amazon (genus *Amazona*, No. 5), were performed by bacteriological (cultural, bacterioscopic) method. Collection of samples of fecal masses in sterile plastic tubes was performed by bird owners. The samples were stored in a refrigerator at a temperature of 4°C for one day, followed by pre-sowing treatment. For decontamination of samples of fecal masses we used a solution of 10% sulfuric acid at exposure for 25 min, followed by washing with sterile distilled water by centrifugation. The precipitate was resuspended in a small amount of sterile saline NaCl and seeded in a volume of 0.3 cm^3 on egg growth medium (pH = 6.0) containing growth factor (alcoholic extract of *M. phlei*) and egg medium for the cultivation of mycobacteria without growth factor (control medium, pH = 7.0). Incubation of crops was performed at a temperature of 37.5–38.0°C. The presence of colony growth was taken into account once a week for four months.

Generic identification, tinctorial and morphological characteristics of the detected microorganisms were determined in smears stained by the method of Ziehl–Neelsen. The species affiliation of the isolated culture of mycobacteria was determined in biochemical (Tween 80 hydrolysis test, amidase and catalase activity, tellurite restoration) and culture (growth rate, growth capacity at 22°C and 45°C, tolerance to 5% sodium chloride in the medium) tests. To do this, a culture suspension at a concentration of 1.0 mg of bacterial mass in 1.0 cm^3 of 0.85% sodium chloride solution was seeded on different for each test medium.

Results and discussion. During the anamnesis it was found that all birds were over 6 years old, all parrots had lethargy and depression, in two grey parrots and one cockatoo (No. 1, 3, 4) — signs of recurrent diarrhea and feather loss, in grey parrot (No. 2) — inflammation of the elbow joint (painful and enlarged), in yellow-crowned amazon (No. 5) in addition to lethargy and depression, other signs of the disease the owner did not observe.

According to the results of cultural analysis of feces from grey parrot No. 2 (with inflamed joint) after 30 days of cultivation on both media were found initially small, round, smooth and shiny bright orange colonies, which eventually merged to form a continuous growth over the entire surface of the medium (Fig. 1). During the microscopy of the isolated culture of microorganisms, acid-fast rods were observed, which were located separately or in clusters (Fig. 2). According to Runyon classification (Runyon, 1959), this culture was classified as slow-growing scotochromogenic atypical mycobacteria (group II). When determining the species affiliation of the isolated culture of mycobacteria, it was found that the optimal temperature for colony growth was 37–38°C, at 22°C the growth of colonies was slower and less intense, at 45°C no growth was observed.

In addition, the growth was not observed on medium containing 5% NaCl. When determining the enzymatic activity in this culture of mycobacteria, positive reactions to nicotinamidase, pyrazinamidase, urea, catalase and negative reactions of Tween 80 hydrolysis and restoration of tellurite from potassium tellurite were established.

Based on cultural-morphological and biochemical characteristics, the culture was classified as *M. scrofulaceum.* Unfortunately, mycobacteriosis caused by *M. scrofulaceum* four months after diagnosis caused the death of the parrot.

In a cultural analysis of the other four samples (No. 1, 3, 4, 5) after 90–120 days of cultivation on medium with growth factor (pH = 6.0) we observed very small, transparent, non-photochromogenic dysgonic colonies smaller than 1.0 mm. Microscopy of scrapings from the surface of this medium revealed a large number of agglomerates of small short acid-fast rods and cocci (Figs. 3–5), in some cases acid-fast rods were detected in the cytoplasm of macrophages (Fig. 6).

Under standard cultivation conditions, that is on a conventional medium for culturing mycobacteria without growth factor and neutral pH, colony growth was not observed, these microorganisms remained nonculturable, but microscopy revealed single small clusters of acid-fast rods and cocci.

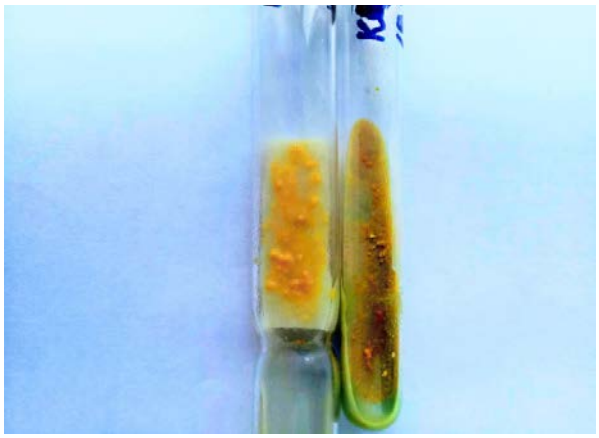


Figure 1. Growth of culture (*M. scrofulaceum*) from grey parrot No. 2 on egg and potato media

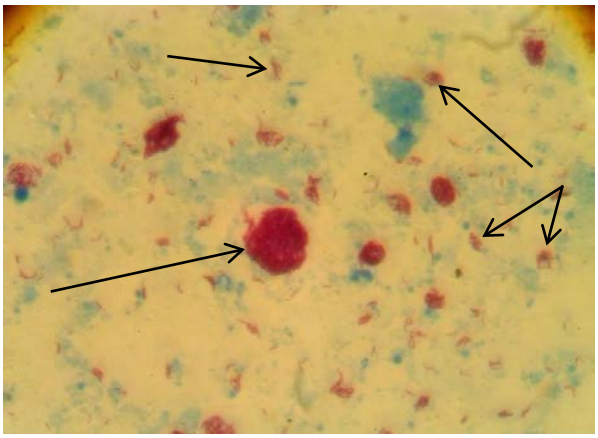


Figure 2. Microscopy of mycobacterial culture *(M. scrofulaceum)* from grey parrot No. 2

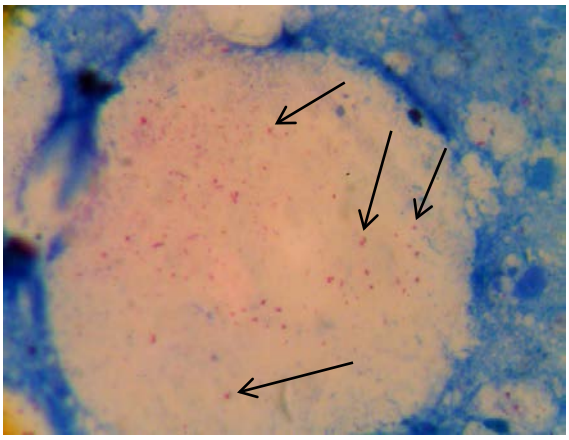


Figure 3. Medium flush (grey parrot No. 1)

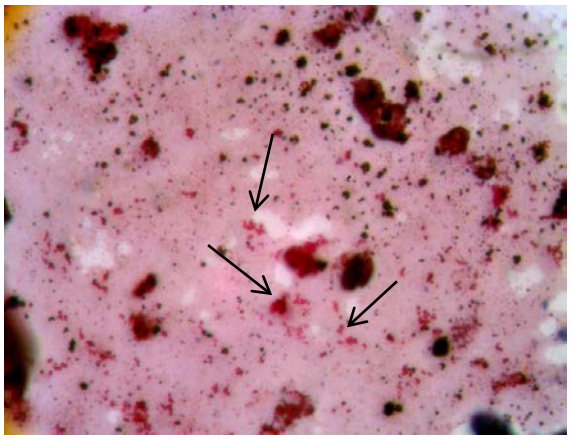


Figure 4. Medium flush (grey parrot No. 3)

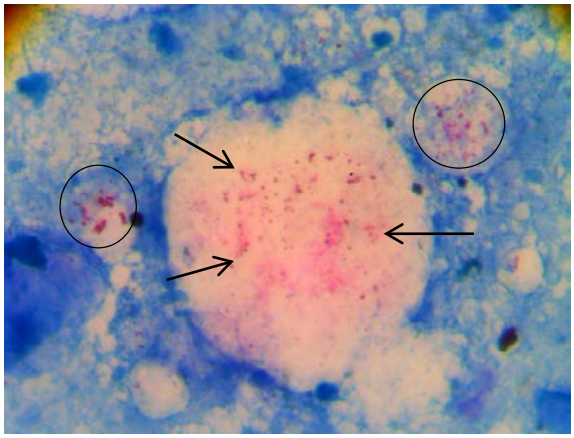


Figure 5. Medium flush (yellow-crowned amazon No. 5)

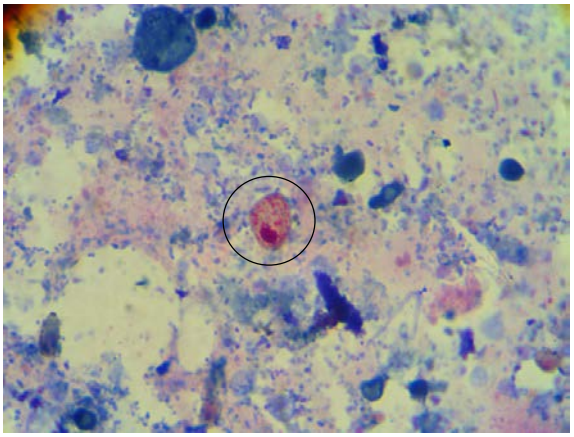


Figure 6. Medium flush (cockatoo No. 4). Macrophage with acid-fast rods

Besides, these mycobacteria did not grow on a medium with 5% NaCl and at a temperature of 25°C and 45°C. Biochemical characteristics were determined only in two cultures (from grey parrot No. 1 and cockatoo). In the other two cultures (from yellow-crowned amazon and grey parrot No. 3) we failed to obtain satisfactory growth of colonies and a sufficient amount of bacterial mass to determine biochemical parameters. Cultures from grey parrot No. 1 and cockatoo hydrolyzed Tween 80 and had positive reactions to pyrazinamidase and urease.

The results obtained, namely: dependence on growth factor, very slow cell replication and colony growth, cell morphology (small cocci), predisposition to acidic environment (pH = 6.0), and high specific sensitivity of *Psittacus* parrots to this species of mycobacteria give reason to believe that the detected microorganisms belong to *M. genavense*. Unfortunately, it was not possible to make a more accurate identification based on molecular-genetic methods. Thus, the isolation of *M. genavense* and *M. scrofulaceum* indicates the circulation and persistence

of these species of mycobacteria among domestic parrots in Ukraine. Given that isolated species of mycobacteria cause lymphadenitis (*M. scrofulaceum*), as well as disseminated infection in immunocompromised individuals, children and the elderly, domestic parrots pose a potential threat to the owners of these birds.

Conclusions. Every parrot with any granulomatous, ocular, sinus, skin lesions, intestinal disorders should be considered as a potential source of mycobacterial infection. To clarify the diagnosis, it is necessary to conduct a bacteriological examination of biological material from such birds.

References

Antinoff, N., Hoefer, H., Kiehn, T. and Bottger, E. (1996) 'Mycobacteriosis caused by *Mycobacterium genavense* in a psittacine bird', *Proceeding of the 17th Annual Conference and Expo of the Association of Avian Veterinarians (AAV) (Tampa, Florida, USA, 28–30 August 1996)*. Bedford, Texas: AAV, pp. 169–170.

Aranaz, A., Liébana, E., Mateos, A. and Dominguez, L. (1997) 'Laboratory diagnosis of avian mycobacteriosis', *Seminars in Avian and Exotic Pet Medicine*, 6(1), pp. 9–17. doi: 10.1016/s1055-937x(97)80036-9.

Dhama, K., Mahendran, M., Tiwari, R., Dayal Singh, S., Kumar, D., Singh, S. and Sawant, P. M. (2011) 'Tuberculosis in birds: insights into the *Mycobacterium avium* infections', *Veterinary Medicine International*, 2011, p. 712369. doi: 10.4061/2011/712369.

Dvorska, L., Matlova, L., Ayele, W., Fischer, O., Amemori, T., Weston, R., Alvarez, J., Beran, V., Moravkova, M. and Pavlik, I. (2007) 'Avian tuberculosis in naturally infected captive water birds of the Ardeideae and Threskiornithidae families studied by serotyping, IS*901* RFLP typing, and virulence for poultry', *Veterinary Microbiology*, 119(2–4), pp. 366–374. doi: 10.1016/j.vetmic.2006.09.010.

Fulton, R. and Sanchez, S. (2013) 'Tuberculosis', in: Swayne, D. E. (ed.) *Diseases of Poultry*. 13th ed. Ames, IA: Wiley-Blackwell, pp. 1008–1017. doi: 10.1002/9781119421481.ch23

Hejlícek, K. and Treml, F. (1995) 'Comparison of the pathogenesis and epizootiologic importance of avian mycobacteriosis in various types of domestic and free-living syntropic birds' [Srovnání patogeneze a epizootologického významu aviární mykobakteriózy různých druhů domestikovaných a volně zijících synantropních ptáků], *Veterinarni Medicina*, 40(6), pp. 187–194. PMID: 7676577. [in Czech].

Hoop, R. K. (1997) 'Public health implications of exotic pet mycobacteriosis', *Seminars in Avian and Exotic Pet Medicine*, 6(1), pp. 3–8. doi: 10.1016/s1055-937x(97)80035-7.

Hoop, R. K., Böttger, E. C. and Pfyffer, G. E. (1996) 'Etiological agents of mycobacterioses in pet birds between 1986 and 1995', *Journal of Clinical Microbiology*, 34(4), pp. 991–992. doi: 10.1128/jcm.34.4.991-992.1996.

Manarolla, G., Liandris, E., Pisoni, G., Moroni, P., Piccinini, R. and Rampin, T. (2007) '*Mycobacterium genavense* and avian polyomavirus co-infection in a European goldfinch (*Carduelis carduelis*)', *Avian Pathology*, 36(5), pp. 423–426. doi: 10.1080/03079450701598390.

Morita, Y., Maruyama, S., Hashizaki, F. and Katsube, Y. (1999) 'Pathogenicity of *Mycobacterium avium* complex serovar 9 isolated from painted quail (*Excalfactoria chinensis*)', *Journal of Veterinary Medical Science*, 61(12), pp. 1309–1312. doi: 10.1292/jvms.61.1309.

OIE (World Organisation for Animal Health) (2018) 'Chapter 3.3.6. Avian tuberculosis', in: *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals (Mammals, Birds and Bees)*. 8th ed. Paris: OIE, pp. 860–870. Available at: http://www.oie.int/fileadmin/Home/eng/Health_standards/tahm/3.03.06_AVIAN_TB.pdf.

Painter, K. S. (1997) 'Avian tuberculosis caused by *Mycobacterium avium* serotype 3 in captive wildfowl', *Veterinary Record*, 140(17), pp. 457–458. doi: 10.1136/vr.140.17.457.

Quaranta, V., Santagada, G., Latorre, L. and Raimondi, P. (1996) 'Serious outbreak of tuberculosis by *Mycobacterium avium* in a pheasant-breeding (*Phasianus colchicus*)' [Grave epidemia di tubercolosi da *Mycobacterium avium* in un allevamento di fagiani (*Phasianus colchicus*)], *Acta Medica Veterinaria*, 42, pp. 209–212. [in Italian].

Runyon, E. H. (1959) 'Anonymous mycobacteria in pulmonary disease', *Medical Clinics of North America*, 43(1), pp. 273–290. doi: 10.1016/S0025-7125(16)34193-1.

Realini, L., De Ridder, K., Hirschel, B. and Portaels, F. (1999) 'Blood and charcoal added to acidified agar media promote the growth of *Mycobacterium genavense*', *Diagnostic Microbiology and Infectious Disease*, 34(1), pp. 45–50. doi: 10.1016/s0732-8893(99)00014-0.

Schmidt, V., Schneider, S., Schlömer, J., Krautwald-Junghanns, M.-E. and Richter, E. (2008) 'Transmission of tuberculosis between men and pet birds: A case report', *Avian Pathology*, 37(6), pp. 589–592. doi: 10.1080/03079450802428901.

Schmitz, A., Korbel, R., Thiel, S., Wörle, B., Gohl, C. and Rinder, M. (2018a) 'High prevalence of *Mycobacterium genavense* within flocks of pet birds', *Veterinary Microbiology*, 218, pp. 40–44. doi: 10.1016/j.vetmic.2018.03.026.

Schmitz, A., Rinder, M., Thiel, S., Peschel, A., Moser, K., Reese, S. and Korbel, R. (2018b) 'Retrospective evaluation of clinical signs and gross pathologic findings in birds infected with *Mycobacterium genavense*', *Journal of Avian Medicine and Surgery*, 32(3), pp. 194–204. doi: 10.1647/2017-261.

Tell, L., Woods, L. and Cromie, R. (2001) 'Mycobacteriosis in birds', *Revue Scientifique et Technique. (International Office of Epizootics)*, 20(1), pp. 180–203. doi: 10.20506/rst.20.1.1273.

Van der Heyden, N. (1997) 'Clinical manifestations of mycobacteriosis in pet birds', *Seminars in Avian and Exotic Pet Medicine*, 6(1), pp. 18–24. doi: 10.1016/s1055-937x(97)80037-0.

Washko, R. M., Hoefer, H., Kiehn, T. E., Armstrong, D., Dorsinville, G. and Frieden, T. R. (1998) '*Mycobacterium tuberculosis* infection in a green-winged macaw (*Ara chloroptera*): Report with public health implications', *Journal of Clinical Microbiology*, 36(4), pp. 1101–1102. doi: 10.1128/jcm.36.4.1101-1102.1998.

UDC 619:576.8:639.371.5.091(285:[477.52/.54+477.62]) **DOI 10.36016/JVMBBS-2020-6-2-2**

FEATURES OF THE PARASITIC SYSTEM FORMATION IN COMMON CARP IN THE AQUACULTURE OF THE NORTH-EASTERN AND EASTERN REGIONS OF UKRAINE

Yevtushenko A. V.

National Scientific Center 'Institute of Experimental and Clinical Veterinary Medicine', Kharkiv, Ukraine, e-mail: aevt76@gmail.com

Summary. The research aimed to determine the peculiarities of the formation of the parasitic system in common carp in the aquaculture of the North-Eastern and Eastern regions of Ukraine. According to the results of the research, 27 species of parasites were found in common carp (12 — protozoa, 6 — monogeneans, 3 — trematodes, 2 — cestodes, 4 — parasitic crustaceans). Three species (11.1%) of registered parasites were invasive. 22 species (81.5%) of detected common carp's parasites develop directly and 5 (18.5%) — with the participation of definitive and intermediate hosts. 26 species (96.3%) of common carp's parasitic fauna were found in crucian carp and 21 species (77.8%) in other coarse fish species (roach, rudd, bleak, perch). It has been established that among the protozoa, pathogens from the genera *Trichodina*, *Chilodonella*, and *Ichthyophthirius* were of epizootic importance for young common carp; *Eimeria carpelli*, *Ichthyobodo necator*, *Myxobolus ellipsoides*, and *Myxobolus dogieli* had relevance. The level of prevalence in common carp fingerlings during outbreaks of chylodonelosis was 72%, two-year-olds — 65%, three-year-olds — 27%, during outbreaks of ichthyoftiriosis — 45%, 56%, and 24%, respectively. Prevalence of *Trichodina acuta* and *Trichodina nigra* in common carp fingerlings was, respectively, 52% and 38%, *Ichthyobodo necator* — 16%, *Eimeria carpelli* — 22%, *Myxobolus ellipsoides*, and *Myxobolus dogieli* — 17–18%. Among the pathogens of helminthic diseases in the aquaculture, monogenetic suckers (*Dactylogyrus anchoratus*, *Dactylogyrus extensus*, *Dactylogyrus vastator*, *Gyrodactylus cyprini*, *and Gyrodactylus katharineri*), as well as cestodes (*Bothriocephalus acheilognathi* and *Khawia sinensis*) were the most epizootic significance for common carp. Prevalence of parasites from the genus *Dactylogyrus* reached 68–87% in fingerlings, from the genus *Gyrodactylus* — 21–36%. The highest level of *Bothriocephalus acheilognathi* infection (82%) was registered in fingerlings. The maximum prevalence of *Khawia sinensis* (62%) was observed in two-year-olds. Outbreaks of diseases caused by crustaceans *Lernaea cyprinacea* and *Argulus foliaceus* with a high level of prevalence have been reported in fish from fingerlings to three-year-olds. The level of prevalence of *Lernaea cyprinacea* was 69%, *Argulus foliaceus* — 22%

Keywords: parasitic system, protozoa, helmints, parasitic crustaceans, common carp, aquaculture

Introduction. The most common object of freshwater aquaculture in Ukraine is the common carp (*Cyprinus carpio* Linnaeus, 1758). It was bred by domestication of wild carp. This species of fish is resistant to adverse factors of the aquatic environment — does not die from the reduction of dissolved oxygen in water to 0.8–1.0 mg/dm^3, can withstand high densities of placing, transportation. Common carp is quite resistant to a number of parasitic and infectious diseases. The temperature optimum for reproduction, nutrition and growth is 18–26°C. In the conditions of fish farms of Ukraine, common carp is grown mainly in the polyculture together with herbivorous species, which allows to make maximum use of the natural fodder base of reservoirs, because under such farming conditions fish species are not food competitors (Hrynzhevskyi, 1998; Andriushchenko and Alymov, 2008). However, high placing density with intensification of farming methods, hydrological links of ponds, the presence of aboriginal fish species, a large number of piscivorous birds provoke outbreaks of parasitic diseases and, as a consequence, reduced fish productivity. It should be noted that coarse fish species (crucian carp, roach, rudd, bleak, perch, etc.) are of great importance in the formation of the center of invasions. These fish species are often reservoir of invasions and can be a source of infection and a cause of disease outbreaks (Davydov and Temnikhanov, 2003). There are many scientific works devoted to the study of parasitocenoses of industrial fish species in inland waters of Ukraine. However, the parasitic fauna of common carp differs in different regions. In addition, seasonal and age dynamics of infection of fish has its own characteristics (Davydov et al., 2005, 2011; Pukalo and Loboiko, 2005; Sachuk and Yuskiv, 2010; Katiukha and Vozniuk, 2016; Mandyhra and Zbozhynska, 2008). Separate studies have focused on the role of non-industrial ichthyofauna in the spread of pathogens (Katyukha and Orel, 2018).

