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 4
Issue 3

KHARKIV
2018

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 № 241, March 9, 2016) and biological sciences (order of the Ministry of Education and Science № 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 № 9 of 30.07.2018)

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) and Index Copernicus (indexcopernicus.com)

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:
КВ № 21398-11198P of June 25, 2015

Part 1. Veterinary medicine

UDC 619:616.98:578.822:57.083:636.7(477.52)

A RETROSPECTIVE STUDY OF CANINE PARVOVIRUS IN PRIVATE VETERINARY CLINIC 'HEALTH', SUMY REGION, UKRAINE (2015–2018)

Tion M. T. [1], Fotina H. A. [2], Saganuwan A. S. [3]

[1] Department of Veterinary Medicine, College of Veterinary Medicine, University of Agriculture, Makurdi, Benue State, Nigeria, e-mail: tions_doc@yahoo.co.uk
[2] Department of Epizootology and Parasitology, Sumy National Agrarian University, Sumy, Ukraine, e-mail: super.annafotina@ukr.net
[3] Department of Veterinary Physiology, Pharmacology and Biochemistry, College of Veterinary Medicine, University of Agriculture, Makurdi, Benue State, Nigeria

Summary. The aim of the research is to conduct retrospective study of canine parvovirus in private veterinary clinic 'Health' in the Sumy region, Ukraine. Because of the widespread nature of the CPV infection and paucity of information on its epidemiology in Ukraine, it is necessary to carry out a retrospective study of the disease in Ukraine. Data on reported and confirmed cases of CPV infection, which was presented to private veterinary clinic 'Health' (Sumy), between 2015 and 2018 was reviewed. The data were sorted according to age, sex, breed, as well as treatment and vaccination status. The overall prevalence rate was 2.1%. Age, sex, breed, vaccination status showed association with the rate of infection. 43 (68.3%) puppies younger than 6 months old had higher incidence rate than those ones older than 6 months. Males (36 individuals, 57.1%) were more affected than females (27, 42.9%), while exotic breeds (43, 68.3%) were more affected than local breeds (20, 31.7%) and the number of recovered dogs (59, 93.7%) was higher than those who died (4, 6.3%). However, the majority of dogs was not vaccinated (46, 73.0%) in comparison to vaccinated dogs (17, 27.0%). The incidence (25, 40.0%) was observed in 2017. In conclusion, there is high prevalence of CPV infection in Ukraine with young, male and exotic breeds being affected much more than old, female and local breeds. Low incidence of death rate among the affected dogs may be attributed to successful immunization against the disease.

Keywords: dogs, canine parvovirus enteritis, epidemiology, prevalence, Ukraine

Introduction. The first mammal domesticated by human was a dog (*Canis familiaris*). They have been living together as companions since that, and they are useful for human as working partners and pets (Birchard and Sherding, 2006). The affection developed by humans towards dogs continues until this time (Daodu, Amosun and Oluwayelu, 2017).

Canine parvovirus (CPV) infection is a highly contagious, fatal disease of dogs, affecting mostly the gastrointestinal tract. The infection has no predilection for age, sex or breed of dogs. However, the Doberman pinscher, the Rottweiler and the German Shepherd Dog are at greater risk of the infection as compared to other breeds (Touihri et al., 2009; Castro et al., 2007; Gombač et al., 2008). It is a major cause of morbidity and mortality in puppies (Goddard and Leisewitz, 2010). It is transmitted from infected to susceptible dogs through the fecal-oral route and exposure to fomites (Decaro et al., 2005; Nivy et al., 2011). CPV infection also transmitted by house flies, flesh flies and blow/bottle flies (Bagshaw et al., 2014). The disease is characterized by anorexia, vomiting, bloody diarrhea, lethargy, myocarditis and leucopoenia (Streck et al., 2009; Tabor, 2011).

CPV is a small, non-enveloped, linear, single-strand DNA virus of the family Parvoviridae (Prittie, 2004; McCaw and Hoskins, 2006).

Three antigenic variants of CPV-2 (2a, 2b, and 2c) have been reported to be in global circulation, and their frequency of occurrence varies according to the geographical location (Bingga et al., 2014; Touihri et al., 2009; Wilson et al., 2014).

The diagnosis is based on clinical signs which include fever, nausea, abdominal pain, vomiting and bloody diarrhea (McCaw and Hoskins, 2006). The final diagnosis is based on electron microscopy, virus isolation, fecal hemagglutination, latex agglutination, counter-immunoelectrophoresis, immunochromatography, and PCR (Macintire and Smith-Carr, 1997; Pollock and Carmichael, 1988; Desario et al., 2005; Oh et al., 2006). PCR has been shown as more sensitive and reliable than other diagnostic methods (Desario et al., 2005; Buonavoglia et al., 2001).

CPV infection is primarily treated using supportive care, fluid therapy (crystalloid fluids, synthetic and natural colloids), a combination of antibiotics, antiemetics,

analgesics, nutritional support, and anthelmintics (Prittie, 2004; Brown and Otto, 2008).

Prevention of the disease is carried out by vaccination, 3 doses given at the age of 6, 9, and 12 weeks old using attenuated or modified live vaccines (Bergman et al., 2006).

However, some commercial CPV vaccines are not able enough to stimulate immunity against CPV-2 infection in puppies with high titers of maternally derived antibodies (Larson and Schultz, 1997).

Maternal antibodies cause failure of vaccination, and their presence should be checked before vaccination (Prittie, 2004). Because of the widespread of CPV infection worldwide, there is need to study prevalence of the disease in Ukraine.