The purpose of the study was to determine the peculiarities of the formation of the parasitic system in common carp in the aquaculture of the North-Eastern and Eastern regions of Ukraine. In this regard, the following tasks were set: to study the species composition of parasites, to determine the level of infection of fish, to study the age dynamics of infection, to identify epizootically significant species of parasites that can cause disease outbreaks.

Materials and methods. Fifteen specimens of each fish species and age groups were studied in a specialized laboratory of the National Scientific Center 'Institute of Experimental and Clinical Veterinary Medicine' (Kharkiv, Ukraine). Ichthyological material was taken in different seasons of the year from spawning, growing, feeding, and wintering ponds in specialized fish farms, as well as agricultural ponds of Kharkiv, Sumy, Poltava, and Donetsk regions.

Ichthyological analysis was performed by the method of incomplete helminthological autopsy according to Bykhovskaya-Pavlovskaya (1985) and Markevich (1951). Species affiliation of parasites was determined by the 'Keys to Parasites of Freshwater Fish of the Fauna of the USSR' (Bauer, 1984, 1985, 1987).

Prevalence of infection (PI, %) was determined by the formula:

$$PI = \frac{x}{y} \times 100\%$$

where: x — the number of fish in which parasites were found; y — the total number of studied fish.

Statistical processing of the obtained results was carried out following the recommendations on biometrics using the parametric Student's *t*-test (Van Emden, 2019).

Results and discussion. According to the results of the research (Table 1), 27 species of parasites were found in common carp: 12 species (44.4%) of protozoa, 6 (22.2%) — monogeneans, 3 (11.1%) — trematodes, 2 (7.4%) — cestodes, 4 (14.8%) — parasitic crustaceans.

Table 1 — Species composition of common carp's, crucian carp's, and coarse fish's parasites and places of their localization in the conditions of aquaculture of the North-Eastern and Eastern regions of Ukraine

No.	Parasite species	Localization	Fish species		
			Common carp	Crucian carp	Other coarse fish (roach, rudd, bleak, perch)
1	* *Cryptobia branchialis* (Nie in Chen, 1956)	gills	+	+	+
2	*Ichthyobodo necator* (= *Costia necatrix*) (Henneguy, 1883)	gills, skin	+	+	+
3	*Eimeria carpelli* Leger et Stankovitch, 1921	intestine	+	+	+
4	*Myxobolus cyprini* Doflein, 1898	kidneys, muscles	+	+	+
5	*Myxobolus carassii* Klokačeva, 1914	gills, abdomen	+	+	+
6	*Myxobolus ellipsoides* Thélohan, 1892	all organs and tissues	+	+	+
7	*Myxobolus dogieli* Bykhovskaya-Pavlovskaya et Bykhovski, 1940	kidneys, intestine wall, gills	+	–	–
8	*Chilodonella piscicola* (Zacharias, 1894) Jankowski, 1980	gills, body surface	+	+	+
9	*Ichthyophthirius multifiliis* Fouquet, 1876	gills, body surface	+	+	+
10	*Trichodina acuta* Lom, 1961	gills, body surface	+	+	+
11	*Trichodina nigra* Lom, 1961	gills, body surface	+	+	+
12	*Trichodinella epizootica* (Raabe, 1950) Sramek-Husek, 1953	gills, body surface	+	+	+
13	*Dactylogyrus anchoratus* (Dujardin, 1845)	gills	+	+	–
14	*Dactylogyrus vastator* Nybelin, 1924	gills	+	+	–
15	*Dactylogyrus extensus* Mueller et Van Cleave, 1932	gills	+	+	–
16	*Gyrodactylus cyprini* Diarova, 1964	gills, body surface	+	+	–
17	*Gyrodactylus kattharineri* Malmberg, 1964	gills, body surface	+	+	+
18	*Diplozoon paradoxum* von Nordmann, 1832	gills	+	+	+
19	*Diplostomum spathaceum* (Rudolphi, 1819) mtc	eyes	+	+	+
20	*Posthodiplostomum cuticola* (Nordmann, 1832) mtc	skin	+	+	+
21	*Ichthyocotylurus variegatus* (= *Tetracotyle variegate*) (Creplin, 1825) Odening, 1969 mtc	abdomen	+	+	+
22	* *Khawia sinensis* Hsü, 1935	intestine	+	+	+
23	* *Bothriocephalus acheilognathi* Yamaguti, 1934	intestine	+	+	+
24	*Philometra sanguinea* (Rudolphi, 1819)	fins	–	+	–
25	*Ergasilus sieboldi* von Nordmann, 1832	gills	+	+	–
26	*Ergasilus briani* Markevich, 1933	gills	+	+	+
27	*Lernaea cyprinacea* Linnaeus, 1758	skin	+	+	+
28	*Argulus foliaceus* (Linnaeus, 1758)	skin	+	+	+

Remarks: * — invasive species, mtc — metacercariae.

Only 3 species (11.1%) from the detected parasites are invasive species. It should be noted that 22 species (81.5%) of detected common carp's parasites develop directly and 5 (18.5%) — with the participation of definitive and intermediate hosts. Herewith, the fish is an additional (second intermediate) host in the life cycle of 3 species (11.1%) of parasites.

In common carp 18 species (66.7%) of the detected pathogens are parasites of the surface of the body, skin and gills, three species are parasites of the intestine, one species — a parasite of the eyes, one species — a parasite of the abdominal cavity. Representatives of the genus *Myxobolus* were found in various organs and tissues.

It was found that only one species from the genus *Myxobolus* showed specificity to the host: *M. dogieli* was found only in common carp. Representatives of the genus *Dactylogyrus* were found only in common carp and crucian carp. *G. katharineri* was found in all studied carp fish, while *G. cyprini* — only in common carp and crucian carp. It should be noted that nematodes of the genus *Philometra* were not detected in common carp, but a species-specific parasite *Ph. sanguinea* was detected in crucian carp.

Thus, according to the obtained data, it was established that 96.3% of common carp's parasitic fauna were found in crucian carp and 77.8% — in other coarse fish species (roach, rudd, bleak, perch). This fact must be taken into account when planning preventive and anti-epizootic measures in the fight against parasitic diseases of common carp in aquaculture.

According to the results of studying the age dynamics of common carp infection with pathogens of protozoa infectious diseases, the data shown in Fig. 1 were obtained.

Thus, the data in Fig. 1 show that outbreaks of diseases caused by parasitic protozoa were recorded mainly fingerlings and two-year-olds. Flagellates *C. branchialis* were found in 8–10% of fingerlings and two-year-olds, and *I. necator* mainly in fingerlings (16%), less often in two-year-olds (7%).

Sporozoa *M. cyprini* and *M. carassii* with almost the same prevalence of infection (7–10%) were registered in fish of both fingerlings and two-year-olds, while *M. ellipsoides* and *M. dogieli* were registered mainly in fingerlings (17–18%).

Outbreaks of eimeriosis (*E. carpelli*) were observed in 22% of common carp fingerlings, and only parasite carriage was recorded in older fish.

It should be noted that among all detected protozoa outbreaks of the diseases among three-year-old fish were registered when infection by pathogens *Ch. piscicola* and *I. multifiliis*. High levels of infection were observed both among fingerlings — 72% and 45%, among two-year-olds — 65% and 56%, and among three-year-olds — 27% and 24%, respectively.

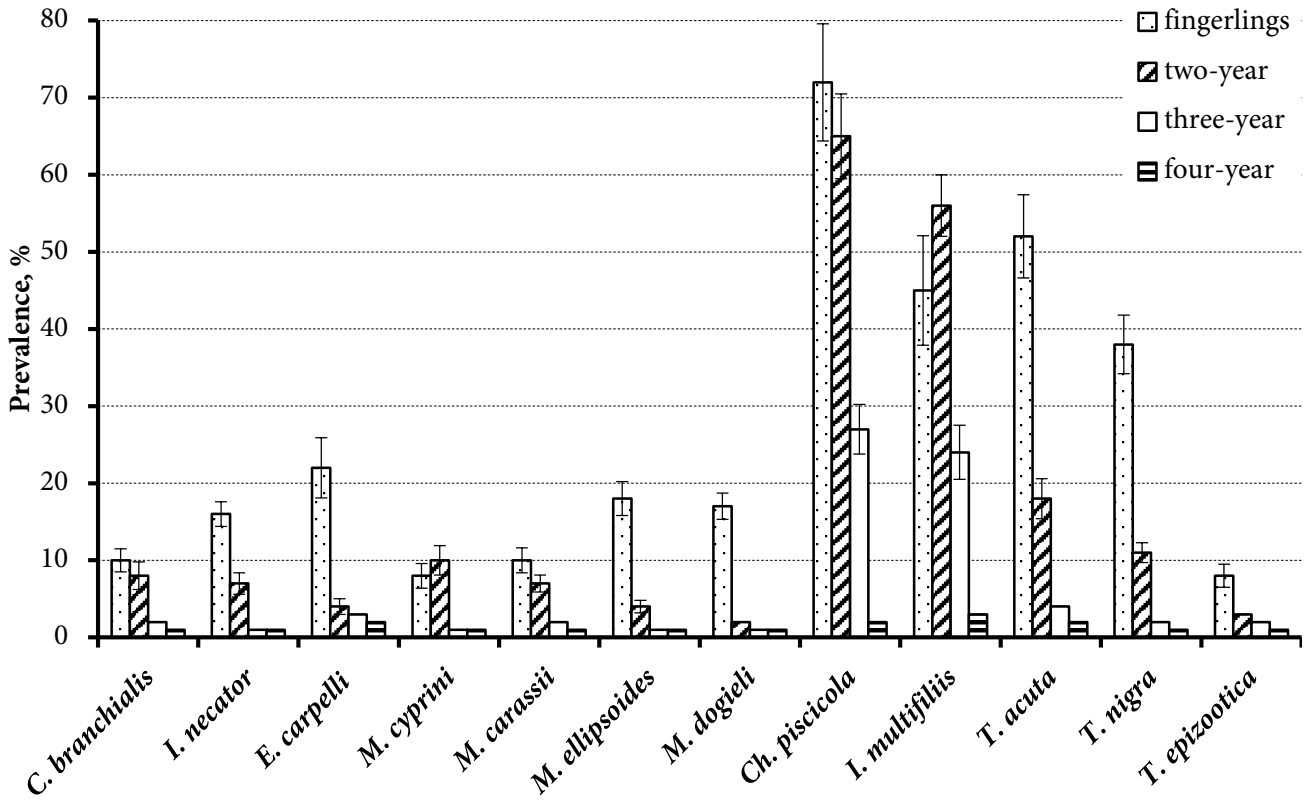


Figure 1. Age dynamics of infection of common carp with pathogens of protozooses during outbreaks of diseases in fish farms of the North-Eastern and Eastern regions of Ukraine

A different picture was observed when infecting fish with pathogens of other ciliaphorosis — *T. acuta*, *T. nigra*, *T. epizootica*: the highest level of infection was registered in fingerlings — 52%, 38%, 8%, and the infection of two-year-olds was much lower — 18%, 11%, 3%, respectively. Infection of four-year-olds with protozoa was practically not registered, and the level of mean intensity testified to parasitic carriers of pathogens.

When studying the seasonal dynamics of fish infection with protozoa, it was found that infection of fingerlings and two-year-old common carp with myxosporidia (*M. cyprini, M. carassii, M. ellipsoides, M. dogieli*) was recorded throughout the summer and the maximum level of prevalence was recorded in autumn. In winter, the level of infection decreased. A similar pattern was observed when infecting fish with the causative agent of eimeriosis — *E. carpelli.*

Among fingerlings, the peak level of infection with parasites *I. necator* was recorded in July. An increase in the infection of fish with *T. nigra* and *T. epizootica* was observed in August. In August–September, the maximum infection of fingerlings and two-year-olds with *C. branchialis* was determined.

Outbreaks of ichthyoftiriosis infection (*I. multifiliis*) were more often registered in spawning ponds when growing young, as well as in the autumn among common carp of different age groups after transferring to winter ponds. The highest level of infection of fish with *C. piscicola* and *T. acuta* was recorded in winter ponds from October to April.

Thus, according to the results of the conducted researches it was established that the pathogens from the genera *Trichodina*, *Chilodonella*, and *Ichthyophthirius* had the greatest epizootic significance for the young common carp; representatives of the genera *Eimeria* and *Ichthyobodo*, as well as some species from the genus *Myxobolus* were relevant. Fish of older age groups were parasitic carriers of protozoa, only species *C. piscicola* and *I. multifiliis* sometimes caused outbreaks of diseases under the high density of placing — in winter ponds.

The age dynamics of common carp infection with helminthiasis and crustaceosis pathogens during disease outbreaks in fish farms in the North-Eastern and Eastern regions of Ukraine is shown in Fig. 2.

The data in Fig. 2 show that outbreaks of dactylogyrosis and gyrodactylosis were recorded primarily in fingerlings, with prevalence by parasites of the genus *Dactylogyrus* reaching 68–87%, while prevalence by parasites of the genus *Gyrodactylus* in fingerlings — 21–36%. Older fish were parasitic carriers of pathogens. *D. paradoxum* monogeneans had no epizootic significance — they were recorded only sporadically.

Metacercariae of trematodes *P. cuticola*, *D. spathaceum*, *I. variegatum* were registered both in fingerlings and two-year-olds, but the level of prevalence ranged from 8–13%.

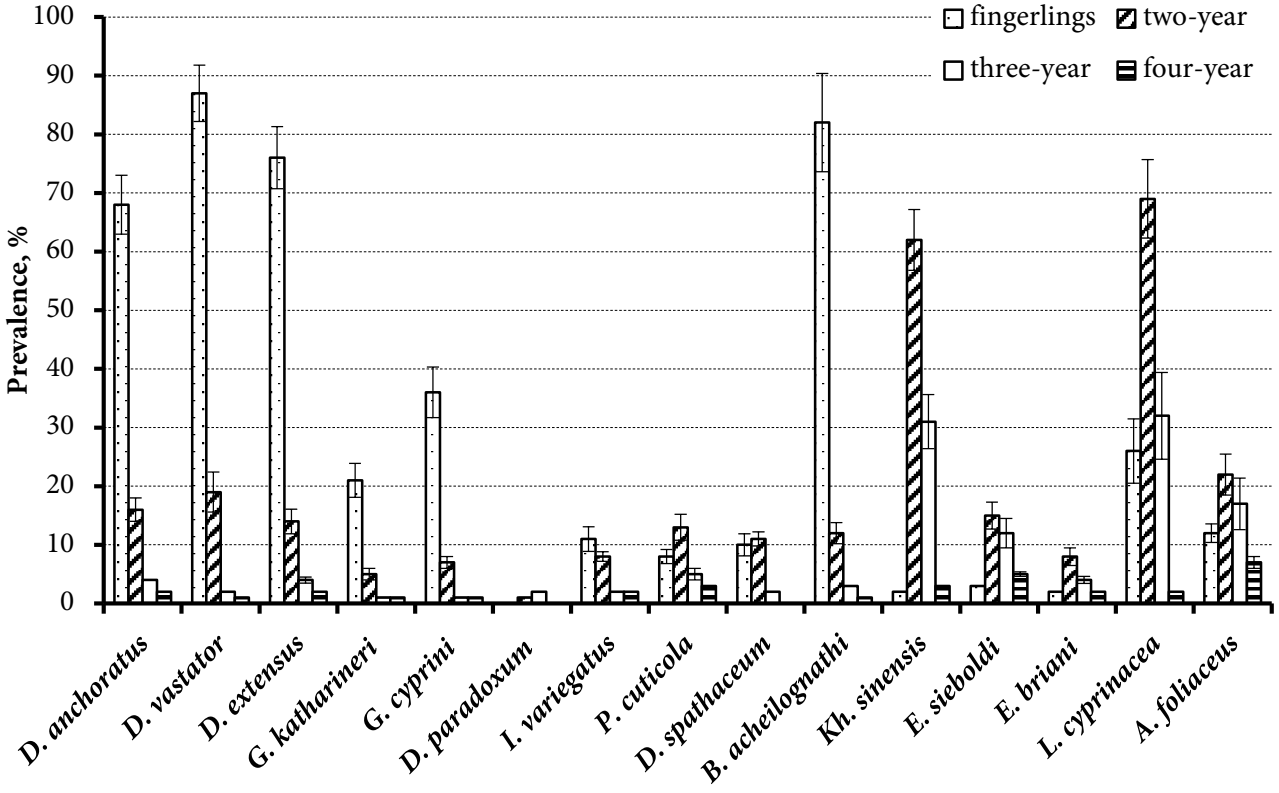


Figure 2. Age dynamics of infection of common carp with helminthic and crustacean pathogens in disease outbreaks in fish farms in the North-Eastern and Eastern regions of Ukraine

The opposite pattern was observed when common carp was infected with cestodes *B. acheilognathi* and *Kh. sinensis*. Thus, the highest level of infection with *B. acheilognathi* was registered in fingerlings — 82%, with an infection of two-year-olds no more than 12%.

Instead, the maximum prevalence with *Kh. sinensis* was registered in two-year-olds — 62%, while among these-year-olds the level of infection did not exceed 2%. A high level of infection with *Kh. sinensis* was registered in three-year-old fish — 31%. These cestodes have a complex cycle of development: for *B. acheilognathi* intermediate hosts are cyclops, and for *Kh. sinensis* — oligochaetes. Common carp in the first months of life has a planktonic type of food, and later — benthic, which explains the difference in the age dynamics of infection.

According to the obtained data, parasitic crustaceans were more often found in two-year-old fish. Thus, the highest level of prevalence of two-year-old common carp *L. cyprinacea* was 69%, *E. sieboldi* — 15%, *E. briani* — 8%, *A. foliaceus* — 22%, and fingerlings — 26%, 3%, 2%, 12%, respectively. High levels of *L. cyprinacea* and *A. foliaceus* infection were reported in unfavorable farms and among three-year-old fish — 32% and 17%, respectively.

The results of the analysis of the seasonal dynamics of fish infection with pathogens of monogenoidosis showed some of its features during the year. Thus, the outbreak of infection of *D. anchoratus*, *D. extensus* were registered among fingerlings in June, in July the level of infection decreased slightly, but in August there was a sharp increase in prevalence to 68% and 76%, respectively. Another picture was observed with *D. vastator* infection: in June, the peak of infection was registered — up to 87%, then the prevalence decreased, but it increased again in September, but not more than 40%. As the water temperature decreased, the prevalence decreased. Pathogens *G. cyprini* and *G. katharineri*, on the contrary, were found mainly in fingerlings in the spring in winter ponds.

Infection with pathogens of trematode infections (*P. cuticola*, *D. spathaceum*, *I. variegatus*) was recorded throughout the growing season. The peak of infection was observed in the autumn. During the winter, the level of *P. cuticola* infection decreased, while the prevalence of *D. spathaceum* and *I. variegatus* did not change.

During the whole summer period, common carp infection with *B. acheilognathi* cestodes was also registered, and the prevalence gradually increased and gained maximum value in August–September. Another picture was observed during infection with cestodes *Kh. sinensis*: the first peak of the infection was observed in fingerlings in the spring, and after re-infection during the spring–summer period, the second peak of the infection was recorded - in two-year-olds, in the autumn.

The highest level of infection with parasitic crustaceans from the genera *Ergasilus* and *Lernaea* was recorded throughout the summer period with the maximum level of prevalence in July–August. Crustaceans *A. foliaceus* were recorded on fish throughout the year, but the peak of infection was observed in June–July.

Thus, the results of the study showed that monogenetic suckers (*D. anchoratus*, *D. extensus*, *D. vastator*, *G. cyprini*, *G. katharineri*), cestodes (*B. acheilognathi*, *Kh. sinensis*), crustaceans (*L. cyprinacea and A. foliaceus*) had the greatest epizootic value in aquaculture for common carp.

Our results on the epizootic significance of the detected pathogens are confirmed by the data of other authors. Significant species diversity of freshwater fish parasites in natural hydroecosystems is considered harmless. However, under the conditions of artificial cultivation, many species of pathogens can cause epizooty, lead to pathologies in the body of fish, lead to a decrease in fish productivity.

First of all, such pathogens include protozoa, monogeneans and parasitic crustaceans (Mikheev, Pasternak and Valtonen, 2003; Wei, Li and Yu, 2013; Bastos Gomes et al., 2017; Molnár, 1996; Molnár and Székely, 2014; Lux, 1990). Invasive parasites play a significant role in the epizootology of parasitic diseases (Scholz, Kuchta and Williams, 2012; Oros, Hanzelová and Scholz, 2009). The increase in the number of piscivorous birds associated with environmental measures is contributing to the increase in the infection of common carp and other pond fish with trematode metacercariae.

Data on the epizootic significance of trematode infections are confirmed by other researchers (Ondracková et al., 2004; Jithila and Prasadan, 2018; Georgieva et al., 2013). The obtained results on the infection of non-industrial fish species with pathogens of parasitic diseases dangerous to common carp, are consistent with the data of O. M. Davydov (Davydov and Temnikhanov, 2003; Davydov et al., 2011, 2012), according to which coarse fish can be a reservoir of a number of dangerous parasitic diseases such as ichthyoftiriosis, chylodonellosis, lerneosis, etc.

Conclusions. 1. In fish farms of the North-Eastern and Eastern regions of Ukraine, 27 species of parasites were found in common carp: 12 — protozoa, 6 — monogeneans, 3 — trematodes, 2 — cestodes, 4 — parasitic crustaceans. Three species (11.1%) of registered parasites were invasive. 22 species (81.5%) of detected common carp's parasites develop directly and 5 (18.5%) — with the participation of definitive and intermediate hosts. 26 species (96.3%) of common carp's parasitic fauna were found in crucian carp and 21 species (77.8%) in other coarse fish species (roach, rudd, bleak, perch).

2. It has been established that among the protozoa, pathogens from the genera *Trichodina*, *Chilodonella*, and *Ichthyophthirius* were of epizootic importance for young common carp; *Eimeria carpelli*, *Ichthyobodo necator*, *Myxobolus ellipsoides*, and *Myxobolus dogieli* had relevance. The level of prevalence in common carp

fingerlings during outbreaks of chylodonelosis was 72%, two-year-olds — 65%, three-year-olds — 27%, during outbreaks of ichthyoftiriosis — 45%, 56%, and 24%, respectively. Prevalence of *Trichodina acuta* and *Trichodina nigra* in common carp fingerlings was, respectively, 52% and 38%, *Ichthyobodo necator* — 16%, *Eimeria carpelli* — 22%, *Myxobolus ellipsoides*, and *Myxobolus dogieli* — 17–18%.

3. Among the pathogens of helminthic diseases in the aquaculture, monogenetic suckers (*Dactylogyrus anchoratus*, *Dactylogyrus extensus*, *Dactylogyrus vastator*, *Gyrodactylus cyprini*, and *Gyrodactylus katharineri*), as well as cestodes (*Bothriocephalus acheilognathi* and *Khawia sinensis*) were the most epizootic significance for common carp. Prevalence of parasites from the genus *Dactylogyrus* reached 68–87% in fingerlings, from the genus *Gyrodactylus* — 21–36%. The highest level of *Bothriocephalus acheilognathi* infection (82%) was registered in fingerlings. The maximum prevalence of *Khawia sinensis* (62%) was observed in two-year-olds.

4. Outbreaks of diseases caused by crustaceans *Lernaea cyprinacea* and *Argulus foliaceus* with a high level of prevalence have been reported in fish from fingerlings to three-year-olds. The level of prevalence of *Lernaea cyprinacea* was 69%, *Argulus foliaceus* — 22%.

Prospects for further research. The obtained results on the age and seasonal dynamics of infection allow the introduction of a set of treatment and prevention measures in the control of pathogens (Dunn and Hatcher, 2015). Thus, to combat monogenetic suckers and parasitic crustaceans, it is recommended to take preventive measures at the beginning of the growing season — in June among fish of all ages.

Therapeutic treatment of fish with larval trematodes is recommended twice — in July and in September among fingerlings and two-year-olds. The most effective period for intestinal cestodes treatment is August–September. Preventive treatment of fish of all ages with parasitic protozoa should be carried out after transferring fish to winter ponds.

References

Andriushchenko, A. I. and Alymov S. I. (2008) *Pond Fish Farming [Stavove rybnytstvo]*. Kyiv: National Agrarian University Publish Center. ISBN 9789668689031. [in Ukrainian].

Bastos Gomes, G., Miller, T. L., Vaughan, D. B., Jerry, D. R., McCowan, C., Bradley, T. L. and Hutson, K. S. (2017) 'Evidence of multiple species of *Chilodonella* (Protozoa, Ciliophora) infecting Australian farmed freshwater fishes', *Veterinary Parasitology*, 237, pp. 8–16. doi: 10.1016/j.vetpar.2017.03.004.

Bauer, O. N. (ed.) (1984) *Keys to Parasites of Freshwater Fish of the Fauna of the USSR. Volume 1. Parasitic Protozoa [Opredelitel' parazitov presnovodnykh ryb fauny SSSR. Tom 1. Paraziticheskie prosteyshie]* (3 vols). Leningrad: Nauka. [in Russian].

Bauer, O. N. (ed.) (1985) *Keys to Parasites of Freshwater Fish of the Fauna of the USSR. Volume 2. Parasitic Metazoa (Chapter 1) [Opredelitel' parazitov presnovodnykh ryb fauny SSSR. Tom 2. Paraziticheskie mnogokletochnye (Pervaya chast')]* (3 vols). Leningrad: Nauka. [in Russian].

Bauer, O. N. (ed.) (1987) *Keys to Parasites of Freshwater Fish of the Fauna of the USSR. Volume 3. Parasitic Metazoa (Chapter 2) [Opredelitel' parazitov presnovodnykh ryb fauny SSSR. Tom 3. Paraziticheskie mnogokletochnye (Vtoraya chast')]* (3 vols). Leningrad: Nauka. [in Russian].

Bykhovskaya-Pavlovskaya, I. Ye. (1985) *Fish Parasites [Parazity ryb]*. Leningrad: Nauka. [in Russian].

Davydov, O. N. and Temnikhanov, Yu. D. (2003) *Diseases of Freshwater Fish [Bolezni presnovodnykh ryb]*. Kiev: Vetinform, 2003. ISBN 9667063119. [in Russian].

Davydov, O. N., Bazeev, R. E., Kurovskaya, L. Ya. and Temnikhanov, Yu. D. (2005) 'Changes in helminth number during introduction of herbivorous fish into Kiev water basin' [Izmeneniya chislennosti gel'mintov pri introduktsii rastitel'noyadnykh ryb v Kievskom vodokhranilishche], *Vestnik zoologii. Supplement*, 19(1), pp. 96–97. Available at: http://mail.izan.kiev.ua/vz-pdf/suppl/Supplement%202005-19-1.pdf. [in Russian].

Davydov, O. N., Neborachek, S. I., Kurovskaya, L. Ya. and Lysenko, V. N. (2011) *Ecology of Fish Parasites in Water-Bodies of Ukraine [Ekologiya parazitov ryb vodoemov Ukrainy]*. Kiev. ISBN 9789660262348. [in Russian].

Davydov, O. N., Lysenko, V. N., Kurovskaya, L. Ya. and Neborachek, S. I. (2012) 'Analysis of the species diversity of the Prussian carp parasites in the Southern Palearctic' [Analiz vidovogo raznoobraziya parazitov karasya serebryanogo Yuzhnoy Palearktiki], *Fisheries Science of Ukraine [Rybohospodarska nauka Ukrainy]*, 3–4, pp. 63–72. Available at: http://nbuv.gov.ua/UJRN/rnu_2012_3-4_16. [in Russian].

Dunn, A. M. and Hatcher, M. J. (2015) 'Parasites and biological invasions: Parallels, interactions, and control', *Trends in Parasitology*, 31(5), pp. 189–199. doi: 10.1016/j.pt.2014.12.003.

Georgieva, S., Soldánová, M., Pérez-del-Olmo, A., Dangel, D. R., Sitko, J., Sures, B. and Kostadinova, A. (2013) 'Molecular prospecting for European *Diplostomum* (Digenea: Diplostomidae) reveals cryptic diversity', *International Journal for Parasitology*, 43(1), pp. 57–72. doi: 10.1016/j.ijpara.2012.10.019.

Hrynzhevskyi, M. V. (1998) *Aquaculture of Ukraine (Organizational and Economic Aspects) [Akvakultura Ukrainy (orhanizatsiino-ekonomichni aspekty)]*. Lviv: Vilna Ukraina. ISBN 96607306194. [in Ukrainian].

Jithila, P. J. and Prasadan, P. K. (2018) 'Description of *Tetracotyle wayanadensis* n. sp. (Digenea: Strigeidae) metacercaria infecting six species of freshwater fishes from Western Ghats, India', *Journal of Parasitic Diseases*, 42(2), pp. 226–231. doi: 10.1007/s12639-018-0988-9.

Katyukha, S. M. and Orel, A. M. (2018) 'The role of aboriginal fish in the emergence of an invasion in invading species' [Rol aboryhennykh ryb u vynyknenni invazii sered ryb-

vselentsiv], *Veterinary Biotechnology [Veterynarna biotekhnolohiia]*, 32(2), pp. 230–235. doi: 10.31073/vet_biotech32(2)-27. [in Ukrainian].

Katiukha, S. M. and Vozniuk, I. O. (2016) 'Spreading of parasitic diseases of fishes in the ponds of the Rivne Oblast' [Poshyrennia invaziinykh khvorob ryb u vodoimakh Rivnenskoi oblasti], *Veterinary Biotechnology [Veterynarna biotekhnolohiia]*, 28, pp. 94–101. Available at: http://nbuv.gov.ua/UJRN/vbtb_2016_28_12. [in Ukrainian].

Lux, E. (1990) 'Population dynamics and interrelationships of some *Dactylogyrus* and *Gyrodactylus* species on *Cyprinus carpio*', *Angewandte Parasitologie*, 31(3), pp. 143–149.

Mandyhra, M. S. and Zbozhynska, O. V. (2008) 'Epizootic situation in fish farms of Rivne Region' [Epizootychna sytuatsiia v rybnytskykh hospodarstvakh Rivnenshchyny], *Veterinary Medicine [Veterynarna medytsyna]*, 90, pp. 311–315. [in Ukrainian].

Markevich, A. P. (1951) *Parasite Fauna of Freshwater Fish of the Ukrainian SSR [Parazitofauna presnovodnykh ryb Ukrainskoy SSR]*. Kiev: Publishing House of the Academy of Sciences of Ukrainian. [in Russian].

Mikheev, V. N., Pasternak, A. F. and Valtonen, E. T. (2003) 'How do fish ectoparasites *Argulus* spp. (Crustacea: Branchiura) match with their hosts at the behavioural and ecological scales?', *Journal of General Biology [Zhurnal Obshchei Biologii]*, 64(3), pp. 238–247.

Molnár, K. (1996) 'Remarks on the morphology, site of infection and validity of some coccidian species from fish', *Acta Veterinaria Hungarica*, 44(3), pp. 295–307.

Molnár, K. and Székely, C. (2014) 'Tissue preference of some myxobolids (Myxozoa: Myxosporea) from the musculature of European freshwater fishes', *Diseases of Aquatic Organisms*, 107(3), pp. 191–198. doi: 10.3354/dao02688.

Ondracková, M., Reichard, M., Jurajda, P. and Gelnar, M. (2004) 'Seasonal dynamics of *Posthodiplostomum cuticola* (Digenea, Diplostomatidae) metacercariae and parasite-enhanced growth of juvenile host fish', *Parasitology Research*, 93(2), pp. 131–136. doi: 10.1007/s00436-004-1123-7.

Oros, M., Hanzelová, V. and Scholz, T. (2009) 'Tapeworm *Khawia sinensis*: Review of the introduction and subsequent decline of a pathogen of carp, *Cyprinus carpio*', *Veterinary Parasitology*, 164(2–4), pp. 217–222. doi: 10.1016/j.vetpar.2009.05.010.

Pukalo, P. Ya. and Loboiko, Yu. V. (2005) 'The most common diseases of pond fish and their prevention' [Naibilsh poshyreni zakhvoriuvannia stavovykh ryb ta yikh profilaktyka], *Farmer [Silskyi hospodar]*, 11/12, pp. 36–37. [in Ukrainian].

Sachuk, R. M. and Yuskiv, I. D. (2010) 'Ecological and parasitological monitoring of carp in fish farms of Rivne Region' [Ekoloho-parazytolohichnyi monitorynh koropa v rybovodnykh hospodarstvakh Rivnenskoi oblasti], *Scientific Messenger of Lviv National University of Veterinary Medicine and Biotechnologies named after S. Z. Gzhytskyj [Naukovyi visnyk Lvivskoho natsionalnoho universytetu veterynarnoi medytsyny ta biotekhnolohii imeni S. Z. Gzhytskoho]*, 12(2), pt. 1, pp. 274–278. Available at: http://nbuv.gov.ua/UJRN/nvlnu_2010_12_2(1)__57. [in Ukrainian].

Scholz, T., Kuchta, R. and Williams, C. (2012) '*Bothriocephalus acheilognathi*', in Woo, P. T. K. and Buchmann, K. (eds.) *Fish Parasites: Pathobiology and Protection*. Wallingford: CABI, pp. 282–297. doi: 10.1079/9781845938062.0282.

Van Emden, H. F. (2019) *Statistics for Terrified Biologists*. 2nd ed. Hoboken, NJ: John Wiley & Sons. ISBN 9781119563679.

Wei, J. Z., Li, H. and Yu, H. (2013) 'Ichthyophthiriasis: emphases on the epizootiology', *Letters in Applied Microbiology*, 57(2), pp. 91–101. doi: 10.1111/lam.12079.

Part 2. Biotechnology

UDC 602.3:619:616.98:578.82/83.085.23:636.22/.28 **DOI 10.36016/JVMBBS-2020-6-2-3**

STUDY OF BIOLOGICAL PROPERTIES OF FIELD ISOLATES OF CATTLE MINOR INFECTIONS AGENTS ON HOMOLOGICAL CELL CULTURES

Gorbatenko S. K. [1], Solodiankin O. S. [1], Lymanska O. Yu. [1], Rudova N. G. [1], Kulyk I. O. [1], Kornieikov O. M. [1], Stegniy M. Yu. [1], Kuznetsova O. V. [1], Dunaeva O. V. [2], Gorbatenko V. P. [3]

[1] National Scientific Center 'Institute of Experimental and Clinical Veterinary Medicine', Kharkiv, Ukraine, e-mail: st.gorbatenko@gmail.com
[2] Kharkiv National Medical University, Kharkiv, Ukraine
[3] Kharkiv State Zooveterinary Academy, Kharkiv, Ukraine

Summary. Biological properties of field isolates of bovine immunodeficiency virus and bovine foamy virus on homological cell cultures (fetal bovine lung and bovine coronary artery endothelial cells) were investigated. Pathogens of bovine slow infections, namely bovine immunodeficiency virus and bovine foamy virus, are able to integrate into cell cultures of homologous to cattle type, which is confirmed by the results of PCR. There has been determined the presence of genetic material of pathogens of bovine immunodeficiency (BIV) and spumavirus infection (BFV) in the cultivation of lymphocytes of field isolates in the culture of bovine coronary artery endothelial cells (BCAEC) at the level of 5th passage, and in the cell culture of fetal bovine lung (FBL) — at the level of 10th passage. In the process of integration of pathogens of immunodeficiency and spumavirus infection of cattle in continuous cell cultures FBL and BCAEC, morphological changes in the state of the monolayer by the principle of syncytiation and vacuolation are observed

Keywords: bovine immunodeficiency virus, bovine foamy virus, cell cultures, PCR, cattle

Introduction. The profitability of animal husbandry in countries with developed livestock industries is hampered by many chronic viral infectious diseases, the causative agents of which, due to immunosuppressive effects, reduce the effectiveness of specific prevention, productivity, and quality of livestock products, general resistance of livestock. These include leukemia, immunodeficiency, spumavirus infection of cattle. The causative agents of the above diseases, namely bovine leukemia virus (BLV), bovine immunodeficiency virus (BIV), and bovine foamy virus (BFV) are genetically and antigenically related retroviruses, which, affecting cattle, cause a slow course of the disease (Mousavi et al., 2014).

In Ukraine, the infectious process, the dynamics of the epizootic process in bovine leukemia, the peculiarities of the disease in livestock farms, the issue of eradication of the disease at the legislative level have been studied. However, the question of the spread and mechanism of the infectious process in slow infections of cattle caused by BIV and BFV, in Ukraine has not been studied (Bhatia, Patil and Sood, 2013). Minor infections, in particular immunodeficiency and spumavirus infection, are widespread in animal husbandry worldwide (Kolotvin, 2007; Materniak-Kornas et al., 2017; Mousavi et al., 2014; Rodrigues et al., 2019; Santos et al., 2019; Meas et al., 2003).

It is known that the causative agents of the above slow infections may not manifest themselves in the infected organism for many years, causing, in most cases, associated, two- or three-variant course of the disease and cause significant damage to livestock both in terms of animal resistance and product quantity and quality (Krasnikova, 2011; Supotnitskiy, 2009; Fedorov and Verkhovsky, 1996; Bhatia, Patil and Sood, 2013; Suarez et al., 1993).

Diseases caused by them are accompanied by non-plastic pathologies and are widespread among vertebrates. Losses from each of the diseases, and especially in the associated course, are due to the death and culling of animals, insufficient quantity and reduced quality of livestock products, loss of gene pool, the cost of anti-epizootic measures (Bhatia, Patil and Sood, 2013; Romen et al., 2007; Pinto-Santini, Stenbak and Linial, 2017).

In connection with the above program of scientific research provides for monitoring analysis of the epizootic state of animal husbandry in Ukraine for slow (minor) infectious diseases, primarily immunodeficiency and spumavirus infection, study the biological properties of pathogens in case of detection so that after the accumulation of biological mass BIV and BFV to design domestic means of retrospective diagnostics of the latter.

The aim of the study was to investigate of biological properties of field isolates of bovine immunodeficiency virus and bovine foamy virus on homological cell cultures (fetal bovine lung and bovine coronary artery endothelial cells).