The aim of the study was to conduct retrospective study of canine parvovirus in private veterinary clinic 'Health' in the Sumy region, Ukraine.

Materials and methods. The data was collected from private veterinary clinic 'Health' located in Sumy region, Ukraine (50° 55′ 17.76″ N, 34° 48′ 1.04″ E).

The records of dogs presented by other diseases and canine parvovirus infection within a period from 2015 to 2018, were collected and sorted according to age, sex, breeds, vaccination status, type of care and treatment. The diagnoses were based on history, clinical signs and laboratory findings using X-ray, ultrasound and SensPERT® Canine Parvovirus Antigen Test Kit. The prevalence and associated risk factors of CPV disease were analyzed and presented using chi square and significant differences were detected at 5% level (Petrie and Watson, 1999).

Results and discussion. Out of 3,010 cases presented to private veterinary clinic 'Health' within the study period, 63 (2.1%) cases were diagnosed of canine parvovirus infection.

Dogs younger than 6-month-old were more affected (43 individuals, 68.3%) than those ones who older than 6-month-old (20, 31.7%).

Females, (27, 42.9%) were less susceptible as compared to males (36, 57.1%).

Local breeds of dogs were also less affected (20, 31.7%) in comparison to exotic breeds (43, 68.3%).

However, unvaccinated dogs (46, 73.0%) were more affected than the vaccinated dogs (17, 27.0%).

The incidence rate was higher in household dogs (47, 74.6%) than in outhouse dogs (16, 25.4%) (Table 1).

The highest prevalence rate (25, 40.0%) was observed in 2017 with a monthly distribution in February and November (9, 14%) (Table 2).

Table 1 — Distribution of canine parvovirus infection

Groups	Total number of diagnosed cases	
	Frequency, n	Percentage, %
Age (Months)		
< 6	43	68.3
> 6	20	31.7
Sex		
Male	36	57.1
Female	27	42.9
Breed		
Local	20	31.7
Exotic	43	68.3
Vaccination Status		
Vaccinated	17	27.0
Unvaccinated	46	73.0
Movement of dog		
In house	47	74.6
Out house (Stray)	16	25.4
Treatment		
Recovered	59	93.7
Dead	4	6.3

Table 2 — Yearly and monthly distribution of canine parvovirus cases at private veterinary clinic 'Health', Sumy between 2015-2018.

Period	Total number of diagnosed cases	
	Frequency, n	Percentage, %
Years		
2015	13	20.6
2016	15	23.8
2017	25	39.7
2018	10	15.9
Months		
January	8	12.7
February	9	14.2
March	2	3.2
April	2	3.2
May	4	6.4
June	2	3.2
July	4	6.4
August	3	4.8
September	6	9.5
October	8	12.7
November	9	14.2
December	6	9.5

This study shows that CPV infection was endemic in Sumy during the period from 2015 to 2018. The prevalence of 2.1% (63 individuals, 3,010 cases) diverges from higher prevalence rate of 3.4% (84 and 2,486 respectively) reported in Slovenia (Gombac et al., 2008).

This might be caused by the higher awareness of dog vaccination by owners and breeders. The incidence of 43 (68.3%) for < 6-month-old dogs as well as 20 (31.7%) for > 6-month-old dogs agrees with the reports that dogs within the age limits (i. e. puppies between 6 weeks and

6 months old) have a higher risk to be infected (McCaw and Hoskins, 2006; Prittie, 2004).

It might be possible due to a decrease of maternally derived antibodies level from vaccinated or naturally infected female dogs before primary vaccination, leaving puppies without protection and therefore vulnerable to CPV infection (Houston, Ribble, and Head, 1996).

The higher prevalence rate (36, 57.1%) in males as compared to females (27, 42.9%) agrees with the findings that male dogs are more affected than female dogs (Shima, Apaa and Mosugu, 2015; Tion et al., 2018), but diverges with the hypothesis that females are more susceptible than males (Umar et al., 2015). However, Castro et al. (2007) reported that the disease does not have predilection for sex. Therefore, sex predilection can occur due to the preference for male dogs as pets or for security, as compared to female dogs that may be needed much more by breeders.

The higher incidence rate (43, 68.3%) of exotic breed compared to local breed (20, 31.7%) as well as highest incidence rate of mongrels (20, 31.7%); the German Shepherd Dog (10, 15.9%); the Siberian Husky (6, 9.5%); the Rottweiler and Labrador Retriever (4, 6.3% each); the Jagdterrier (3, 4.8%); the American Bulldog, the Schnauzer, and the Jack Russell Terrier (2, 3.2% each); the Bernese Mountain Dog, the Borzoi, the American Staffordshire Terrier, the Chihuahua, the Cane Corso, the East European Shepherd, the Central Asian Shepherd Dog, the American Pit Bull Terrier, the King Charles Spaniel, and the Samoyed (1, 1.6% each) as shown in Table 3 meet reports indicating that certain breeds are at an increased risk of severe CPV-2 infection.

Table 3 — The prevalence of canine parvovirus enteritis among various breeds of dogs at private veterinary clinic 'Health', Sumy, Ukraine (2015–2018)

No.	Breed	Number of affected dogs	Percentage, %
1	Bernese Mountain Dog	1	1.6
2	Mongrels	20	31.7
3	German Shepherd Dog	10	15.9
4	Borzoi	1	1.6
5	Siberian Husky	6	9.5
6	American Staffordshire Terrier	1	1.6
7	American Bulldog	2	3.2
8	Jack Russell Terrier	2	3.2
9	Chihuahua	1	1.6
10	Jagdterrier	3	4.8
11	Cane Corso	1	1.6
12	Rottweiler	4	6.3
13	Labrador Retriever	4	6.3
14	Schnauzer	2	3.2
15	East European Shepherd	1	1.6
16	Central Asian Shepherd Dog	1	1.6
17	American Pit Bull Terrier	1	1.6
18	King Charles Spaniel	1	1.6
19	Samoyed	1	1.6

The most affected breeds are the Rottweiler, the Doberman, the American Pit Bull Terrier, the Labrador Retriever, and the German Shepherd Dog (Glickman et al., 1985; Houston, Ribble and Head, 1996; Castro et al., 2007; Shima et al., 2015). The reason for the breed's susceptibility remains unknown.

The incidence rate 73.0% (46) of unvaccinated dogs as compared to the vaccinated dogs (17, 27.0%) agrees with the report indicating that some dogs may lack the ability to stimulate immune response and overcome interference of vaccination by maternal antibodies (Nandi and Kumar, 2010; Coyne, 2000; Meers et al., 2007). Young unvaccinated or incompletely vaccinated dogs are most susceptible to the disease (Kahn and Line, 2010; Parrish, 2017). Other dogs might be affected due to poor quality and improper handling of vaccines that may lack the ability to stimulate immune response capable to protect puppy from the disease (Tizard and Ni, 1998; Schultz, 2000).

The all-year-round incidence of infection agrees with the report of Kalli et al. (2010) indicating that the breed predisposition and seasonal variation are among the risk factors of CPV infection. The recovery of 59 (93.7%) from the infection may be due to the effects of polypharmacy (Prittie, 2004; Macintire and Smith-Carr, 1997; Brown and Otto, 2008).

Conclusion. CPV infection is endemic in Sumy with a prevalence rate of 2.1% affecting young, male, exotic breed, unvaccinated and homeless more than adult, female, local, vaccinated and household dogs.

The disease occurs every month of the year affecting mongrels, the German Shepherd Dog, the Siberian Husky, the Rottweiler, the Labrador Retriever, the Jagdterrier, the American Bulldog, the Schnauzer, the Jack Russell Terrier, the Bernese Mountain Dog, the Borzoi, the American Staffordshire Terrier, the Chihuahua, the Cane Corso, the East European Shepherd, the Central Asian Shepherd Dog, the American Pit Bull Terrier, the King Charles Spaniel, and the Samoyed.

Acknowledgement. The authors are grateful to the entire staff and management of private veterinary clinic 'Health', Sumy, for the opportunity and support during the period of this study.

Conflict of interest. The authors declare that there is no conflict of interest.

References

Bagshaw, C., Isdell, A. E., Thiruvaiyaru, D. S., Brisbin, I. L. and Sanchez, S. (2014) 'Molecular detection of canine parvovirus in flies (Diptera) at open and closed canine facilities in the eastern United States', *Preventive Veterinary Medicine*, 114(3–4), pp. 276–284. doi: 10.1016/j.prevetmed.2014.02.005.

Bergman, J. G. H. E., Muniz, M., Sutton, D., Fensome, R., Ling, F. and Paul, G. (2006) 'Comparative trial of the canine parvovirus, canine distemper virus and canine adenovirus type 2 fractions of two commercially available modified live vaccines', *Veterinary Record*, 159(22), pp. 733–736. doi: 10.1136/vr.159.22.733.

Bingga, G., Liu, Z., Zhang, J., Zhu, Y., Lin, L., Ding, S. and Guo, P. (2014) 'High resolution melting curve analysis as a new tool for rapid identification of canine parvovirus type 2 strains', *Molecular and Cellular Probes*, 28(5–6), pp. 271–278. doi: 10.1016/j.mcp.2014.08.001.

Birchard, S. J. and Sherding, R. G. (2006) *Saunders Manual of Small Animal Practice*. 3rd ed. St. Louis, Missouri: Saunders Elsevier. doi: 10.1016/B0-7216-0422-6/X5001-3.

Brown, A. J. and Otto, C. M. (2008) 'Fluid therapy in vomiting and diarrhea', *Veterinary Clinics of North America: Small Animal Practice*, 38(3), pp. 653–675. doi: 10.1016/j.cvsm.2008.01.008.

Buonavoglia, C., Martella, V., Pratelli, A., Tempesta, M., Cavalli, A., Buonavoglia, D., Bozzo, G., Elia, G., Decaro, N. and Carmichael, L. (2001) 'Evidence for evolution of canine parvovirus type 2 in Italy', *Journal of General Virology*, 82(12), pp. 3021–3025. doi: 10.1099/0022-1317-82-12-3021.

Castro, T. X., Miranda, S. C., Labarthe, N. V., Silva, L. E. and Cubel Garcia, R. C. N. (2007) 'Clinical and epidemiological aspects of canine parvovirus (CPV) enteritis in the State of Rio de Janeiro: 1995–2004', *Arquivo Brasileiro de Medicina Veterinária e Zootecnia*, 59(2), pp. 333–339. doi: 10.1590/S0102-09352007000200010.

Coyne, M. J. (2000) 'Efficacy of two canine parvovirus vaccines for inducing seroconversion in Rottweiler and Doberman pinscher pups with various levels of maternally derived antibodies', *Veterinary Therapeutics: Research in Applied Veterinary Medicine*, 1(1), pp. 35–42. PMID: 19757563.