Materials and methods. Donor animals infected with BIV and BFV were selected. Molecular genetic studies have confirmed the presence of BIV and BFV in lymphocytes, so we used a suspension of leukocyte fraction of animal blood (Hachiya et al., 2018; Materniak et al., 2013). Donor blood, collected in sterile vials with anticoagulant in the amount of 50 cm^3, was processed to isolate lymphocytes. To do this, the blood sample was diluted two times with buffered saline with the addition of penicillin 100 IU/cm^3, pH 7.2, then 8 cm^3 each was layered on a solution of triombrast (sodium amidotrizoate) with a density of 1.075, pre-poured into centrifuge tubes of 2 cm^3.

After centrifugation at 1,000 rpm for 40 min we took a layer of lymphocytes in a separate tube, washed once with buffered saline at 1,000 rpm for 10 min and one time with Eagle's medium by the above mode. The volume of the lymphocyte suspension was adjusted to 10 cm^3 with Eagle's medium to count the number of living cells with the addition of 0.1% trypan blue solution.

The concentration of live lymphocytes was adjusted to 1–3×10^6 cells/cm^3 in culture medium, which consists of 90% Eagle's medium, 10% native serum and 100 IU/cm^3 of penicillin.

To stimulate the production of the virus, short-term cultivation of lymphocytes that were isolated from the donor's blood was previously performed. For this purpose, the lymphocyte suspension was incubated for 48 h at a temperature of 37 ± 0.5°C, after that the number of living cells in the suspension of short-term cultured lymphocytes was determined.

The resulting suspension was tested for sterility by seeding on bacteriological growth media (nutrient agar and nutrient broth, enriched with glucose; Sabouraud and thioglycollate media) to exclude the presence of bacterial and fungal contamination.

Checking for the presence of BIV and BFV in lymphocytes after cultivation and the degree of their accumulation was assessed by polymerase chain reaction (PCR).

Two types of continuous monolayer cell cultures were used for infection, namely fetal bovine lung (FBL) and bovine coronary artery endothelial cells (BCAEC). Cell cultures for infection were taken at the level of 24–48 h of growth with 50–75% fulfillment of the monolayer. Reseeding of continuous cell cultures was performed in a nutrient medium consisting of 45% Eagle's medium, 45% of medium 199, 10% of bovine native serum, penicillin 100 IU/cm^3 and gentamicin 40 mg/cm^3.

A monolayer of FBL and BCAEC cell cultures with lymphocytes was incubated for 72 h at a conventional temperature 37 ± 0.5°C, after which it was washed twice with Hanks' solution, followed by re-replacement of the growth medium. Reseeding of cultures was carried out by the completion of the monolayer, on average every 3–4 days with a growth medium, the composition of which is indicated above.

Containers with uninfected monolayer of FBL and BCAEC cell cultures were used as controls. Each passage was monitored daily visually and using light microscopy. At the level of each third and fifth passages, samples were subjected to molecular genetic testing (PCR) to detect the genetic material of pathogens.

Primer systems Int 1-Int 2 (outer pair, length of amplified product is 430 bp) and Int 3-Int 4 (inner pair, length of amplified product 221 bp) were used to detect BFV proviral DNA by 'nested' version of PCR according to the recommendations of the developers (Materniak et al., 2013).

To detect BIV proviral DNA, a pair of primers RT_+(–) flanking the conserved reverse transcriptase domain (PCR product length 495 bp) and a pair of primers BIV_Pol_+(–) flanking the *pol* gene of bovine immunodeficiency virus (PCR product length is 235 bp) were used. Amplification was performed by standard PCR according to the recommendations of the developers (Moody et al., 2002).

Results and discussion. Microscopic studies of the continuous cultures after infection showed that the addition of short-term cultured lymphocytes did not cause destructive changes in the morphology of cells of both lines.

The cells of the monolayer were located densely, with clearly defined borders, a small number of vacuoles was observed in the cytoplasm, the nuclei had a typical oval shape.

After 1st passage, lymphocytes were still partially found, but after 2nd passages, lymphocytes were not detected during microscopy.

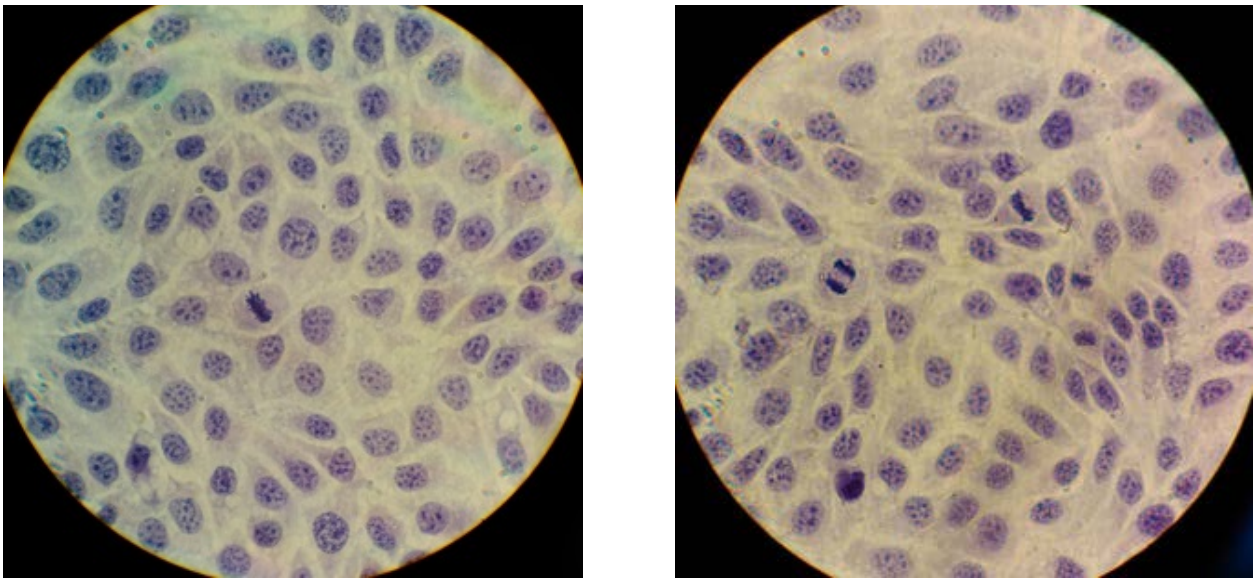
Observations of the state of the monolayer of cell cultures (FBL+BIV) and (FBL+BFV), at the level of 1st, 2nd, and 3rd passages established satisfactory filling of the monolayer, morphologically the cells of the experimental culture were similar to control, PCR at the level of 3rd passages showed the presence of BIV and BFV genetic material in monolayer cells.

At 4th–6th passages in the experimental cell culture morphological destruction of cells with signs of symplast formation was observed — in the culture there were enlarged cells with two or three nuclei, monolayer cells were removed from the glass with difficulty using trypsin-EDTA solution.

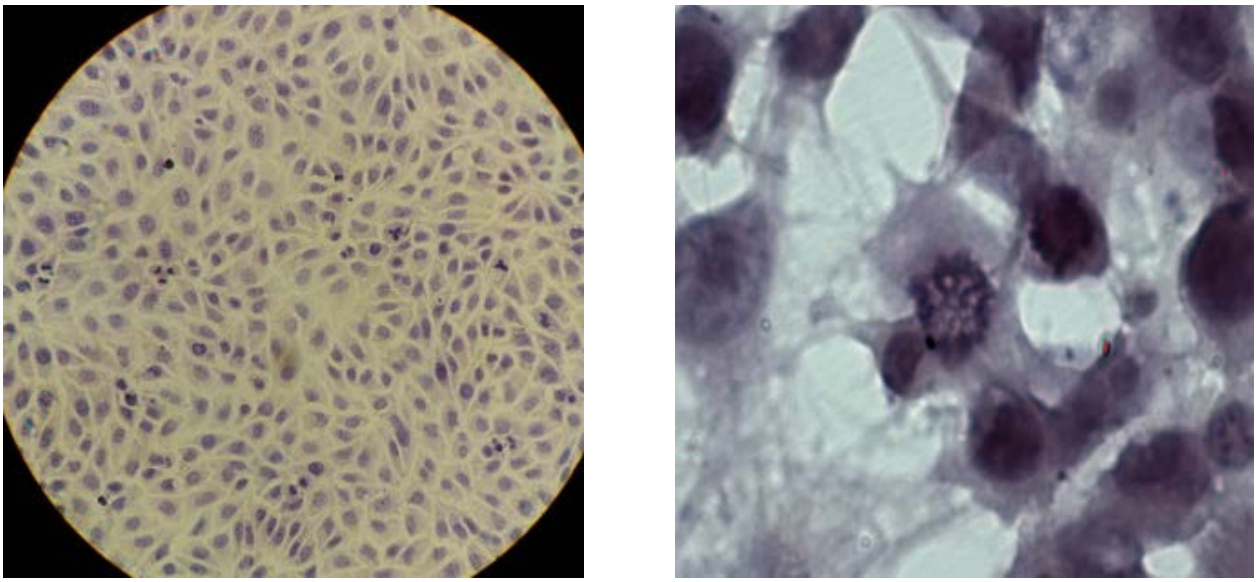
At the level of 7th–8th passages, the picture of the state of the monolayer remained similar, with a sharp increase in the number of dead cells in the culture fluid.

For a detailed study of the morphology of infected FBL cells, the cell suspension was seeded in test tubes with cover slides according to the conventional method, after the formation of a monolayer, culture cells were stained by Giemsa, and more intense staining was observed in the perinuclear zone.

Fig. 1 shows the changes in monolayer cells observed during the implementation of the research program.



Normal cell monolayer



Tripolar mitosis | Hollow metaphase

Figure 1. Changes in monolayer cells.

A total of 15 passages of culture (FBL+BIV) and (FBL+BFV) each were performed. By PCR genetic material of pathogens of immunodeficiency and spumavirus infection of cattle was still recorded at the level of 10th passage. Molecular-genetic study of cell culture in PCR at the level of 13th and 15th passages showed a negative result.

The gel picture (Fig. 2) shows the results of detection of genetic material of pathogens of immunodeficiency and spumavirus infection of cattle in the next passages of the culture of FBL cells.

A study on the possibility of integrating the field forms of pathogens of slow infections (BIV and BFV) in the continuous cell culture of BCAEC revealed a lower susceptibility of this culture cells to viruses of the Retroviridae family.

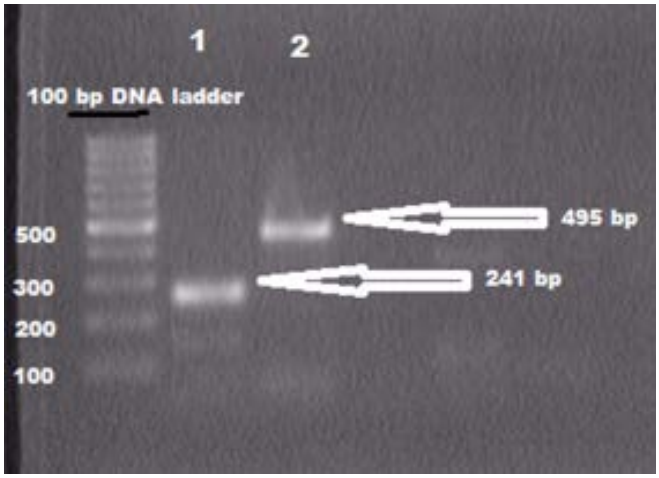


Figure 2. Gel picture of positive blood samples for BFV and BIV (100 bp DNA ladder — molecular weight marker, 1 — BFV positive sample, 2 — BIV positive sample).

According to the results of PCR at the level of 3rd passage, BIV and BFV genetic material in the cells of the monolayer of BCAEC culture was noted and already at this level of cultivation numerous vacuolation in infected monolayer cells (BCAEC+BFV) and destructive changes in the monolayer, which were expressed in its partial destruction with the formation of a numerical number of dead cells, were observed. At the level of 4th and 5th passages vacuolation and syncytial formation were observed in most (70–80%) of monolayer cells. At the level of 5th passage, PCR results showed the presence of genetic material of pathogens of retroviral infections, and material of 7th passage gave a negative result.

Conclusions. 1. Pathogens of bovine slow infections, namely bovine immunodeficiency virus and bovine foamy virus, are able to integrate into cell cultures of homologous to cattle type, which is confirmed by the results of PCR.

2. There has been determined the presence of genetic material of pathogens of bovine immunodeficiency (BIV) and spumavirus infection (BFV) in the cultivation of lymphocytes of field isolates in the culture of bovine coronary artery endothelial cells (BCAEC) at the level of 5th passage, and in the cell culture of fetal bovine lung (FBL) — at the level of 10th passage.

3. In the process of integration of pathogens of immunodeficiency and spumavirus infection of cattle in continuous cell cultures FBL and BCAEC, morphological changes in the state of the monolayer by the principle of syncytiation and vacuolation are observed.

References

Bhatia, S., Patil, S. S. and Sood, R. (2013) 'Bovine immunodeficiency virus: A lentiviral infection', *Indian Journal of Virology*, 24(3), pp. 332–341. doi: 10.1007/s13337-013-0165-9.

Fedorov, Yu. N. and Verkhovsky, O. A. (1996) 'Bovine immunodeficiency virus' [Virus immunodefitsita krupnogo rogatogo skota], *Veterinary* [*Veterinariya*], 10, pp. 17–19. [in Russian].

Hachiya, Y., Kimura, K., Oguma, K., Ono, M., Horikita, T. and Sentsui, H. (2018) 'Isolation of bovine foamy virus in Japan', *Journal of Veterinary Medical Science*, 80(10), pp. 1604–1609. doi: 10.1292/jvms.18-0121.

Kolotvin, V. V. (2007) *Bovine Immunodeficiency Virus: Indication of Infection and Prevalence in Farms of the Russian Federation [Virus immunodefitsita krupnogo rogatogo skota: indikatsiya infektsii i rasprostranennost' v khozyaystvakh Rossiyskoy Federatsii]*. The dissertation thesis for the scientific degree of the candidate of biological sciences. Moscow: All-Russian Scientific Research Institute of Experimental Veterinary Medicine named after Ya. R. Kovalenko. Available at: https://search.rsl.ru/ru/record/01003053744. [in Russian].

Krasnikova, E. S. (2011) 'Epizootic situation on Viral immunodeficiency of cattle in Saratov and the Saratov Region' [Epizooticheskaya situatsiya po virusnomu immunodefitsitu krupnogo rogatogo skota v gorode Saratove i Saratovskoy oblasti], *Vestnik Veterinarii*, 4, pp. 70–71. Available at: https://www.elibrary.ru/item.asp?id=17069902. [in Russian].

Materniak, M., Hechler, T., Lochelt, M. and Kuzmak, J. (2013) 'Similar patterns of infection with bovine foamy virus in experimentally inoculated calves and sheep', *Journal of Virology*, 87(6), pp. 3516–3525. doi: 10.1128/JVI.02447-12.

Materniak-Kornas, M., Osiński, Z., Rudzki, M. and Kuźmak, J. (2017) 'Development of a recombinant protein-based ELISA for detection of antibodies against bovine foamy virus', *Journal of Veterinary Research*, 61(3), pp. 247–252. doi: 10.1515/jvetres-2017-0034.

Meas, S., Yilmaz, Z., Usui, T., Torun, S., Yesilbag, K., Ohashi, K. and Onuma, M. (2003) 'Evidence of bovine immunodeficiency virus in cattle in Turkey', *The Japanese Journal of Veterinary Research*, 51(1), pp. 3–8. doi: 10.14943/jjvr.51.1.3.

Moody, C. A., Pharr, G. T., Murphey, J., Hughlett, M. B., Weaver, C. C., Nelson, P. D. and Coats, K. S. (2002) 'Confirmation of vertical transmission of bovine immunodeficiency virus in naturally infected dairy cattle using the polymerase chain reaction', *Journal of Veterinary Diagnostic Investigation*, 14(2), pp. 113–119. doi: 10.1177/104063870201400204.

Mousavi, S., Haghparast, A., Mohammadi, G. and Tabatabaeizadeh, S.-E. (2014) 'Prevalence of bovine leukemia virus (BLV) infection in the northeast of Iran', *Veterinary Research Forum*, 5(2), pp. 135–139. PMID: 25568707.

Pinto-Santini, D. M., Stenbak, C. R. and Linial, M. L. (2017) 'Foamy virus zoonotic infections', *Retrovirology*, 14(1), p. 55. doi: 10.1186/s12977-017-0379-9.

Rodrigues, A. P. S., Fonseca Júnior, A. A., Lima, G. K., Bicalho, J. M., Leite, R. C. and Reis, J. K. P. (2019) 'Molecular detection of bovine immunodeficiency virus (BIV) in bovines from the state of Minas Gerais, Brazil', *Arquivo Brasileiro de Medicina Veterinária e Zootecnia*, 71(2), pp. 711–714. doi: 10.1590/1678-4162-10495.

Romen, F., Backes, P., Materniak, M., Sting, R., Vahlenkamp, T. W., Riebe, R., Pawlita, M., Kuzmak, J. and Löchelt, M. (2007) 'Serological detection systems for identification of cows shedding bovine foamy virus via milk', *Virology*, 364(1), pp. 123–131. doi: 10.1016/j.virol.2007.03.009.

Santos, A. F., Cavalcante, L. T. F., Muniz, C. P., Switzer, W. M. and Soares, M. A. (2019) 'Simian foamy viruses in Central and South America: A new world of discovery', *Viruses*, 11(10), p. 967. doi: 10.3390/v11100967.

Suarez, D. L., VanDerMaaten, M. J., Wood, C. and Whetstone, C. A. (1993) 'Isolation and characterization of new wild-type isolates of bovine lentivirus', *Journal of Virology*, 67(8), pp. 5051–5055. doi: 10.1128/JVI.67.8.5051-5055.1993.

Supotnitskiy, M. V. (2009) *Evolutionary Pathology. On the Question of the Place of HIV Infection and HIV/AIDS Pandemic Among Other Infectious, Epidemic and Pandemic Processes [Evolyutsionnaya patologiya. K voprosu o meste VICh-infektsii i VICh/SPID-pandemii sredi drugikh infektsionnykh, epidemicheskikh i pandemicheskikh protsessov]*. Moscow: Vuzovskaya kniga. ISBN 9785950203787. Available at: http://supotnitskiy.ru/book/supotnitskiy_evolucionnaya_patologia.pdf. [in Russian].

Part 3. Biosafety

UDC 619:615.284/.285.076.9.099.036.11:636.932.028:636.7 **DOI 10.36016/JVMBBS-2020-6-2-4**

DETERMINATION OF ACUTE TOXICITY PARAMETERS OF THE DRUG 'MEGASTOP FOR DOGS' ON WHITE RATS AND MICE

Orobchenko O. L. [1], Romanko M. Ye. [1], Yaroshenko M. O. [1], Gerilovych I. O. [1], Zhukova N. A. [2], Sapko S. A. [2], Maslyuk A. V. [3]

[1] National Scientific Center 'Institute of Experimental and Clinical Veterinary Medicine', Kharkiv, Ukraine, e-mail: toxi-lab@ukr.net
[2] Research and Production Enterprise 'Suziria' LLC, Kharkiv, Ukraine, e-mail: zhukova.n@priroda.ua
[3] State Scientific and Research Institute of Laboratory Diagnostics and Veterinary and Sanitary Expertise, Kyiv, Ukraine, e-mail: maslychok@ukr.net

Summary. The experiments were performed on 58 males of nonlinear white rats 3–4 months old and weighing 180–200 g and 64 females of nonlinear white mice 2.5–3 months old and weighing 18–22 g. In the main experiment on rats, six experimental groups were formed, the animals of which were injected intragastrically with the drug 'MEGASTOP for dogs' (by absolute weight) in doses of 1,000.0, 2,000.0, 3,000.0, 4,000.0, 5,000.0, and 6,000.0 mg/kg body weight; in the main experiment on mice, seven experimental groups were formed, the animals of which were administered the drug in doses of 100.0, 500.0, 1,000.0, 1,500.0, 2,000.0, 2,500.0, and 3,000.0 mg/kg body weight. Control rats and mice were injected with 2.0 cm^3 and 0.2 cm^3 of polyethylene glycol-400, respectively. Clinical symptoms of poisoning with the drug 'MEGASTOP for dogs' of white rats (at doses of 2,000.0–6,000.0 mg/kg body weight) and mice (at doses of 1,000.0–3,000.0 mg/kg body weight) were refusals of food and water, loss of coordination, sitting in one place, a dose-dependent increase in depression with subsequent complete depression, lack of response to external stimuli and death on the first or fourth day after administration. During autopsy in rats and mice that died as a result of poisoning with the drug 'MEGASTOP for dogs', we recorded pallor of the mucous membranes of the mouth, trachea, pharynx, and esophagus; increase in heart volume, atrial blood supply; pulmonary hyperemia; uncoagulated blood; increase in liver volume, dark cherry color, flabby consistency; catarrhal inflammation of the mucous membrane of the small intestine. According to the results of determining the parameters of acute toxicity of the drug 'MEGASTOP for dogs' in the case of a single intragastric injection, LD_{50} for male rats is 3,384.98 ± 444.94 mg/kg, and for female mice — 2,025.88 ± 279.46 mg/kg body weight, which allows to classify it to class IV by the toxicity — low-toxic substances (LD_{50} — 501–5,000 mg/kg) and by the degree of danger to class III— moderately dangerous substances (LD_{50} — 151–5,000 mg/kg)

Keywords: acute toxicity, male rats, female mice, dose, intragastric administration, 'MEGASTOP for dogs'

Introduction. Ectoparasitic and invasive diseases of dogs are widespread both in the world and in Ukraine (Havryk, 2015; Diakou et al., 2019; Hasib et al., 2020), so effective control is possible only with the use of highly effective and available drugs, which determines the relevance of the development of new antiparasitic veterinary drugs.