Daodu, O. B., Amosun, E. A. and Oluwayelu, D. O. (2017) 'Antibiotic resistance profiling and microbiota of the upper respiratory tract of apparently healthy dogs in Ibadan, South West, Nigeria', *African Journal of Infectious Diseases*, 11(1), pp. 1–11. doi: 10.21010/ajid.v11i1.1.

Decaro, N., Elia, G., Martella, V., Desario, C., Campolo, M., Trani, L. D., Tarsitano, E., Tempesta, M. and Buonavoglia, C. (2005) 'A real-time PCR assay for rapid detection and quantitation of canine parvovirus type 2 in the feces of dogs', *Veterinary Microbiology*, 105(1), pp. 19–28. doi: 10.1016/j.vetmic.2004.09.018.

Desario, C., Decaro, N., Campolo, M., Cavalli, A., Cirone, F., Elia, G., Martella, V., Lorusso, E., Camero, M. and Buonavoglia, C. (2005) 'Canine parvovirus infection: which diagnostic test for virus?', *Journal of Virological Methods*, 126(1–2), pp. 179–185. doi: 10.1016/j.jviromet.2005.02.006.

Glickman, L. T., Domanski, L. M., Patronek, G. J. and Visintainer, F. (1985) 'Breed-related risk factors for canine parvovirus enteritis', *Journal of the American Veterinary Medical Association*, 187(6), pp. 589–594. Available at: https://www.researchgate.net/publication/20153295.

Goddard, A. and Leisewitz, A. L. (2010) 'Canine parvovirus', *Veterinary Clinics of North America: Small Animal Practice*, 40(6), pp. 1041–1053. doi: 10.1016/j.cvsm.2010.07.007.

Gombač, M., Švara, T., Tadić, M. and Pogačnik, M. (2008) 'Retrospective study of canine parvovirosis in Slovenia', *Slovenian Veterinary Research*, 45(2), pp. 73–78. Available at: http://www2.vf.uni-lj.si/ZB/SlovVetRes_45_(2)_pp73-78.pdf.

Houston, D. M., Ribble, C. S. and Head, L. L. (1996) 'Risk factors associated with parvovirus enteritis in dogs: 283 cases (1982–1991)', *Journal of the American Veterinary Medical Association*, 208(4), pp. 542–546. PMID: 8603904.

Kahn, C. M. and Line, S. (eds.) (2010) *The Merck Veterinary Manual*. 10th ed. White House Station, NJ, USA: Merck and Co., Inc. ISBN 9780911910933

Kalli, I., Leontides, L. S., Mylonakis, M. E., Adamama-Moraitou, K., Rallis, T. and Koutinas, A. F. (2010) 'Factors affecting the occurrence, duration of hospitalization and final outcome in canine parvovirus infection', *Research in Veterinary Science*, 89(2), pp. 174–178. doi: 10.1016/j.rvsc.2010.02.013.

Larson, L. J. and Schultz, R. D. (1997) 'Comparison of selected canine vaccines for their ability to induce protective immunity against canine parvovirus infection', *American Journal of Veterinary Research*, 58(4), pp. 360–363. PMID: 9099379.

Macintire, D. K. and Smith-Carr, S. (1997) 'Canine parvovirus. Part II. Clinical signs, diagnosis, and treatment', *The Compendium on Continuing Education for the Practicing Veterinarian*, 19(3), pp. 291–302. Available at: https://scribd.com/document/23747918.

McCaw, D. L. and Hoskins, J. D. (2006) 'Canine viral enteritis', in Green, C. E. (ed.) *Infectious Diseases of the Dog and Cat*. 4th ed. St. Louis, MO: Saunders Elsevier, pp. 63–73. ISBN 9781416061304.

Meers, J., Kyaw-Tanner, M., Bensink, Z. and Zwijnenberg, R. (2007) 'Genetic analysis of canine parvovirus from dogs in Australia', *Australian Veterinary Journal*, 85(10), pp. 392–396. doi: 10.1111/j.1751-0813.2007.00206.x.

Nandi, S. and Kumar, M. (2010) 'Canine parvovirus: current perspective', *Indian Journal of Virology*, 21(1), pp. 31–44. doi: 10.1007/s13337-010-0007-y.

Nivy, R., Hahn, S., Perl, S., Karnieli, A., Karnieli, O. and Aroch, I. (2011) 'A fatal outbreak of parvovirus infection: first detection of canine parvovirus type 2c in Israel with secondary *Escherichia coli* septicemia and meningoencephalitis', *Israel Journal of Veterinary Medicine*, 66(3), pp. 96–102. Available at: http://www.ijvm.org.il/node/172.

Oh, J.-S., Ha, G.-W., Cho, Y.-S., Kim, M.-J., An, D.-J., Hwang, K.-K., Lim, Y.-K., Park, B.-K., Kang, B. and Song, D.-S. (2006) 'One-step immunochromatography assay kit for detecting antibodies to canine parvovirus', *Clinical and Vaccine Immunology*, 13(4), pp. 520–524. doi: 10.1128/CVI.13.4.520-524.2006.

Parrish, C. R. (2017) 'Parvoviridae', in MacLachlan, N. J. and Dubovi, E. J. (eds.) *Fenner's Veterinary Virology*. 5th ed. Academic Press, pp. 245–257. doi: 10.1016/B978-0-12-800946-8.00012-X.

Petrie, A. and Watson, P. (1999) *Statistics for Veterinary and Animal Science*. Oxford, UK: Blackwell Science. ISBN 9780632050253.

Pollock, R. V. and Carmichael, L. E. (1988) 'Canine viral enteritis', in Barlough, J. E. (ed.) *Manual of Small Animal Infectious Diseases*. New York: Churchill Livingston, pp. 101–107. ISBN 9780443085080.

Prittie, J. (2004) 'Canine parvoviral enteritis: a review of diagnosis, management, and prevention', *Journal of Veterinary Emergency and Critical Care*, 14(3), pp. 167–176. doi: 10.1111/j.1534-6935.2004.04020.x.

Schultz, R. D. (2000) 'Considerations in designing effective and safe vaccination programs for dogs', in Carmichael, L. E. (ed.) *Recent Advances in Canine Infectious Diseases*, IVIS Document Number A0110.0500. Available at: http://www.ivis.org/advances/Infect_Dis_Carmichael/schultz/chapter.asp.

Shima, F. K., Apaa, T. T. and Mosugu, J. I. T. (2015) 'Epidemiology of canine parvovirus enteritis among hospitalized dogs in Effurun/Warri Metropolitan Region of Delta State, Nigeria', *Open Access Library Journal*, 2(1), p. e1208. doi: 10.4236/oalib.1101208.

Streck, A. F., Souza, C. K. de, Gonçalves, K. R., Zang, L., Pinto, L. D. and Canal, C. W. (2009) 'First detection of canine parvovirus type 2c in Brazil', *Brazilian Journal of Microbiology*, 40(3), pp. 465–469. doi: 10.1590/S1517-83822009000300008.

Tabor, B. (2011) 'Canine parvovirus', *Veterinary Technician*, 32(5), pp. E1–E10. Available at: http://vetfolio-vetstreet.s3.amazonaws.com/2a/c3dfa0a9a611e087120050568d3693/file/VT0511_Tabor_CE.pdf.

Tion, M. T., Apaa, T. T., Saganuwan, A. S., Nwankwo, C. H., Tughgba, T., Anumtyo, T. M., Amine, A. A., Nguetyo, S. A., Igoh, F. A. and Akpehe-Ishor, W. (2018) 'The epidemiology of canine parvovirus enteritis in dogs of Makurdi, Benue State, Nigeria', World's Veterinary Journal, 8(3), pp. 48–54. Available at: http://wvj.science-line.com/vol-8--no-3-sep-2018.html.

Tizard, I. and Ni, Y. (1998) 'Use of serologic testing to assess immune status of companion animals', *Journal of the American Veterinary Medical Association*, 213(1), pp. 54–60. PMID: 9656025.

Touihri, L., Bouzid, I., Daoud, R., Desario, C., El Goulli, A. F., Decaro, N., Ghorbel, A., Buonavoglia, C. and Bahloul, C. (2009) 'Molecular characterization of canine parvovirus-2 variants circulating in Tunisia', *Virus Genes*, 38(2), pp. 249–258. doi: 10.1007/s11262-008-0314-1.

Umar, S., Ali, A., Younus, M., Maan, M. K., Ali, S., Khan, W. A. and Irfan, M. (2015) 'Prevalence of canine parvovirus infection at different pet clinics in Lahore, Pakistan', *Pakistan Journal of Zoology*, 47(3), 657–663. Available at: http://zsp.com.pk/pdf47/657-663 (8) PJZ-2148-14 21-4-15 2nd revised copy 8-3-15 Ali-et-al.-2014-Canine-Parvo-_.pdf.

Wilson, S., Illambas, J., Siedek, E., Stirling, C., Thomas, A., Plevová, E., Sture, G. and Salt, J. (2014) 'Vaccination of dogs with canine parvovirus type 2b (CPV-2b) induces neutralising antibody responses to CPV-2a and CPV-2c', *Vaccine*, 32(42), pp. 5420–5424. doi: 10.1016/j.vaccine.2014.07.102.

UDC 619:616.68-073:636.082.31

REMOTE-NONCONTACT AND NON-INVASIVE DIAGNOSTICS OF GONADODYSTROPHY IN MALES

Naumenko S. V., Koshevoy V. I.

Kharkiv State Zooveterinary Academy, Kharkiv, Ukraine,
e-mail: frolka001@gmail.com, vsevolod_koshevoy@yahoo.com

Summary. The results of the development and implementation of remote-noncontact and noninvasive diagnostic methods of gonadodystrophy in males are presented in the article. These methods include the use of thermal imager and ultrasound scanner. It is quite simple in implementation and can be used in veterinary medicine practice for rapid determination of the reproductive function, functional state of testes, detection or exclusion of pathological processes. The results are used to make a diagnosis and for differential diagnostics of andrological pathologies and in computer-based speculation programs for the recovery of reproductive function in males.

Keywords: males, diagnostics, gonadodystrophy, thermal imager, thermography, ultrasound scanner, sonography

Introduction. Various factors of the external and internal medium can have a negative influence on the structure and function of the testes. Among such factors, the major ones are hypokinesis, deficient state of organism, imbalance in the prooxidant-antioxidant system, toxic substances, ionizing radiation and other pathogens (Kharuta et al., 2012; Koshevoy et al., 2015).

Andrological medical examination which includes general clinical examination, biochemical (determination of homoeostasis indexes such as protein, vitamins, mineral substances, hormones) and andrological examinations (genitals state, in particular testes (consistence, temperature, pain reaction), determination of sexual reflexes activity), determination of indexes of sperm quality are obligatory in veterinary medicine practice (Yablonskyi, 2002; Koshevoy et al., 2017).