Besides, it is necessary to rotate (combine) the active substances to prevent their resistance in parasites (Tucker, Kaufman and Weeks, 2019; Klafke et al., 2020; Kumar, Klafke and Miller, 2020). Some of the promising active ingredients of antiparasitic drugs in this regard today are imidacloprid and ivermectin (Sheele and Ridge, 2016; Gomez and Picado, 2017).

Imidacloprid belongs to the group of chloronicotinyl compounds. It blocks postsynaptic cholinergic receptors that are sensitive to nicotine and are located in ectoparasites in the central nervous system. Interruption of nerve impulse transmission leads to paralysis and death of the parasite. Imidacloprid has a low level of penetration through the blood-brain barrier, so it has virtually no effect on the central nervous system of mammals. Ivermectin belongs to the compounds produced by microorganisms of *Streptomyces avermitilis* (Elbert, Nauen and Leicht, 1998).

Ivermectin has a pronounced antiparasitic effect on larval and mature forms of nematodes. The mechanism of action of ivermectin on the parasite is that it stimulates the release of the inhibitory neurotransmitter, gamma-aminobutyric acid, in presynaptic neurons, which binds to special receptors on nerve endings, increasing the permeability of membranes for chlorine ions and blocking the transmission of nerve-muscle pulses. This leads to impaired nerve impulse transmission, paralysis and death of parasites (Laing, Gillan and Devaney, 2017).

Thus, Research and Production Enterprise 'Suziria' LLC offered a new drug — 'MEGASTOP for dogs'. One milliliter of the drug contains active substances:

imidacloprid — 100.0 mg; ivermectin — 25.0 mg, and excipients: N-methylpyrrolidone, dimethyl sulfoxide, polyethylene glycol-400.

The drug 'MEGASTOP for dogs' is used for the prevention and treatment of animals affected by fleas (*Ctenocephalides* spp.), acariform mites (*Otodectes cynotis, Notoedres cati, Sarcoptes sapis*), mites of the genus Demodex; prevention of heartworm disease (effective against 3rd and 4th stages larvae of *Dirofilaria immitis*).

The 1st stage of toxicological research of new drugs is to determine their acute toxicity, the purpose of which is to obtain information on the danger of the test substance in short-term conditions and as a result of which it is expected to obtain data on lethal doses and symptoms of acute poisoning.

Therefore, **the aim of the work** was to determine the parameters of acute toxicity of the veterinary drug 'MEGASTOP for dogs' manufactured by Research and Production Enterprise 'Suziria' LLC under conditions of a single oral administration to white rats and mice.

Materials and methods. The experiments were performed on 58 males of nonlinear white rats 3–4 months old and weighing 180–200 g and 64 females of nonlinear white mice 2.5–3 months old and weighing 18–22 g, kept under optimal vivarium conditions (Zapadnyuk et al., 1983; Kotsiumbas et al., 2006; Karkishchenko and Grachev, 2010): room temperature was 18 ± 2 °C, relative humidity 60–70%, lighting cycle day — night, during the experiment, was 10–14 hours, and also a 10-fold change in air volume in the vivarium room per hour was provided.

For feeding rats and mice used complete feed for rodents. The animals had free access to water and food.

Before the start of the study, each animal was weighed. Doses administered were calculated individually according to the weight of each animal, and the volume of drug administered intragastrically at one time did not exceed 2.5 cm^3 and 1.0 cm^3 for rats and mice, respectively. Determination of the dose range for the main experiment was performed in a previous experiment.

In a previous experiment on rats on the principle of analogues control and three experimental groups were formed of four animals each (n = 4). The drug was administered in doses of 1,000.0, 3,000.0, and 6,000.0 mg/kg body weight in absolute weight of the drug once orally using a esophageal gastric tube. Animals in the control group were administered polyethylene glycol-400.

After accounting the results of the previous experiment in the main experiment, six experimental groups were formed, rats which were administered the drug in doses of 1,000.0, 2,000.0, 3,000.0, 4,000.0, 5,000.0, and 6,000.0 mg/kg body weight, as well as the control group, animals which were injected with polyethylene glycol-400 in a volume of 2.0 cm^3 according to the same regulations. There were six animals in each group (n = 6).

In a previous experiment on mice on the principle of analogues control and three experimental groups were formed of four animals each (n = 4). The drug was administered in doses of 200.0, 1,000.0, and 5,000.0 mg/kg body weight in absolute weight of the drug once orally using an esophageal gastric tube. Animals in the control group were administered polyethylene glycol-400.

After accounting the results of the previous experiment in the main experiment, seven experimental groups were formed, mice which were administered the drug in doses of 100.0, 500.0, 1,000.0, 1,500.0, 2,000.0, 2,500.0, and 3,000.0 mg/kg body weight, as well as a control group, the animals of which were injected with polyethylene glycol-400 in a volume of 0.2 cm^3 according to the same regulations. There were six animals in each group (n = 6).

Experiments on animals were carried out in accordance with the rules of the 'European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes' (CE, 1986) and Council Directive 86/609/EEC (CEC, 1986).

The clinical condition of the experimental animals was observed for 14 days, noting the appearance and development of clinical signs of poisoning, the time of death or recovery to physiological norm. During the clinical examination of rats we paid attention to behavior, reaction to external stimuli, appetite, skin condition, color of mucous membranes, frequency of respiration and defecation, changes in color and consistency of feces, etc. (Kotsiumbas et al., 2006).

After the death of the animals we performed a pathological autopsy. The macroscopic method of research was used to establish pathological changes (Zharov, Ivanov and Strel'nikov, 2003). Pathological autopsy was performed according to the following scheme: at the 1st stage the external inspection was carried out, noting the condition of the coat and mucous membranes; at the 2nd stage we performed autopsy and examination of body cavities and internal organs, such as pharynx, trachea, larynx, heart, lungs, liver, spleen, kidneys, stomach, intestines, noting changes in color, consistency, pattern and shape of organs.

According to the results of death, LD_{10}, LD_{16}, LD_{50}, LD_{84}, LD_{90}, LD_{100}, and LD_{50} error were calculated by the method of probit analysis in the modification of Prozorovskiy (Prozorovskiy, 2007). Toxicometric parameters of the drug were calculated by the method of least squares for probit analysis of mortality curves. The percentage of lethality, probits (Y), weights of probits (Z) were established. To plot the abscissa, the values of the drug doses (mg/kg) were plotted, and the value of the effect (%) was plotted on the ordinate axis.

The obtained results were processed by methods of variation statistics using the software package StatPlus v. 5.9.8.5. Data were presented as mean values with a standard deviation of 95% confidence level by the Student's *t*-test (Van Emden, 2019).

Results and discussion. In a previous experiment, rats were administered the drug 'MEGASTOP for dogs' in doses of 1,000.0, 3,000.0, and 6,000.0 mg/kg body weight. Clinical observations showed that intragastric administration of the drug to rats of the experimental group I (1,000.0 mg/kg body weight) 1–2 h after drug administration, caused a slight depression, which persisted during the 1st day after administration, however, the animals ate feed and drank water well. On the 2nd day, the condition of animals in this group returned to normal.

In rats of the experimental group II (3,000.0 mg/kg body weight) 40–60 min after administration of the drug a slight suppression was recorded, which increased during the 1st day after administration, the animals were reluctant to take food and water, moved slowly around the cage, the reaction to external stimuli was reduced. Then in two rats an increase in depression was observed, the animals sat in one place, breathing hard, the fur was puffy. On the 2nd–4th days, the condition of two animals from this group was normalized, and in two animals complete depression was observed, lack of response to external stimuli; and on the 2nd and 3rd day they died (Table 1).

Table 1 — Dynamics of rat death in a previous experiment to determine the acute toxicity of the drug 'MEGASTOP for dogs' (n = 16)

Terms of rat death, in	Groups of rats and doses, mg/kg			
	Control	Experimental		
		I (1,000.0)	II (3,000.0)	III (6,000.0)
4–8 hours	–	–	–	–
1–1.5 days	–	–	–	4
1.5–3 days	–	–	2	–
4–14 days	–	–	–	–
Total died	–	–	2	4

In rats of the experimental group III, which were administered at a dose of 6,000.0 mg/kg body weight, during the 1st day after administration, more and more depression was observed, the animals refused food and water, then loss of coordination was observed, the animals were reluctant to move around the cage, mostly sat in one place. One day after administration, complete depression was observed in animals, rats did not respond to external stimuli, lying on their stomachs. In addition, there was a specific, unpleasant odor, the death of animals occurred within 1–1.5 days after administration of the drug (Table 1).

In the main experiment, rats were administered the drug 'MEGASTOP for dogs' in doses of 1,000.0, 2,000.0, 3,000.0, 4,000.0, 5,000.0, and 6,000.0 mg/kg body weight. During the observation of animals of the experimental group I in 30–60 min after administration of the drug a slight depression was recorded, which persisted during the day after administration, however, food and water during the day the animals took well.

In rats of the experimental group II, the depressed state was observed for 2–3 days after drug administration, appetite was also reduced, but thirst was noted. In one animal of this group increasing depression was observed during 4 days after administration, and on the 4th day it died (Table 2). The picture of poisoning was similar in rats of the experimental group III, but on the 3rd and 4th days one rat died.

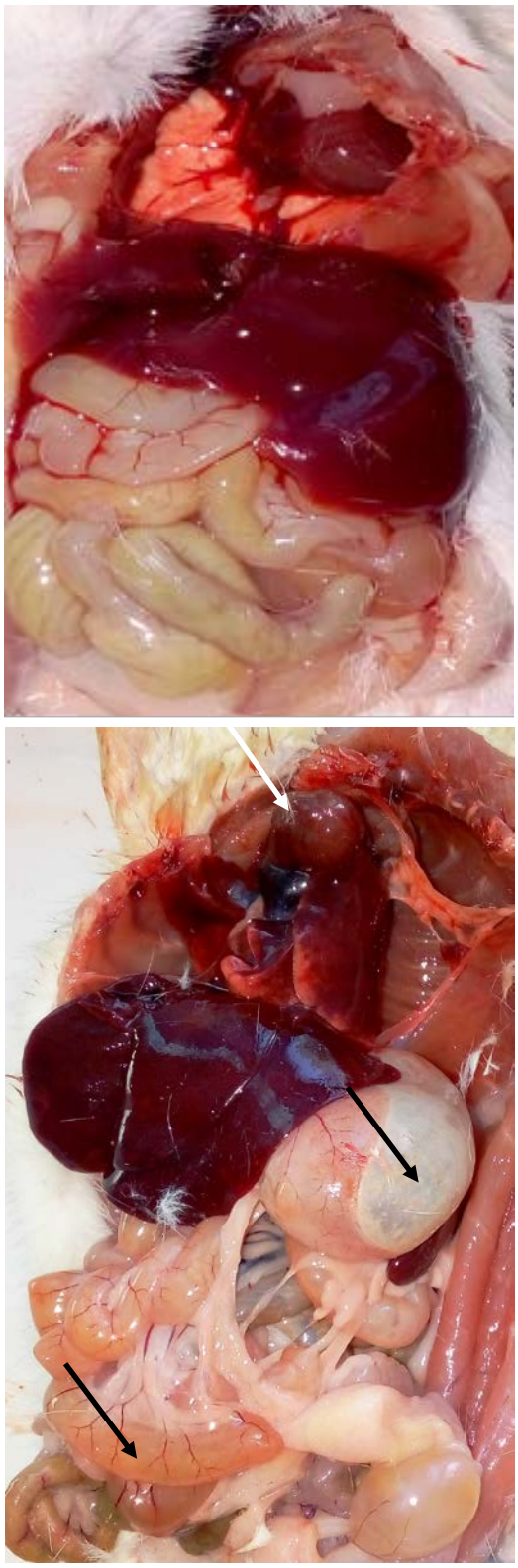
Table 2 — Dynamics of rat death in the main experiment to determine acute toxicity of the drug 'MEGASTOP for dogs' (n = 42)

Groups of rats and doses, mg/kg body weight		Terms of rat death, in					
		1 day	2 days	3 days	4 days	5–14 days	Total died
Control		–	–	–	–	–	–
Experimental	I (1,000.0)	–	–	–	–	–	–
	II (2,000.0)	–	–	–	1	–	1
	III (3,000.0)	–	–	1	1	–	2
	IV (4,000.0)	–	3	1	–	–	4
	V (5,000.0)	4	2	–	–	–	6
	VI (6,000.0)	6	–	–	–	–	6

In animals of the experimental group VI in 30–60 minutes after administration of the drug there was an increase in depression, the animals sat in one place, breathing hard, the hair was puffy. On the 4th day, the condition of two animals from this group was normalized, while in four ones, starting from the 2nd day after administration, complete depression was observed, no reaction to external stimuli; and on the 2nd and 3rd days they died. In rats of experimental groups V and VI during the 1st day after introduction there was an increasing depression, the animals refused food and water, sat in one place, breathed heavily, the coat was puffy. The death of animals occurred on the 1st–2nd day after administration of the drug (Table 2).

After the death of rats we performed a pathological autopsy. During the external examination of the carcasses of the experimental animals, it was found that the fur was disheveled, dull; leaks from the mouth, nasal cavity, eyes, and anus were not observed; however, we noted the pallor of the visible mucous membranes.

At autopsy in rats recorded pallor of the mucous membranes of the mouth, trachea, pharynx, and esophagus; bloating and drug residues were observed in the stomach; the heart is enlarged, the atria are full of blood; lungs hyperemic, with greater severity in animals of experimental groups V and VI, from pink-marble to dark burgundy, the pattern in the section is blurred; blood is not clotted; liver enlarged, dark cherry color, flabby consistency; spleen and pancreas unchanged; the bladder is filled with urine, the kidneys are light brown, not enlarged; catarrhal inflammation of the mucous membrane was found in the small intestine (Fig. 1).



A

B

Figure 1. Pathological changes in the internal organs of rats under a single intragastric injection of the drug 'MEGASTOP for dogs' (doses of 5,000.0 and 6,000.0 mg/kg body weight): A — control group (liver and intestines without pathological changes); B — experimental group (atria are blood-filled, the liver is enlarged, dark cherry color, bloating in stomach, catarrhal inflammation in the small intestine)

The next step in studying the toxicological characteristics of the drug 'MEGASTOP for dogs' was to determine the average lethal dose and its standard error (LD_{50}, LD_{10}, LD_{16}, LD_{84}, LD_{90}, LD_{100}).

A graphical representation of the dose-effect curve for rats is shown in Fig. 2.

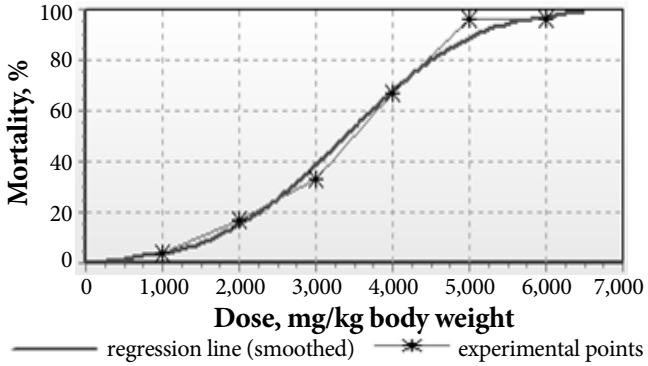


Figure 2. Mortality curve of male rats under conditions of a single oral administration of the drug 'MEGASTOP for dogs' (probit analysis method)

The results of calculating the average lethal dose of the drug for rats under oral administration are shown in Table 3.

Table 3 — The results of the calculation of lethal doses of the drug 'MEGASTOP for dogs' under conditions of a single oral administration to male rats

Dose, mg/kg body weight	Percentage (%)	N	Probit (Y)	Weighting factor (Z)
1,000	0.0417	6	3.2680	1.5359
2,000	0.1667	6	4.0326	3.5653
3,000	0.3333	6	4.5697	4.5697
4,000	0.6667	6	5.4303	4.5697
5,000	0.9583	6	6.7320	1.5359
6,000	0.9583	6	6.7320	1.5359
Regression statistics				
LD_{50}	3,384.98	LD_{50} SE	444.94	
LD_{10}	1,674.12	LD_{16}	2,050.17	
LD_{84}	4,719.78	LD_{90}	5,095.83	
LD_{100}	5,387.18			

According to the results of research, it was found that the LD_{50} of the drug 'MEGASTOP for dogs' under the conditions of its single oral administration to male rats is 3,384.98 ± 444.94 mg/kg, LD_{10} — 1,674.12 mg/kg, LD_{16} — 2,050.17 mg/kg, LD_{84} — 4,719.78 mg/kg, LD_{90} — 5,095.83 mg/kg, LD_{100} — 5,387.18 mg/kg body weight, respectively.

In a previous experiment, mice were injected with the drug 'MEGASTOP for dogs' in doses of 200.0, 1,000.0, and 5,000.0 mg/kg body weight. Clinical observations showed that intragastric administration of the drug to mice of the

experimental group I (200.0 mg/kg body weight) 1–2 h after drug administration, caused a slight depression, which persisted during the 1st day after administration, however, food and water during the day the animals took well. On the 2nd day, the condition of animals in this group returned to normal. Mice deaths were not observed in this group (Table 4).

Table 4 — Dynamics of death of mice in a previous experiment to determine the acute toxicity of the drug 'MEGASTOP for dogs' (n = 16)

Terms of mice death, in	Groups of rats and doses, mg/kg			
	Control	Experimental		
		I (200.0)	II (1,000.0)	III (5,000.0)
4–8 hours	–	–	–	–
9–24 hours	–	–	–	4
2 days	–	–	1	–
3–14 days	–	–	–	–
Total died	–	–	1	4

In mice of the experimental group II (1,000.0 mg/kg body weight) in 40–60 min after administration of the drug a slight inhibition was recorded, which increased during the 1st day after administration, the animals were reluctant to take food and water, moved slowly through the cell, the reaction on external stimuli was reduced. Then one mouse had an increase in depression, the animal was sitting in one place, breathing hard, the fur was puffy. On 2nd–4th days, the condition of three animals from this group was normalized, and one mouse died on the 2nd day after administration (Table 4).

In mice of the experimental group III, which were administered the drug at a dose of 5,000.0 mg/kg body weight, during the 1st day after administration there was an increase in depression, the animals refused food and water, then incoordination was observed, the animals were reluctant to move around the cage, mostly sitting in one place. In 22 h after administration, the complete depression in animals was observed, mice did not respond to external stimuli, lying on their stomachs. In addition, there was a specific, unpleasant odor, the death of animals occurred within 24 h after administration of the drug (Table 4).

In the main experiment, mice were administered the drug 'MEGASTOP for dogs' in doses of 100.0, 500.0, 1,000.0, 1,500.0, 2,000.0, 2,500.0, and 3,000.0 mg/kg body weight. During the observation of animals of the experimental group I no clinical picture of poisoning was observed, the animals were active and took food and water well. In the experimental group II during the day after drug administration there was a decrease in the response to external stimuli, which disappeared during the 2nd day of the experiment. The death of mice in the experimental groups I and II within 14 days was not detected.

In mice of the experimental groups III–V in 40–60 min after administration, dose-dependent depression was observed, which persisted for 2–3 days after drug administration, appetite was also reduced, but thirst was noted. In one animal from the experimental group III and two ones from the experimental groups IV and V within two days after administration, increasing depression and death were observed on the 1st–3rd days after administration. (Table 5).

Table 5 — Dynamics of death of mice in the main experiment to determine the acute toxicity of the drug 'MEGASTOP for dogs' (n = 48)

Groups of mice and doses, mg/kg body weight		Terms of rat death, in				
		1 day	2 days	3 days	4–14 days	Total died
Control		–	–	–	–	–
Experimental	I (100.0)	–	–	–	–	–
	II (500.0)	–	–	–	–	–
	III (1,000.0)	–	–	1	–	1
	IV (1,500.0)	–	1	1	–	2
	V (2,000.0)	1	1	–	–	2
	VI (2,500.0)	2	2	–	–	4
	VII (3,000.0)	6	–	–	–	6

The most pronounced was the poisoning in mice of the experimental groups VI–VII, in 15–30 min after drug administration, an increase in depression was observed, the animals sat in one place, had difficulty breathing, the hair was puffy. The death of animals was observed on 1st–2nd days after administration of the drug (Table 5).

After the death of the mice, a pathological autopsy was performed. During the external examination of the carcasses of the experimental animals, it was found that the fur was disheveled, dull; leaks from the mouth, nasal cavity, eyes, and anus were not observed; however, we noted the pallor of the visible mucous membranes.

At autopsy in mice we recorded pallor of the mucous membranes of the mouth, trachea, pharynx, and esophagus; bloating and drug residues were observed in the stomach; the heart is enlarged, the atria are full of blood; lungs hyperemic, with greater severity in animals of the experimental groups VI–VII, from pink-marble to dark burgundy, the pattern in the section is blurred; blood is not clotted; liver enlarged, dark cherry color, flabby consistency; spleen and pancreas unchanged; the bladder is filled with urine, the kidneys are light brown, not enlarged; catarrhal inflammation of the mucous membrane was found in the small intestine.

The next step in studying the toxicological characteristics of the drug 'MEGASTOP for dogs' was to determine the average lethal dose and its standard error (LD_{50}, LD_{10}, LD_{16}, LD_{84}, LD_{90}, LD_{100}).

A graphical representation of the dose-effect curve for mice is shown in Fig. 3.

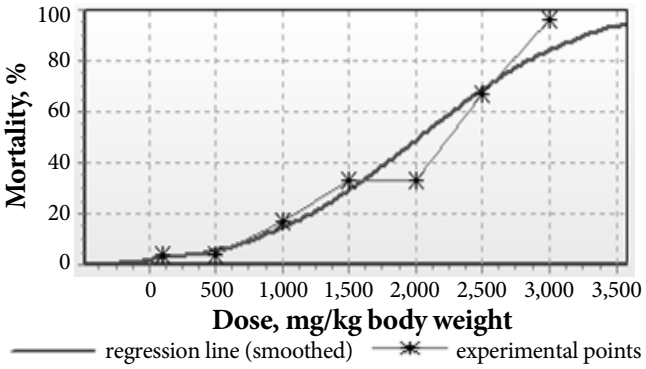


Figure 3. Mortality curve of female mice under conditions of a single oral administration of the drug 'MEGASTOP for dogs' (probit analysis method)

The results of calculating the average lethal dose of the drug for mice under conditions of a single oral administration are shown in Table 6.

Table 6 — The results of the calculation of lethal doses of the drug 'MEGASTOP for dogs' under conditions of a single oral administration to female mice

Dose, mg/kg body weight	Percentage (%)	N	Probit (Y)	Weighting factor (Z)
100	0.0417	6	3.2680	1.5359
500	0.0417	6	3.2680	1.5359
1,000	0.1667	6	4.0326	3.5653
1,500	0.3333	6	4.5697	4.5697
2,000	0.3333	6	4.5697	4.5697
2,500	0.6667	6	5.4303	4.5697
3,000	0.9583	6	6.7320	1.5359
Regression statistics				
LD_{50}	2,025.88	LD_{50} SE		279.46
LD_{10}	785.05	LD_{16}		1,057.79
LD_{84}	2,993.98	LD_{90}		3,266.72
LD_{100}	3,478.02			

According to the results of research, it was found that the LD_{50} of the drug 'MEGASTOP for dogs' under the conditions of its single oral administration to female mice is 2,025.88 ± 279.46 mg/kg, LD_{10} — 785.05 mg/kg, LD_{16} — 1,057.79 mg/kg, LD_{84} — 2,993.98 mg/kg, LD_{90} — 3,266.72 mg/kg, LD_{100} — 3,478.02 mg/kg body weight, respectively.

Therefore, the drug 'MEGASTOP for dogs' in terms of toxicity can be classified as class IV — low-toxic substances (LD_{50} 501–5,000 mg/kg) (Kotsiumbas et al., 2006), and by the degree of danger to class III — moderately dangerous substances (LD_{50} 151–5,000 mg/kg) by GOST 12.1.007-76 (Gosstandart, 1976).

Conclusions. 1. Clinical symptoms of poisoning of white rats and mice with the drug 'MEGASTOP for dogs' were refusal of food and water, incoordination, sitting in one place, dose-dependent increase in depression with subsequent complete suppression, lack of response to external stimuli and death on the 1st–4th days after administration.

2. During autopsy in rats and mice killed by poisoning with the drug 'MEGASTOP for dogs', we recorded pallor of the mucous membranes of the mouth, trachea, pharynx, and esophagus; bloating and drug residues were observed in the stomach; the heart is enlarged, the atria are full of blood; lungs hyperemic, with greater severity with increasing dose, from pink-marble to dark burgundy, the pattern in the section is blurred; blood is not clotted; liver enlarged, dark cherry color, flabby consistency; spleen and pancreas unchanged; the bladder is filled with urine, the kidneys are light brown, not enlarged; catarrhal inflammation of the mucous membrane was found in the small intestine.

3. According to the results of determining the parameters of acute toxicity of the drug 'MEGASTOP for dogs' in the case of a single intragastric injection of LD_{50} for male rats is 3,384.98 ± 444.94 mg/kg, and for female mice — 2,025.88 ± 279.46 mg/kg body weight, which allows to classify it by the toxicity to class IV — low-toxic substances (LD_{50} 501–5,000 mg/kg) and by the degree of danger to class III — moderately dangerous substances (LD_{50} 151–5,000 mg/kg).

References

CE (The Council of Europe). (1986) *European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes*. (European Treaty Series, No. 123). Strasbourg: The Council of Europe. Available at: https://conventions.coe.int/treaty/en/treaties/html/123.htm.

CEC (The Council of the European Communities). (1986) 'Council Directive 86/609/EEC of 24 November 1986 on the approximation of laws, regulations and administrative provisions of the Member States regarding the protection of animals used for experimental and other scientific purposes', *The Official Journal of the European Communities*, L 358, pp. 1–28. Available at: http://data.europa.eu/eli/dir/1986/609/oj.

Diakou, A., Di Cesare, A., Morelli, S., Colombo, M., Halos, L., Simonato, G., Tamvakis, A., Beugnet, F., Paoletti, B. and Traversa, D. (2019) 'Endoparasites and vector-borne pathogens in dogs from Greek islands: Pathogen distribution and zoonotic implications', *PLOS Neglected Tropical Diseases*, 13(5), p. e0007003. doi: 10.1371/journal.pntd.0007003.

Elbert, A., Nauen, R. and Leicht, W. (1998) 'Imidacloprid, a novel chloronicotinyl insecticide: Biological activity and agricultural importance', in Ishaaya, I. and Degheele, D. (eds.) *Insecticides with Novel Modes of Action*. Berlin, Heidelberg: Springer, pp. 50–73. doi: 10.1007/978-3-662-03565-8_4.

Gomez, S. A. and Picado, A. (2017) 'Systemic insecticides used in dogs: potential candidates for phlebotomine vector control?', *Tropical Medicine and International Health*, 22(6), pp. 755–764. doi: 10.1111/tmi.12870.

Gosstandart (The USSR State Committee of Standards) (1976) GOST 12.1.007-76. *Occupational Safety Standards System. Noxious Substances. Classification and General Safety Requirements [Sistema standartov bezopasnosti truda. Vrednye veshchestva. Klassifikatsiya i obshchie trebovaniya bezopasnosti]*. Moscow: Izdatel'stvo standartov. [in Russian].

Hasib, Y., Kabir, H., Barua, S., Akter, S. and Chowdhury, S. (2020) 'Frequency and prevalence of clinical conditions and therapeutic drugs used in dog and cat at Teaching Veterinary Hospital, Chattogram Veterinary and Animal Sciences University', *Journal of Advanced Veterinary and Animal Research*, 7(1), p. 156–163. doi: 10.5455/javar.2020.g405.

Havryk, K. A. (2015) 'Influence of demodicosis and otodectosis pathogens on blood serum biochemical parameters of sick dogs' [Vplyv zbudnykiv demodekozu ta otodektozu na biokhimichni pokaznyky syrovatky krovi khvorykh sobak], *Scientific Bulletin of Veterinary Medicine [Naukovyi visnyk veterynarnoi medytsyny]*, 1, pp. 68–71. Available at: http://nbuv.gov.ua/UJRN/nvvm_2015_1_17. [in Ukrainian].

Karkishchenko, N. N. and Grachev, S. V. (eds.) (2010) *The Guide to Laboratory Animals and Alternative Models in Biomedical Researches [Rukovodstvo po laboratornym zhivotnym i al'ternativnym modelyam v biomeditsinskikh tekhnologiyakh]*. Moscow: Profil'. ISBN 9785903950102. [in Russian].

Klafke, G. M., Moreno, H. C., Tidwell, J. P., Miller, R. J., Thomas, D. B., Feria-Arroyo, T. P. and Pérez de León, A. A. (2020) 'Partial characterization of the voltage-gated sodium channel gene and molecular detection of permethrin resistance in *Rhipicephalus annulatus* (Say, 1821)', *Ticks and Tick-Borne Diseases*, 11(3), p. 101368. doi: 10.1016/j.ttbdis.2019.101368.

Kotsiumbas, I. Ya., Malyk, O. H., Patereha, I. P., Tishyn, O. L., Kosenko, Yu. M., Chura, D. O., Kotsiumbas, H. I., Piatnychko, O. M., Brezvyn, O. M., Zasadna, Z. S. and Chaikovska, O. I. (2006) *Preclinical Studies of Veterinary Drugs [Doklinichni doslidzhennia veterynarnykh likarskykh zasobiv]*. Lviv: Triada plus. ISBN 9667596648. [in Ukrainian].

Kumar, R., Klafke, G. M. and Miller, R. J. (2020) 'Voltage-gated sodium channel gene mutations and pyrethroid resistance in *Rhipicephalus microplus*', *Ticks and Tick-Borne Diseases*, 11(3), p. 101404. doi: 10.1016/j.ttbdis.2020.101404.

Laing, R., Gillan, V. and Devaney, E. (2017) 'Ivermectin — old drug, new tricks?', *Trends in Parasitology*, 33(6), pp. 463–472. doi: 10.1016/j.pt.2017.02.004.

Prozorovskiy, V. B. (2007) 'Statistic processing of data of pharmacological investigations' [Statisticheskaya obrabotka rezul'tatov farmakologicheskikh issledovaniy], *Psychopharmacology and Biological Narcology [Psikhofarmakologiya i biologicheskaya narkologiya]*, 7(3–4), pp. 2090–2120. Available at: https://www.elibrary.ru/item.asp?id=11691694. [in Russian].

Sheele, J. M. and Ridge, G. E. (2016) 'Toxicity and potential utility of ivermectin and moxidectin as xenointoxicants against the common bed bug, *Cimex lectularius* L.', *Parasitology Research*, 115(8), pp. 3071–3081. doi: 10.1007/s00436-016-5062-x.

Tucker, N. S. G., Kaufman, P. E. and Weeks, E. N. I. (2019) 'Identification of permethrin and etofenprox cross-tolerance in *Rhipicephalus sanguineus* sensu lato (Acari: Ixodidae)', *Pest Management Science*, 75(10), pp. 2794–2801. doi: 10.1002/ps.5391.

Van Emden, H. F. (2019) *Statistics for Terrified Biologists*. 2nd ed. Hoboken, NJ: John Wiley & Sons. ISBN 9781119563679.

Zapadnyuk, I. P., Zapadnyuk, V. I., Zakhariya, E. A. and Zapadnyuk, B. V. (1983) *Laboratory animals. Breeding, Keeping, Use in the Experiment [Laboratornye zhivotnye. Razvedenie, soderzhanie, ispol'zovanie v eksperimente]*. 3rd ed. Kiev: Vishcha shkola. [in Russian].

Zharov, A. V., Ivanov, I. V. and Strel'nikov, A. P. (2003) *Autopsy and Pathomorphological Diagnosis of Animal Diseases [Vskrytie i patomorfologicheskaya diagnostika bolezney zhivotnykh]*. Moscow: KolosS. ISBN 5953200927. [in Russian].

UDC 619:614.31:615.28:661.3/.4:637.5.06/.07 **DOI 10.36016/JVMBBS-2020-6-2-5**

APPLICATION OF EXPRESS METHODS FOR DETECTION OF SLAUGHTER ANIMALS' MEAT ADULTERATION BY TREATMENT WITH ALKALINE DETERGENTS AND DISINFECTANTS

Bogatko N. M.

Bila Tserkva National Agrarian University, Bila Tserkva, Ukraine, e-mail: nadiyabogatko@ukr.net

Summary. The article presents the application of the developed patented express methods, which have reliability in tests of 99.9%, and can be used to control dangerous chemical factors to detect adulteration of meat of slaughter animals with sodium bicarbonate solution and alkaline detergents and disinfectants at production facilities and both meat and meat products. According to these express methods, the number of samples for processing the meat of slaughter animals with a solution of sodium bicarbonate using an alcoholic solution of chrome dark blue (0.5%): beef (n = 2), pork (n = 2) due to the application of meat ≤ 5.0% and beef (n = 4), pork (n = 4) — ≥ 5.1%; for treatment with alkaline detergents when using an alcoholic solution of bromothymol blue (0.04%): beef (n = 3) due to the application on the surface of meat ≤ 5.0% and pork (n = 4) and goat (n = 4) — ≥ 5.1%; for treatment with alkaline disinfectants when using an alcoholic solution of rosolic acid (0.25%): goat (n = 4) due to the application on the surface of meat ≤ 5.0% and beef (n = 5) and pork (n = 4) — ≥ 5.1%; for treatment with alkaline disinfectants when using an alcoholic solution of chrome dark blue (0.3%): beef (n = 5) and pork (n = 4); for treatment with alkaline detergents when using an alcoholic solution of bromocresol green (0.01%): pork (n = 5), beef (n = 4) and lamb (n = 3)

Keywords: beef, pork, lamb, goat, meat control, adulteration, alkaline detergents and disinfectants

Introduction. State risk-based control involves the detection of adulteration of meat of slaughter animals for the establishment of a dangerous chemical factor, namely alkaline detergents and disinfectants due to violations of temperature conditions and shelf life for the production and circulation of raw meat (Amaral et al., 2016).

Therefore, it is necessary to adhere to the temperature regimes for the storage and sale of food products following established regulatory requirements (Aksu, Kaya and Ockerman, 2005). Violation of the shelf life of slaughter meat increases the contamination with microorganisms, which leads to spoilage and loss of consumer properties of the food product (Odewade, Oyelami and Fasogbon, 2018). The adulteration of meat from slaughter animals with detergents and disinfectants is a social problem due to the violation of sanitary and hygienic requirements for production and circulation at facilities (Manning and Soon, 2014). The HACCP system prevents the control of the occurrence of hazards in the food chain by establishing critical control points (Fotina et al., 2016).

These issues have become especially relevant for the implementation of HACCP, VACCP, TACCP systems at the facilities for the production and circulation of meat of slaughter animals — production facilities, wholesale bases, supermarkets, agri-food markets (Milios, Drosinos and Zoiopoulos, 2012; Hulebak and Schlosser, 2002).

Every year the problem of recognizing the safety of meat of slaughter animals becomes more urgent, so it is necessary to develop new methods of identification of this raw material (Aida et al., 2005; Carr, Scheffler and Johnson, 2017).

Therefore, scientists need to develop rapid methods for controlling the safety and quality of slaughter meat during production and circulation, so that the consumer is confident in choosing high-quality food.

Therefore, our research on the detection of adulteration of slaughter meat in agri-food markets and supermarkets using the developed express methods is relevant.

The work aimed to develop and apply express methods for the detection of adulteration of meat of slaughter animals with sodium bicarbonate solution and alkaline detergents and disinfectants.

Materials and methods. The research material was beef, pork, lamb, and goat, which were selected from slaughterhouse meat production facilities, wholesale bases, and sales in agri-food markets and supermarkets. Determination of adulteration of meat of slaughter animals by treatment with sodium bicarbonate solution and alkaline detergents and disinfectants was performed according to the patented in Ukraine methods (Bogatko et al., 2017a, 2017b; Bogatko, Fotina and Yatsenko, 2019a, 2019b, 2019c).

Results and discussion. Express methods are designed to establish the safety and quality of meat of slaughter animals — beef, pork, lamb, goat for detection of treatment with sodium bicarbonate solution and alkaline detergents and disinfectants for elimination of signs of spoilage and reduction of microflora contamination to extend the shelf life in production laboratories, facilities for the production, processing, sale and storage of meat of slaughter animals, supermarkets, wholesale depots, shops, state laboratories of veterinary medicine and testing laboratories of veterinary and sanitary examination in agri-food markets using chemical reagents. The reliability of the developed patented methods is 99.9%.

The essence of the method of detecting intentional treatment with sodium bicarbonate solution of meat of slaughter animals is to use an alcoholic solution of chrome dark blue with a mass concentration of 0.5%, which when interacting with sodium bicarbonate forms a compound from light purple to dark purple depending on the amount treated solution, respectively — ≤ 5.0% and ≥ 5.1%, which will ensure the reliability of the results for determining the safety and quality of meat (Bogatko, Fotina and Yatsenko, 2019a, 2019b, 2019c).

The task of the developed express method was solved by applying using a graduated pipette 0.1–0.2 cm^3 of an alcoholic solution of chrome dark blue with a mass concentration of 0.5% to the surface of pork, beef, lamb, goat with an area of 2.0×2.5 cm, and after 1–2 s we detected the presence of pale pink color (negative reaction) — in the absence of treatment of meat with sodium bicarbonate or the presence of light purple to dark purple color (positive reaction) — depending on the amount of sodium bicarbonate used for treatment, respectively — ≤ 5.0% and ≥ 5.1%.

The stability of indicators on the intensity of the violet color of varying intensity depending on the quantity of addition of sodium bicarbonate: light violet color — ≤ 5.0% (positive reaction); dark purple color — ≥ 5.1% (positive reaction) or the presence of pale pink color (negative reaction) when establishing the treatment of meat of slaughter animals with sodium bicarbonate solution was 99.9%. Also, more reliable data — in 99.0–99.5% were obtained in comparison with the method of determining the content of amino-ammonia nitrogen and in 98.9–99.9% — in the method of determining the content of microorganisms in meat (Bogatko, Konstantinov and Bogatko, 2016; SDVMMAPU, 2002).

Studies have shown that meat from slaughter animals was treated with sodium bicarbonate solution according to color intensity in 31 samples: 10 samples of pork, 12 samples of beef, 4 samples of lamb, 5 samples of goat. The research results are presented in the Table 1.