The processes of andro- and spermiogenesis depend on the optimum parameters of the structure and functioning of the testes. These interdependent processes are vulnerable. Nowadays the conducted research does not allow defining the state of reproductive function in males objectively. Methods of diagnostics of andrological pathologies, in particular gonadodystrophy, must be improved (Kharuta et al., 2009; Stelletta et al., 2013; Draaisma, 2015).

The aim of the study was to develop the methods of the use of ultrasonic scanner and thermal imager in order to determine the functional state of the testes and preventive diagnostics of gonadodystrophy in males.

Materials and methods. The research was carried out in the Department of Veterinary Reproduction and in the Scientific Practical Center of Plant Breeding and Animal Husbandry of the Kharkiv State Zooveterinary Academy and on some farms of Kharkiv and Dnepropetrovsk Regions.

Twelve bulls, seventeen boars and twenty-one rabbits were examined for the research.

Animals were divided into two groups: I — with full appearance of sexual function, clinically healthy, without reproductive pathologies; II — with gonadodystrophy.

Clinical, andrological, ultrasonic, and thermographic investigations, and also some biochemical and hormonal methods of the research were used.

To determine the internal structure and consistence of the gonads the ultrasonic scanner SLE-150 was applied. The thermographic investigations of external privy parts were carried out by thermal imager TI-120 model.

The analysis of the thermogram was conducted with the help of special program 'IR Analysis Software'.

The methods of the preventive remote diagnostics of inflammatory processes in the external privy parts of males were approved in animals with normal condition and gonadodystrophy.

During the research, the temperature of stock-raising apartments was 15–17 °C, relative humidity — 60%. Digital data was worked out by biometric method.

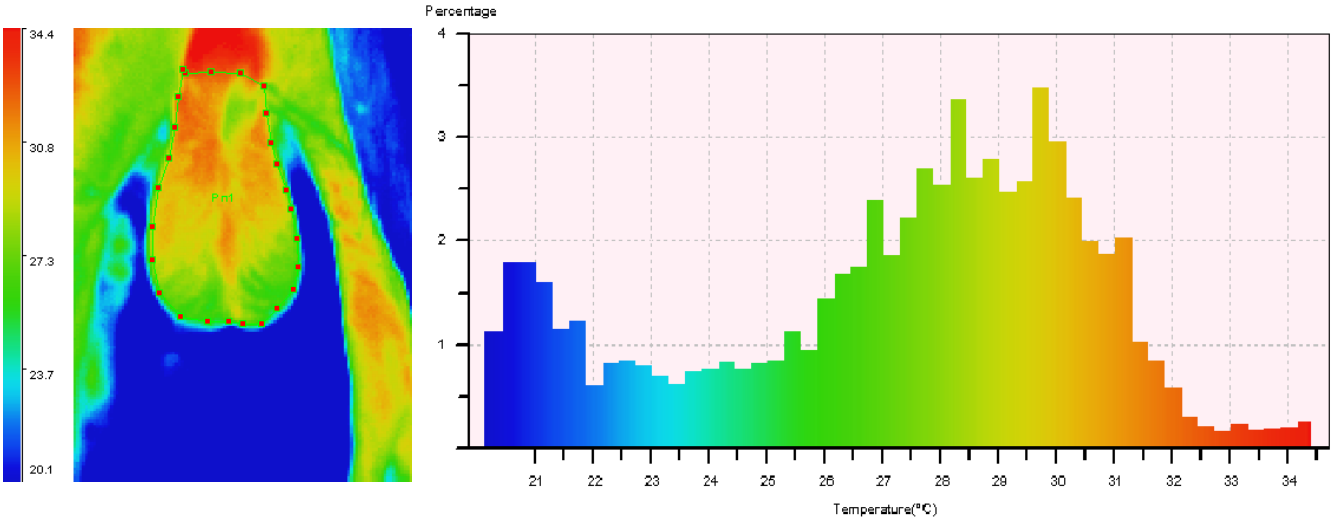
Results. The dependence of temperature gradients of the testes from their morphological condition in bulls in thermographic research was determined. It was defined that animals with gonadodystrophy had decreased temperature gradient compared to animals with full reproductive function (Table 1).

Table 1 — Indexes of distance-project thermography of the testes of males

Species	Groups of animals	
	with a full reproductive function, M ± m	with gonadodystrophy, M ± m
Bulls	29.5 ± 0.250	28.8 ± 0.124***
Boars	30.9 ± 0.113	29.2 ± 0.185*
Rabbits	31.5 ± 0.256	30.2 ± 0.352**