Table 1 — Detection of meat of slaughter animals treated with a solution of sodium bicarbonate by color intensity using chrome dark blue (n = 31)

Type of meat	Indicators of color intensity		
	adulteration (positive reaction)		no adulteration (negative reaction)
	light purple color (≤ 5.0%)	dark purple color (≥ 5.1%)	pale pink color
Pork, n = 10	n = 2	n = 4	n = 4
Beef, n = 12	n = 2	n = 4	n = 5
Lamb, n = 4	n = 1	n = 1	n = 2
Goat, n = 5	n = 1	n = 1	n = 3

Studies have shown that the largest number of samples treated with sodium bicarbonate solution by applying ≤ 5.0% was in beef (n = 2) and pork (n = 2), and by applying ≥ 5.1% was in pork (n = 4) and beef (n = 4).

In the absence of adulteration with alkaline detergents — the absence of purple color of varying intensity, but in the presence of pale pink color (negative reaction) in 5 samples of beef, 4 samples of pork, 3 samples of goat, and 2 samples of lamb.

The essence of the method of detection of intentional treatment with alkaline disinfectants of meat of slaughter animals is to use an alcoholic solution of chrome dark blue with a mass concentration of 0.3%, which when interacting with disinfectants forms a light purple compound that will ensure the reliability of safety results and meat quality (Bogatko, Fotina and Yatsenko, 2019b).

The task of developing the express method was solved by the fact that the cut surface of the muscle tissue of pork, beef, lamb, goat, in the amount of 2.0–2.1 g was crushed with scissors, then placed in a flask, poured distilled water in the amount of 8.0–8.1 cm^3 (ratio 1:4). The meat-water extract was infused for 5–6 min, filtered through an ashless filter. The test tube was filled with filtered meat and water extract in the amount of 2.0–2.5 cm^3; 0.1–0.2 cm^3 of an alcohol solution of chrome dark blue with a mass concentration of 0.3% was introduced with a graduated pipette, the content of the tube was shaken and after 2–3 s, the presence of a light purple color (positive reaction) was established — in the presence of treatment of the meat with alkaline disinfectants or light pink color (negative reaction) — in the absence of treatment of the meat with alkaline disinfectants.

The stability of the indicators for establishing the intensity of the presence of a light purple color (positive reaction) or the presence of a light pink color (negative reaction) during the establishment of the treatment of meat of slaughter animals with alkaline disinfectants was 99.9%. Also, more reliable data — in 99.2–99.8% were obtained in comparison with the method of determining the content of amino-ammonia nitrogen and in 99.0–99.7% — in the method of determining the content of microorganisms in the meat of slaughter animals (Bogatko, Konstantinov and Bogatko, 2016; SDVMMAPU, 2002).

Studies have shown treatment of slaughter meat with alkaline disinfectants according to the intensity of color in 33 samples: 10 samples of pork, 12 samples of beef, 6 samples of lamb, 5 samples of goat. The research results are presented in the Table 2.

Studies have shown that the largest number of samples treated with alkaline disinfectants (the presence of light purple color) was in beef (n = 5) and in pork (n = 4).

In the absence of adulteration with alkaline disinfectants, the presence of light pink color (negative reaction) was noted: in 7 samples of beef; in 6 samples of pork; in 4 samples of lamb and goat.

Table 2 — Detection of meat of slaughter animals treated with alkaline disinfectants by color intensity using chrome dark blue (n = 33)

Type of meat	Indicators of color intensity	
	adulteration (positive reaction)	no adulteration (negative reaction)
	light purple color	light pink color
Pork, n = 10	n = 4	n = 6
Beef, n = 12	n = 5	n = 7
Lamb, n = 6	n = 2	n = 4
Goat, n = 5	n = 1	n = 4

Table 3 — Detection of meat of slaughter animals treated with alkaline detergents by color intensity using bromocresol green (n = 36)

Type of meat	Indicators of color intensity	
	adulteration (positive reaction)	no adulteration (negative reaction)
	blue color	green color
Pork, n = 12	n = 5	n = 7
Beef, n = 12	n = 4	n = 8
Lamb, n = 6	n = 3	n = 3
Goat, n = 6	n = 2	n = 4

The essence of the method of detecting intentional treatment with alkaline detergents of slaughter animals is to use an alcoholic solution of bromocresol green with a mass concentration of 0.01%, which when interacting with alkaline detergents forms a blue compound that will ensure the reliability of safety and quality meat (Bogatko, Fotina and Yatsenko, 2019c).

The task of the developed express method was solved by applying to the surface of muscle tissue of pork, beef, lamb, goat with an area of 2.0×2.5 cm with a graduated pipette 0.1–0.2 cm^3 of an alcohol solution of bromocresol green with a mass concentration of 0.01% and after 1–2 s the presence of a blue color (positive reaction) — in the presence of treatment of the meat with alkaline detergents or the presence of a green color (negative reaction) — in the absence of treatment of the meat with alkaline detergents was set.

The reliability of the developed method for determining the adulteration of meat of slaughter animals and poultry by treatment with alkaline detergents using bromocresol green when determining the intensity of the presence of blue color (positive reaction) or the presence of green color (negative reaction) was 99.9%.

The stability of the indicators for determining the intensity of the presence of a blue color (positive reaction) or the presence of a green color (negative reaction) during the establishment of the treatment of the meat of slaughter animals with alkaline detergents was 99.9%. Also, more reliable data — in 98.9–99.7% obtained in comparison with the method of determining the content of amino-ammonia nitrogen and in 99.1–99.8% — in the method of determining the content of microorganisms in the meat of slaughter animals (Bogatko, Konstantinov and Bogatko, 2016; SDVMMAPU, 2002).

Studies have revealed the treatment of the meat of slaughter animals with alkaline disinfectants according to the intensity of color in 36 samples: 12 samples of pork, 12 samples of beef, 6 samples of lamb, 6 samples of goat. The results of the developed method are presented in the Table 3. Studies have shown that the largest number of samples treated with alkaline detergents (blue) was in pork (n = 5), beef (n = 4) and lamb (n = 3).

In the absence of adulteration with alkaline detergents, the presence of green color (negative reaction) was noted: in 8 samples of beef; in 7 samples of pork; in 4 samples of goat, and in 3 samples of lamb.

The essence of the method of detection of intentional treatment with alkaline detergents of meat of slaughter animals is to use an alcoholic solution of bromothymol blue with a mass concentration of 0.04%, which when interacting with alkaline detergents forms a dark blue compound of varying intensity depending on the level of treatment, respectively — ≤ 5.0% and ≥ 5.1%, which will ensure the reliability of the results for determining the safety and quality of meat (Bogatko et al., 2017b).

The task of the developed express method was solved by applying on the surface of the muscle tissue of pork, beef, lamb, goat with an area of 2.0×2.5 cm, with a graduated pipette 0.2–0.3 cm^3 alcohol solution of bromothymol blue with mass concentration of 0.04%, and after 2–3 s we set the presence of light yellow color (negative reaction) or the presence of dark blue color of different intensity depending on the amount of alkaline detergents: light blue color (positive reaction) — the presence of alkaline detergents funds on the surface of muscle tissue ≤ 5.0%; dark blue color (positive reaction) — the presence of alkaline detergents on the surface of muscle tissue ≥ 5.1%.

The stability of indicators on intensity of dark blue color of various intensity depending on the amount of alkaline detergents to ≤ 5.0% and ≥ 5.1% (positive reactions) or existence of light yellow color (negative reaction) when determining processing of meat of slaughter animals by alkaline detergents was 99.9%. Also, more reliable data — in 98.5–99.6% obtained in comparison with the method of determining the content of amino-ammonia nitrogen and in 98.7–99.5% — in the method of determining the content of microorganisms in the meat of slaughter animals (Bogatko, Konstantinov and Bogatko, 2016; SDVMMAPU, 2002).

Studies have revealed the treatment of meat of slaughter animals with alkaline detergents according to the intensity of color in 36 samples: 12 samples of pork, 12 samples of beef, 6 samples of lamb, 6 samples of goat.

The research results are presented in the Table 4. Studies have shown that the largest number of samples treated with alkaline detergents by their applying ≤ 5.0% was in beef (n = 3) and by applying ≥ 5.1% was in pork (n = 4) and goat (n = 4).

Table 4 — Detection of meat of slaughter animals treated with alkaline detergents by color intensity using bromothymol blue (n = 36)

Type of meat	Indicators of color intensity		
	adulteration (positive reaction)		no adulteration (negative reaction)
	light blue color (≤ 5.0%)	dark blue color (≥ 5.1%)	light yellow color
Pork, n = 12	n = 2	n = 4	n = 6
Beef, n = 12	n = 3	n = 1	n = 8
Lamb, n = 6	n = 1	n = 3	n = 2
Goat, n = 6	n = 1	n = 4	n = 1

In the absence of adulteration with alkaline detergents — the absence of dark blue color of varying intensity, but in the presence of light yellow (negative reaction) in 6 samples of pork, 8 samples of beef, 2 samples of lamb, 1 sample of goat.

The essence of the method of detecting treatment with alkaline disinfectants of meat of slaughter animals is to use an alcoholic solution of rosolic acid with a mass concentration of 0.25%, which when interacting with alkaline disinfectants forms a crimson red compound of varying intensity depending on the number of treatments ≤ 5.0% and ≥ 5.1%, which will ensure the reliability of the results for determining the safety and quality of meat (Bogatko et al., 2017a).

The task of the developed express method was solved by applying on the surface of the muscle tissue of pork, beef, lamb, goat with an area of 2.0×2.5 cm, using a graduated pipette 0.1–0.2 cm^3 of alcoholic solution of rosolic acid with mass concentration of 0.25%, and after 1–2 s we set the presence of light yellow or brown yellow color (negative reaction) or the presence of crimson red color of different intensity depending on the amount of alkaline disinfectants: light crimson color (positive reaction) — the presence of alkaline disinfectants on the surface of muscle tissue ≤ 5.0%; crimson red color (positive reaction) — the presence of alkaline disinfectants on the surface of muscle tissue ≥ 5.1%.

The stability of indicators of crimson red color intensity depending on the amount of alkaline disinfectants ≤ 5.0% and ≥ 5.1% (positive reactions) or presence of light yellow or brown yellow color (negative reaction) when detecting the treatment of meat of slaughter animals with alkaline disinfectants was 99.9%. Also, more reliable data — in 98.3–99.1% obtained in comparison with the method of determining the content of amino-ammonia nitrogen and in 98.4–99.5% — in the method of determining the content of microorganisms in the meat of slaughter animals (Bogatko, Konstantinov and Bogatko, 2016; SDVMMAPU, 2002).

Studies have revealed treatment of slaughter animals' meat with alkaline disinfectants according to color intensity in 33 samples: 10 samples of pork, 12 samples of beef, 6 samples of lamb, 5 samples of goat. The research results are presented in the Table 5.

Table 5 — Detection of meat of slaughter animals treated with alkaline disinfectants by color intensity using rosolic acid (n = 33)

Type of meat	Indicators of color intensity		
	adulteration (positive reaction)		no adulteration (negative reaction)
	light crimson color (≤ 5.0%)	crimson red color (≥ 5.1%)	light yellow or brown yellow color
Pork, n = 10	n = 2	n = 4	n = 4
Beef, n = 12	n = 1	n = 5	n = 6
Lamb, n = 6	n = 1	n = 2	n = 3
Goat, n = 5	n = 4	n = 0	n = 1

Studies have shown that the largest number of samples treated with alkaline disinfectants by applying ≤ 5.0% (the presence of light crimson color) was in goat (n = 4), and the application of ≥ 5.1% (the presence of crimson red color) was in beef (n = 5) and pork (n = 4).

In the absence of adulteration with alkaline disinfectants — the absence of crimson red color of varying intensity, but in the presence of light yellow or brown yellow color (negative reaction) in 6 samples of beef; in 4 samples of pork; in 3 samples of lamb and in 1 sample of goat.

Conclusions. When treating the meat of slaughter animals with sodium bicarbonate solution using an alcoholic solution of chrome dark blue (0.5%), the number of samples was established: beef (n = 2), pork (n = 2) due to the application of ≤ 5.0% and beef (n = 4), pork (n = 4) — ≥ 5.1%; for treatment with alkaline detergents when using an alcoholic solution of bromothymol blue (0.04%): beef (n = 3) due to the application of ≤ 5.0%, and pork (n = 4) and goat (n = 4) — ≥ 5.1%; for treatment with alkaline disinfectants when using an alcoholic solution of rosolic acid (0.25%): goat (n = 4) due to the application of ≤ 5.0%, and beef (n = 5) and pork (n = 4) — ≥ 5.1%; for treatment with alkaline disinfectants when using an alcoholic solution of chrome dark blue (0.3%): beef (n = 5) and pork (n = 4); for treatment with alkaline detergents when using an alcoholic solution of bromocresol green

(0.01%): pork (n = 5), beef (n = 4) and lamb (n = 3). Developed patented express techniques with a test accuracy of 99.9% can be used to control hazardous chemical factors — the establishment of adulteration of meat of slaughter animals with a solution of sodium bicarbonate and alkaline detergents and disinfectants at facilities for the production and circulation of meat and meat products.

References

Aida, A. A., Che Man, Y. B., Wong, C. M. V. L., Raha, A. R. and Son, R. (2005) 'Analysis of raw meats and fats of pigs using polymerase chain reaction for Halal authentication', *Meat Science*, 69(1), pp. 47–52. doi: 10.1016/j.meatsci.2004.06.020.

Aksu, M. I., Kaya, M. and Ockerman, H. W. (2005) 'Effect of modified atmosphere packaging and temperature on the shelf life of sliced pastirma produced from frozen/thawed meat', *Journal of Muscle Foods*, 16(3), pp. 192–206. doi: 10.1111/j.1745-4573.2005.08404.x.

Amaral, J., Meira, L., Oliveira, M. B. P. P. and Mafra, I. (2016) 'Advances in authenticity testing for meat speciation', in Doewney, G. (ed.) *Advances in Food Authenticity Testing*. Elsevier, pp. 369–414. doi: 10.1016/B978-0-08-100220-9.00014-X.

Bogatko, N. M., Fotina, T. I. and Yatsenko, I. V. (2019a) *Method for Detection of Slaughter Animals' Meat Adulteration by Treatment with Sodium Bicarbonate Using Chrome Dark Blue [Sposib vyznachennia falsyfikatsii miasa zabiinykh tvaryn za obrobky rozchynom hidrokarbonatu natriiu iz zastosuvanniam khromovoho temno-synoho]*. Patent no. UA 132813. Available at: https://base.uipv.org/searchINV/search.php?action=viewdetails&IdClaim=256491. [in Ukrainian].

Bogatko, N. M., Fotina, T. I. and Yatsenko, I. V. (2019b) *Method for Detection of Slaughter Animals' Meat Adulteration by Treatment with Alkaline Disinfectants Using Chrome Dark Blue [Sposib vyznachennia falsyfikatsii miasa zabiinykh tvaryn za obrobky luzhnymy dezinfikuiuchymy zasobamy iz zastosuvanniam khromovoho temno-synoho]*. Patent no. UA 132814. Available at: https://base.uipv.org/searchINV/search.php?action=viewdetails&IdClaim=256492. [in Ukrainian].

Bogatko, N. M., Fotina, T. I. and Yatsenko, I. V. (2019c) *Method for Detection of Slaughter Animals' Meat Adulteration by Treatment with Alkaline Detergents Using Bromocresol Green [Sposib vyznachennia falsyfikatsii miasa zabiinykh tvaryn za obrobky luzhnymy myinymy zasobamy iz zastosuvanniam bromkrezolovoho zelenoho]*. Patent no. UA 132815. Available at: https://base.uipv.org/searchINV/search.php?action=viewdetails&IdClaim=256493. [in Ukrainian].

Bogatko, N. M., Konstantinov, P. D. and Bogatko, L. M. (2016) *Veterinary and Sanitary Examination of Meat of Slaughter Animals, Poultry, Rabbits: Guidelines [Veterynarno-sanitarna ekspertyza miasa zabiinykh tvaryn, sviiskoi ptytsi, kroliv: metodychni rekomendatsii]*. Bila Tserkva: Bila Tserkva National Agrarian University. [in Ukrainian].

Bogatko, N. M., Melnik, A. Yu., Serdiukov, Ya. K., Bogatko, L. M. and Bogatko, A. F. (2017a) *Method for Detection of Adulteration of Meat of Slaughter Animals and Poultry by Treatment with Alkaline Disinfectants [Sposib vyznachennia falsyfikatsii miasa zabiinykh tvaryn ta ptytsi za obrobky luzhnymy dezinfikuiuchymy zasobamy]*. Patent no. UA 116830. Available at: https://base.uipv.org/searchINV/search.php?action=viewdetails&IdClaim=236143. [in Ukrainian].

Bogatko, N. M., Melnik, A. Yu., Serdiukov, Ya. K., Bogatko, L. M. and Bogatko, A. F. (2017b) *Method for Detection of Adulteration of Meat of Slaughter Animals and Poultry by Treatment with Alkaline Detergents [Sposib vyznachennia falsyfikatsii miasa zabiinykh tvaryn ta ptytsi za obrobky luzhnymy myinymy zasobamy]*. Patent no. UA 116831. Available at: https://base.uipv.org/searchINV/search.php?action=viewdetails&IdClaim=236144. [in Ukrainian].

Carr, C. C., Scheffler, J. M. and Johnson, D. D. (2017) 'Categorizing processing via the Meat Science Lexicon', *Animal Frontiers*, 7(4), pp. 19–24. doi: 10.2527/af.2017.0438.

Fotina, T. I., Zapara, S. I., Fotina, H. A. and Fotin, A. V. (2016) 'HACCP system as pass for quality and safety of poultry products', *Journal for Veterinary Medicine, Biotechnology and Biosafety*, 2(3), pp. 19–22. Available at: http://jvmbbs.kharkov.ua/archive/2016/volume2/issue3/oJVMBBS_2016023_019-022.pdf.

Hulebak, K. L. and Schlosser, W. (2002) 'Hazard Analysis and Critical Control Point (HACCP) history and conceptual overview', *Risk Analysis*, 22(3), pp. 547–552. doi: 10.1111/0272-4332.00038.

Manning, L. and Soon, J. M. (2014) 'Developing systems to control food adulteration', *Food Policy*, 49(1), pp. 23–32. doi: 10.1016/j.foodpol.2014.06.005.

Milios, K., Drosinos, E. H. and Zoiopoulos, P. E. (2012) 'Factors influencing HACCP implementation in the food industry', *Journal of the Hellenic Veterinary Medical Society*, 63(4), pp. 283–290. doi: 10.12681/jhvms.15442.

Odewade, J. O., Oyelami, L. O. and Fasogbon, A. O. (2018) 'Microbial analysis of processed foods stored in domestic refrigerators of selected in Ile-Ife, Osun State, Nigeria', *American Journal of Bioscience and Bioengineering*, 6(3), pp. 21–26. doi: 10.11648/j.bio.20180603.11.

SDVMMAPU (The State Department of Veterinary Medicine of the Ministry of Agrarian Policy of Ukraine) (2002) *Rules of Antemortem Veterinary Inspection of Animals and Veterinary-Sanitary Examination of Meat and Meat Products [Pravyla peredzabiinoho veterynarnoho ohliadu tvaryn i veterynarno-sanitarnoi ekspertyzy miasa ta miasnykh produktiv]*: approved by the Order of the State Department of Veterinary Medicine of the Ministry of Agrarian Policy of Ukraine No. 28 of June 7, 2002; registered in the Ministry of Justice of Ukraine with No. 524/6812 of on June 21, 2002. Available at: https://zakon.rada.gov.ua/laws/z0524-02. [in Ukrainian].

UDC 619:614.484:615.28.076.9:576.895.132.8 **DOI 10.36016/JVMBBS-2020-6-2-6**

STUDY OF DISINVASIVE PROPERTIES OF INNOVATIVE ALDEHYDE DISINFECTANT

Paliy A. P. [1], Sumakova N. V. [1], Mashkey A. M. [1], Gontar V. V. [1], Palii A. P. [2], Yurchenko D. A. [3]

[1] National Scientific Center 'Institute of Experimental and Clinical Veterinary Medicine', Kharkiv, Ukraine, e-mail: paliy.dok@gmail.com
[2] Kharkiv Petro Vasylenko National Technical University of Agriculture, Kharkiv, Ukraine
[3] Luhansk National Agrarian University, Starobilsk, Luhansk Region, Ukraine

Summary. The pollution rate of environment by pathogens of invasive diseases and contamination of manure, soil, water, and other objects by them are constantly changing depending on the prevalence and intensity of invasion among farm animals. Prevention and control of invasive animal diseases are essential to prevent their spread, as well as to obtain high-quality sanitary products for livestock production. The preservation of pathogens in the environment depends on the intensity of exposure to natural and artificial factors and their resistance to chemicals that are used for disinfection. A large number of disinfectants, both domestic and foreign, have been proposed for disinfection, but they are not always effective under industrial conditions for conducting forced or preventive disinfestation. The introduction of disinfectants into practice is impossible without a preliminary laboratory assessment of their disinvasive properties. The aim of our work was to study the disinvasive properties of a new aldehyde disinfectant on the test models of *Ascaris suum* eggs. The studies were carried out in the Laboratory of Veterinary Sanitation and Parasitology of the National Scientific Center 'Institute of Experimental and Clinical Veterinary Medicine' in accordance with the methodological recommendations 'Methods to Identify and Evaluate Safety Parameters and Quality of Disinfectants, Detergent-Disinfectants Used During Production, Storage, Transportation and Sale of Products of Animal Origin' (2010). As a result of the studies, it was found that the aldehyde disinfectant exhibits disinvasive properties to the test culture of *Ascaris suum* eggs when applied at a concentration of 4.0% at room temperature (18–20 ± 0.5°C) and exposure of 3 h. The disinfectant can be used for preventive and forced disinvasions of animal holding facilities and other veterinary control facilities

Keywords: disinfectant, disinvasive properties, *Ascaris suum*, test culture, concentration, exposure

Introduction. Biological pollution of environment is increasingly being attributed to many major veterinary and ecological problems of our time, one of the aspects of which is transfer and wide spread of helminth eggs and larvae in the environment (Daugschies, Bangoura and Lendner, 2013). Inspection of rural soil in the Kharkiv Region and soil in Kharkiv and Balakliia has revealed its contamination with different morphotypes' helminth eggs. The average level of rural soil pollution is 12.5%. The soil in urban park zones is contaminated with helminth exogenous stages from 5.0 to 55.5%, and urban residential zones — from 20 to 23.3% (Paliy et al., 2019).

Helminthiases of animals in livestock farms and complexes are spread among the susceptible population when non-compliance with veterinary and sanitary regulations and norms is observed (Paliy et al., 2018b), which in turn requires the use of highly effective disinfectants that have a wide range of biocidal effects (Paliy et al., 2015; Paliy, 2018).

Today, composite preparations consisting of several active substances from different groups of chemical compounds are preferred, which, due to the synergism of the components, have a broader range of activity and are effective at much lower concentrations, are more economically advantageous (Zavgorodniy et al., 2013; Koski, Anttila and Kuusela, 2015; Paliy and Paliy, 2019).

However, it should be noted that not all disinfectants, including commercial preparations, are effective against the helminth exogenous stages. Some agents are able to delay or stop the embryogenesis of helminth eggs, but do not completely destroy them (Oh et al., 2016). Other disinfectants are highly effective in contact with a pure helminth egg culture (Bessat and Dewair, 2019). Therefore, before widespread use of disinfectants in order to destroy the causative agents of animal helminthiasis in the environment, it is imperative to determine their disinvasive properties in the laboratory (Campbell et al., 1982; Shalaby et al., 2011).

One of the key conditions that affect the validity of the results obtained from the test of the disinfectant is the use of reference test cultures. In order to evaluate the disinvasive effect of drugs, it is advisable to use the eggs of *Ascaris suum* (Pecson and Nelson, 2005; Yu et al., 2014). This test culture is used to test not only chemicals but also to evaluate the ovicidal activity of physical factors (Brownell and Nelson, 2006).

Glutaraldehyde is most common active substance of most modern disinfectants (Rajan and Ripple, 2009) used in both human medicine (Akamatsu et al., 1997; Burgess et al., 2017) and veterinary practices (Paliy et al., 2016; Zazharskyi et al., 2018).

The purpose of our work was to study the disinvasive properties of a new aldehyde disinfectant on *A. suum* eggs as the test model.

Material and methods. The study of the disinvasive properties of the disinfectant was carried out in the Laboratory of Veterinary Sanitation and Parasitology of the National Scientific Center 'Institute of Experimental and Clinical Veterinary Medicine' in accordance with the methodological recommendations 'Methods to Identify and Evaluate Safety Parameters and Quality of Disinfectants, Detergent-Disinfectants Used During Production, Storage, Transportation and Sale of Products of Animal Origin' (2010).

The object of research was a disinfectant comprising a mixture of quaternary ammonium compounds (25.0%), glutaraldehyde (11.0%), isopropyl alcohol, nonionic surfactants. The sanitizer is a liquid transparent yellowish liquid with a characteristic odor, well soluble in water. 5.0% sodium hydroxide solution was used as a negative control.

To obtain the *A. suum* egg culture, feces of spontaneously invaded pigs were used, in which at least 2–3 eggs in the field of view of the microscope at low magnification were detected by the flotation. One part of the obtained test material was stored in a refrigerator at a temperature of 3°C, and the other part was cultured in an incubator at a temperature of 26–28°C for 20–30 days. In laboratory experiments used helminth eggs that were at protoplast (before blastomer cleavage) and larval stages. To confirm the invasiveness of *A. suum* larvae, a biological test was performed on white rats.

Results and discussions. Preliminary experiments to determine the disinvasive properties of the disinfectant were performed on *A. suum* egg test culture using concentrations of 0.5, 1.0, 2.0, 3.0, 3.5, and 4.0% at a temperature of 20 ± 0.5°C and exposures 1, 3, 6, and 24 h. The obtained results are presented in Table 1.

Analyzing the results in Table 1, it should be noted that the treatment of the test culture with disinfectant solutions with 0.5, 1.0, and 2.0% concentrations at a temperature of 20 ± 0.5°C and exposures 3, 6, and 24 h did not affect *A. suum* egg development.

Treatment of the test culture with 2.0% solution within 24 h caused the death of eggs only on the 28th day.

Along with this, the concentrations of 3.0, 3.5, and 4.0% influenced the delay in the development of test culture eggs and caused their death, that is, they showed a disinvasive property.

The disinfectant exhibited a higher disinvasive activity with 3.5% concentration at exposures 6 and 24 h.

Table 1 — Disinvasive activity rate of the disinfectant to *Ascaris suum* egg culture

Concentration of the disinfectant, %	Terms for determining the viability of *Ascaris suum* eggs, days														
	3			6			14			21			28		
	Exposures, h														
	3	6	24	3	6	24	3	6	24	3	6	24	3	6	24
0.5	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
1.0	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
2.0	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–
3.0	+	+	+	+	+	–	+	+	–	+	–	–	–	–	–
3.5	+	+	+	+	–	–	+	–	–	–	–	–	–	–	–
4.0	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–
Positive control	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Negative control	+	+	+	+	+	–	+	+	–	+	+	–	+	+	–

Notes: '–' — egg death, '+' — egg development.

The results of the disinfectant ovicidal activity determination are presented in Table 2.

Analyzing the results in Table 2, it should be noted that the ovicidal efficacy of the disinfectant depends on the exposure. Thus, on the protoplast stage of *A. suum* eggs it is 96.00% at a concentration of 3.5% and an exposure of 3 h, and 99.22% — at a exposure of 24 h. The disinvasive effect of the disinfectant on *A. suum* eggs at the larval stage was slightly higher (97.64%) at a concentration of 3.5% and an exposure of 3 h. With increasing concentration and the same exposure, *A. suum* eggs at the larval stage were more vulnerable to disinfectant and died 7 days earlier; disinvasive efficacy was also higher (97.64%).

Based on the preliminary experience, to determine the disinfecting concentrations the sanitizer were tested at concentrations of 3.5 and 4.0% on the surfaces of the test objects made of tile, not painted wood, and metal plates (Table 3). When conducting studies to determine the disinvasive properties of the disinfectant relative to *A. suum* egg culture applied on the test objects, it was found that 3.5 and 4.0% solutions had a disinvasive effect on all surfaces. It should be noted that when studying the susceptibility of *A. suum* eggs to the disinfectant during treatment, it is necessary to take into account the physical characteristics of the surfaces, which determines the efficacy of decontamination. In particular, according to

the results (Table 3), the disinfectant at 3.5 and 4.0% concentrations for all exposures showed disinvasive properties on the surfaces made of tile and metal plates and had lower disinvasive activity on the unpainted wood. That is, the rougher the surface of the object being processed, the lower the efficacy of disinvasion.

Table 2 — Ovicidal activity of the disinfectant

Test culture	Concentrations, %	Exposures, h	Terms for death of eggs in experimental cultures, day	Ovicidal efficacy, %
Ascaris suum at the protoplast stage	3.0	3	28	90.00
		6	21	90.86
		24	21	91.10
	3.5	3	21	96.00
		6	6	98.78
		24	6	99.22
	4.0	3	14	99.60
		6	6	99.64
		24	6	99.70
Ascaris suum at the larval stage	3.0	3	21	91.64
		6	14	96.22
		24	6	96.64
	3.5	3	14	97.64
		6	6	98.92
		24	6	99.64
	4.0	3	6	99.22
		6	3	99.64
		24	3	99.86

Table 3 — Disinvasive activity rate of the disinfectant to *Ascaris suum* egg culture applied on the test objects (6^{th} day after treatment)

Concentrations, %	Test object	Exposures, h		
		3	6	24
		Average ovicidal activity for three experiments		
3.5	tile	80.44 ± 0.01	80.89 ± 0.01	84.33 ± 0.01
	wood	67.22 ± 0.01	79.78 ± 0.01	80.67 ± 0.01
	metal	84.33 ± 0.01	85.33 ± 0.01	93.87 ± 0.01
4.0	tile	91.10 ± 0.01	99.64 ± 0.01	99.70 ± 0.01
	wood	80.44 ± 0.01	90.60 ± 0.02	90.85 ± 0.02
	metal	99.55 ± 0.01	99.64 ± 0.01	99.67 ± 0.01
Positive control	tile	–	–	–
	wood	–	–	–
	metal	–	–	–
Negative control	tile	–	–	90.70 ± 0.01
	wood	–	–	53.70 ± 0.02
	metal	–	–	90.70 ± 0.01

Notes: '–' — no ovicidal efficacy; $p < 0.05$.

Comparing the results of the experiments, it should be noted that the exposure affects the disinvasive activity of the sanitizer, that is, the level of inhibition of the development of test cultures and their death. At least 3 h will be required during disinfection measures.

The efficacy of the disinvasive activity of the disinfectant on *A. suum* egg test culture was tested by bioassay on white rats. Animals were divided into three groups of 15 rats each: a research group and two control groups.

White rats of the research group were given 100 eggs of *A. suum* at the larval stage in suspension with physiological saline treated with a disinfectant (4.0%, 3 h). Animals of the 1^{st} control group were given 100 eggs of

A. suum at the larval stage from a culture obtained from invaded pigs. The 2nd control group received physiological saline. Observations of animals were carried out for 6 days. Five rats were euthanized in each group on 1st, 3rd, and 6th days, and the contents of the intestines, intestines, liver, and lungs were examined. In the study of the internal organs of laboratory animals by the compressor method obtained the results presented in Table 4.

When analyzing the results in Table 4, it was found that in animals of the experimental group, eggs and larvae of *A. suum* were found only in the intestinal contents for up to 3rd day, which means that the larvae lost invasiveness. In animals of the 1st control group, live larvae were found in the intestinal mucousa, liver, and lungs, which confirms their invasiveness. In the 2nd control group, the animals remained intact.

Table 4 — Results of bioassay test on white rats (n = 15)

Day	Group of animals								
	Experimental			Control 1			Control 2		
	Number of live *A. suum* larvae in the internal organs of animals								
	intestines	liver	lungs	intestines	liver	lungs	intestines	liver	lungs
1	–	–	–	25.0 ± 0.6	8.2 ± 0.6	–	–	–	–
3	–	–	–	6.2 ± 0.4	12.4 ± 1.4	–	–	–	–
6	–	–	–	–	4.0 ± 0.6	16.0 ± 0.6	–	–	–

Note. '–' — no larvae and eggs.

According to the research results, a method was developed for disinvasing surfaces contaminated with *A. suum* eggs, including mechanical cleaning, disinvasion by irrigation with a disinfectant comprising a mixture of quaternary ammonium compounds (0.5–1.0%), glutaraldehyde (0.22–0.44%), isopropyl alcohol (0.16–0.32%), nonionic surfactants (0.1–0.2%), deionized water (99.02–98.04%) with an exposure of 3–24 h at a consumption rate of 500 ml/m^2 (Paliy et al., 2018a).

Conclusions. During laboratory tests, the disinvasive properties of the innovative aldehyde disinfectant in relation to *Ascaris suum* egg test culture were determined.

The innovative aldehyde disinfectant exhibits disinvasive properties with respect to *Ascaris suum* egg test culture when applied at a concentration of 4.0% at room temperature (18–20 ± 0.5°C) and exposure for 3 hours.

The disinfectant can be used for preventive and forced disinvasions of animal holding facilities and other veterinary control facilities.

References

Akamatsu, T., Tabata, K., Hironaga, M. and Uyeda, M. (1997) 'Evaluation of the efficacy of a 3·2% glutaraldehyde product for disinfection of fibreoptic endoscopes with an automatic machine', *Journal of Hospital Infection*, 35(1), pp. 47–57. doi: 10.1016/S0195-6701(97)90167-5.

Bessat, M. and Dewair, A. (2019) 'Assessment of the inhibitory effects of disinfectants on the embryonation of *Ascaridia columbae* eggs', *PLoS ONE*, 14(5), p. e0217551. doi: 10.1371/journal.pone.0217551.

Brownell, S. A. and Nelson, K. L. (2006) 'Inactivation of single-celled *Ascaris suum* eggs by low-pressure UV radiation', *Applied and Environmental Microbiology*, 72(3), pp. 2178–2184. doi: 10.1128/AEM.72.3.2178-2184.2006.

Burgess, W., Margolis, A., Gibbs, S., Duarte, R. S. and Jackson, M. (2017) 'Disinfectant susceptibility profiling of glutaraldehyde-resistant nontuberculous mycobacteria', *Infection Control and Hospital Epidemiology*, 38(7), pp. 784–791. doi: 10.1017/ice.2017.75.

Campbell, I., Tzipori, A., Hutchison, G. and Angus, K. (1982) 'Effect of disinfectants on survival of cryptosporidium oocysts', *Veterinary Record*, 111(18), pp. 414–415. doi: 10.1136/vr.111.18.414.

Daugschies, A., Bangoura, B. and Lendner, M. (2013) 'Inactivation of exogenous endoparasite stages by chemical disinfectants: Current state and perspectives', *Parasitology Research*, 112(3), pp. 917–932. doi: 10.1007/s00436-013-3324-4.

Koski, P., Anttila, P. and Kuusela, J. (2015) 'Killing of *Gyrodactylus salaris* by heat and chemical disinfection', *Acta Veterinaria Scandinavica*, 58, p. 21. doi: 10.1186/s13028-016-0202-y.

Oh, K.-S., Kim, G.-T., Ahn, K.-S. and Shin, S.-S. (2016) 'Effects of disinfectants on larval development of *Ascaris suum* eggs', *The Korean Journal of Parasitology*, 54(1), pp. 103–107. doi: 10.3347/kjp.2016.54.1.103.

Paliy, A. P. (2018) 'Antibacterial effect of 'Ecocide C' disinfectant against mycobacteria' [Efektyvnist antybakterialnoi dii dezinfikuiuchoho zasobu «Ekotsyd S» shchodo mikobakterii], *Ukrainian Journal of Ecology*, 8(1), pp. 141–147. doi: 10.15421/2018_198. [in Ukrainian].

Paliy, A. P. and Paliy, A. P. (2019) *Technic and Technological Innovations in Dairy Cattle [Tekhniko-tekhnolohichni innovatsii u molochnomu skotarstvi]*. Kharkiv: Miskdruk. ISBN 9786176192077. [in Ukrainian].

Paliy, A., Zavgorodniy, A., Stegniy, B. and Gerilovych, A. (2015) 'A study of the efficiency of modern domestic disinfectants in the system of TB control activities', *Agricultural Science and Practice*, 2(2), pp. 26–31. doi: 10.15407/agrisp2.02.026.

Paliy, A., Stegniy, B., Muzyka, D., Gerilovych, A. and Korneykov, O. (2016) 'The study of the properties of the novel virucidal disinfectant', *Agricultural Science and Practice*, 3(3), pp. 41–47. doi: 10.15407/agrisp3.03.041.

Paliy, A. P., Sumakova, N. V., Mashkey, A. M., Stegniy, B. T., Guzhvynska, S. O. and Rodionova, K. O. (2018a) *A Method for Disinvasion of Surfaces Contaminated with Ascaris Suum Eggs [Sposib dezinvazii poverkhon, kontaminovanykh yaitsiamy Ascaris suum]*. Patent no. UA 130430. Available at: https://base.uipv.org/searchINV/search.php?action=viewdetails&IdClaim=253529. [in Ukrainian].

Paliy, A. P., Sumakova, N. V., Mashkey, A. M., Petrov, R. V., Paliy, A. P. and Ishchenko, K. V. (2018b) 'Contamination of animal-keeping premises with eggs of parasitic worms', *Biosystems Diversity*, 26(4), pp. 327–333. doi: 10.15421/011848.

Paliy, A., Sumakova, N., Petrov, R., Shkromada, O., Ulko, L. and Palii, A. (2019) 'Contamination of urbanized territories with eggs of helmiths of animals', *Biosystems Diversity*, 27(2), pp. 118–124. doi: 10.15421/011916.

Pecson, B. M. and Nelson, K. L. (2005) 'Inactivation of *Ascaris suum* eggs by ammonia', *Environmental Science and Technology*, 39(20), pp. 7909–7914. doi: 10.1021/es050659a.

Rajan, J. S. and Ripple, S. D. (2009) 'Efficacy and safety of glutaraldehyde-based high level disinfectant products', *American Journal of Infection Control*, 37(5), p. e22. doi: 10.1016/j.ajic.2009.04.022.

Shalaby, H. A., Abdel-Shafy, S., Ashry, H. M. and El-Moghazy, F. M. (2011) 'Efficacy of hydrogen peroxide and dihydroxy benzol mixture (disinfectant) on *Toxocara canis* eggs', *Research Journal of Parasitology*, 6(4), pp. 144–150. doi: 10.3923/jp.2011.144.150.

Yu, Y.-M., Kim, J.-W., Na, W.-S., Youn, Y.-N., Choi, I.-W. and Lee, Y.-H. (2014) 'Effects of some pesticides on development of *Ascaris suum* eggs', *The Korean Journal of Parasitology*, 52(1), pp. 111–115. doi: 10.3347/kjp.2014.52.1.111.

Zavgorodniy, A. I., Stegniy, B. T., Paliy, A. P., Gorzheiev, V. M. and Smirnov, A. M. (2013) *Scientific and Practical Aspects of Disinfection in Veterinary Medicine [Naukovi ta praktychni aspekty dezinfektsii u veterynarii]*. Kharkiv: FOP Brovin O. V. ISBN 9789662445596. [in Ukrainian].

Zazharskyi, V. V., Davydenko, P., Kulishenko, O., Chumak, V., Kryvaya, A., Biben, I. A., Tishkina, N. M., Borovik, I., Boyko, O. O. and Brygadyrenko, V. V. (2018) 'Bactericidal, protistocidal and nematodicidal properties of mixtures of alkyldimethylbenzyl ammonium chloride, didecyldimethyl ammonium chloride, glutaraldehyde and formaldehyde', *Regulatory Mechanisms in Biosystems*, 9(4), pp. 540–545. doi: 10.15421/021881.

Contents

Part 1. Veterinary medicine

Part 2. Biotechnology

Part 3. Biosafety