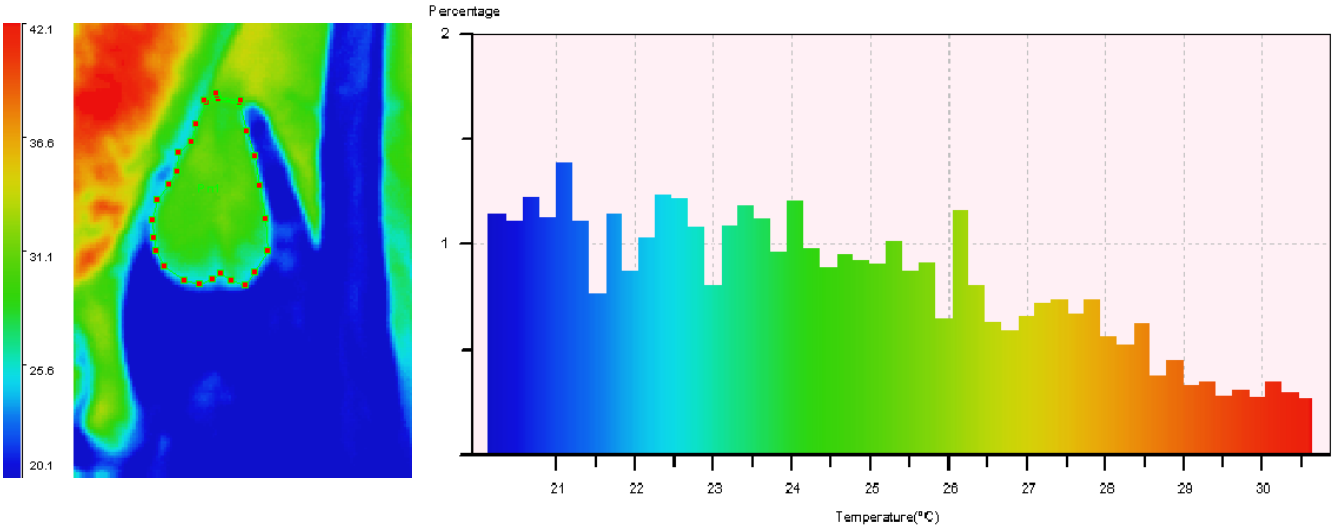
Notes: * — $P < 0.001$, ** — $P < 0.02$, *** — $P < 0.037$ compared to full reproductive function.

In addition, differences in the indexes of thermograms were defined. In thermogram of the testes of bulls with a full reproductive function dominated 'warm' colors of the palette (red and orange) (Fig. 1). On the other hand, the thermographic images of the testes of males with gonadodystrophy had expressive thermo-spotted area with predominance of 'cold' colors that is typical for blood circulation disorders (Fig. 2).



a b

Figure 1. Thermogram of the testes of bulls with a full reproductive function: a — color palette, b — the program of read-out of indexes

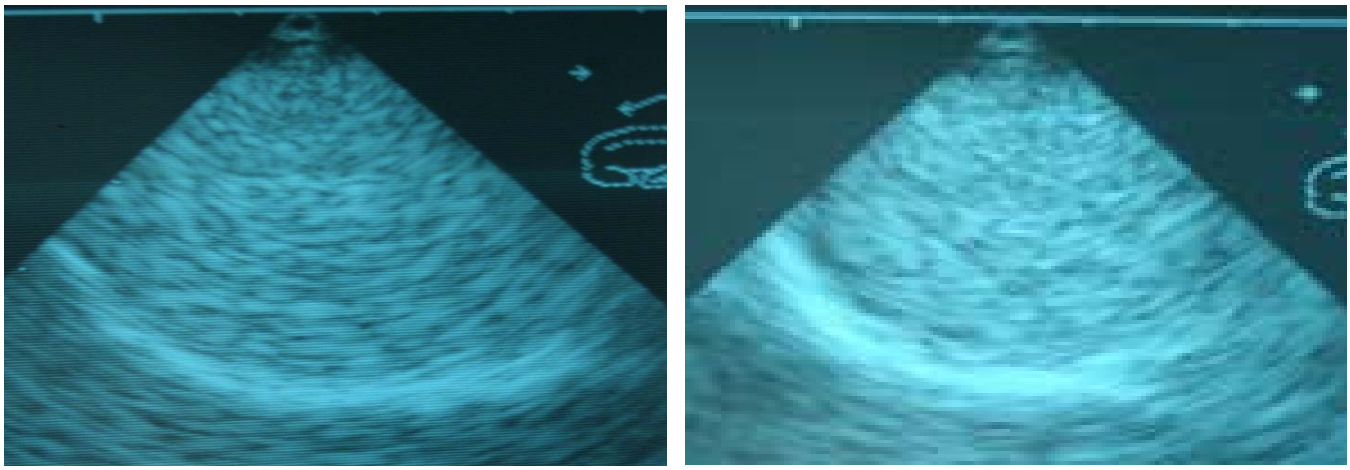


a b

Figure 2. Thermogram of the testes of bulls with gonadodystrophy: a — color palette, b — the program of read-out of indexes

Taking into account the findings, it can be concluded that decline of temperature gradient and insignificant hypothermia area are typical for gonadodystrophy in males. Ultrasonographic method of research of the testes includes determination of increased echogenicity. The echograms of gonads were characterized by definite alternative features. Hyperechoic structure is defined by bright white spots on a black background. These spots show the surfaces with high reflecting ability, such as bones, gases, and collagen. Hypoechoic structure is revealed by dissipated dark grey points, which show the reflection of waves from soft tissues. Non-echogenic structure is black one and appears during complete passing of waves through the medium (liquid). A computer program developed on Object Pascal was used to determine the consistence of tissue. Ultrasonograms of testes of boars are presented on Fig. 3.

In dystrophic processes in the testes tissues the increase of their size, decline of parenchyma echogenicity with maintaining of homogeneity were observed.



a b

Figure 3. Ultrasonograms of testes of boars: a — with full reproductive function, b — with gonadodystrophy

Conclusion. Methods of remote-noncontact and non-invasive estimation of the gonad endostructure in males are simple in implementation and can be used in the practice of veterinary medicine for rapid determination of full reproductive function, functional state of the testes, and the detection or exclusion of pathological processes.

References

Draaisma, H. S. (2015) *Application of infrared scrotal thermography (IRST) under field conditions in bulls extensively managed in tropical Costa Rica, and its relationship with spermiogramme, clinical variables and final breeding soundness classification*. The Master thesis. Utrecht: Utrecht University. Available at: https://dspace.library.uu.nl/handle/1874/315633.

Kharuta, G. G., Podvaliuk, D. V., Lototskyi, V. V. and Baban, O. A. (2009) 'The use of sonography in livestock and veterinary medicine' [Vykorystannia sonohrafii u tvarynnytstvi i veterynarnii medytsyni], *Veterinary Practice [Veterynarna praktyka]*, 5, pp. 24–26. [in Ukrainian].

Kharuta, G. G., Velbivets, M. V., Volkov, S. S. and Vlasenko, S. A. (2012) *Reproduction of livestock animals [Vidtvorennia silskohospodarskykh tvaryn]*. Bila Tserkva: Bila Tserkva National Agrarian University. ISBN 9789662122268. [in Ukrainian].

Koshevoy, V. P., Naumenko, S. V., Koshevoy, V. I., Maliukin, Yu. V., Klochkov, V. K. and Kavok, N. S. (2015) 'Computer monitoring of the indicators of structural and functional conditions of the reproductive system organs in males at deficiency of carotene (vitamin A) and zinc' [Kompiuternyi monitorynh pokaznykiv strukturno-funktsionalnoho stanu orhaniv reproduktyvnoi systemy u samtsiv pry defitsyti karotynu (vitaminu A) ta Tsynku], *Problems of Zooengineering and Veterinary Medicine [Problemy zooinzhenerii ta veterynarnoi medytsyny]*, 31(2), pp. 62–70. Available at: http://nbuv.gov.ua/UJRN/pzvm_2015_31(2)__16. [in Ukrainian].

Koshevoy, V. P., Naumenko, S. V., Koshevoy, V. I. and Skliarov, P. M. (2017) *'Distance non-contact and noninvasive diagnostics of pathological processes in male gonads: methodological recommendations' [Dystantsiino-bezkontaktna ta neinvaziina diahnostyka patolohichnykh protsesiv u honadakh samtsiv: metodychni rekomendatsii]*. Kharkiv: Kharkiv State Zooveterinary Academy. [in Ukrainian].

Stelletta, C., Vencato, J., Fiore, E. and Gianesella, M. (2013) 'Infrared thermography in reproduction', in Luzi, F., Mitchell, M., Nanni Costa, L. and Redaelli, V. (eds.) *Thermography: Current Status and Advances in Livestock Animals and in Veterinary Medicine*. Brescia, Italy: Fondazione Iniziative Zooprofilattiche e zootecniche, pp. 113–125. Available at: https://www.fondiz.it/download/96/quaderni/17047/092-2013-thermography-current-status-and-advances-in-livestock-animals-and-in-veterinary-medicine.pdf.

Yablonskyi, V. A. (2002) *Practical obstetrics, gynecology and animal reproduction biotechnology with the basics of andrology [Praktychne akusherstvo, hinekolohiia ta biotekhnolohiia vidtvorennia tvaryn z osnovamy androlohii]*. Kyiv: Meta. ISBN 9667947033. [in Ukrainian].

UDC 619:579.869.1:615.281:615.33

DETERMINATION *LISTERIA* SPP. (*L. WELSHIMERI, L. GRAYI, L. MURRAYI, L. INNOCUA*) SENSITIVITY TO ANTIBIOTICS

Vygovska L. M.

Ukrainian Laboratory of Quality and Safety of Agricultural Products of the National University of Life and Environmental Sciences of Ukraine, Kyiv, Ukraine, e-mail: lnvygovska@gmail.com

Summary. This research was conducted to determine the sensitivity of *Listeria* spp. (*L. welshimeri*, *L. grayi*, *L. murrayi*, *L. innocua*) to following antibiotic groups: penicillins, cephalosporins, carbapenems, aminoglycosides, macrolides, lincosamides, tetracyclines, quinolones, nitrofurans, chloramphenicol, and vancomycin. Studying cultures were identified with microbiological analysis of soil samples, raw materials of plant origin, and rodent feces. The study and interpretation of the results were carried out using diffusive method in a comparative aspect with the reference strains of *Listeria* spp. appropriate species. As a result, the sensitivity studied groups *Listeria* spp. to antibiotics was determined.

Keywords: *Listeria* spp., reference strains, antibiotics, sensitivity, resistance

Introduction. For today, international requirements regulate the study of animal food products to detect *Listeria monocytogenes* — the listeriosis causative agent. However, cases of people infected by the *Listeria* spp., which are considered as non-pathogenic, have been recently reported. In this regard, it is relevant to study the sensitivity of *Listeria* spp. to antibiotics, in particular those that were considered as non-pathogenic until recently.

Bacteria of the *Listeria* genus are widespread in nature. They have different adaptive capabilities and, apart from humans and animals, they can live in the environment. *Listeria*, as facultative psychrophile, grows in a wide temperature range of 1–45 °C, capable of accumulation and virulence persistence in environmental objects at low temperatures (soil, water, plants), and even become more virulent under favorable conditions. This leads to increasing of *Listeria* concentration in environmental objects in spring and autumn, but it significantly decreases in the soil in summer (Euzéby and Parte, 2018; EFSA and ECDC, 2010). Winter freezing of the soil does not have negative impact on their viability. The viability and reproductive activity are significantly affected by water balance and optimal, close to neutral, pH values. They are characterized by high metabolic ductility, the ability to transform from saprophytic to parasitic lifestyle and back (Euzéby and Parte, 2018; EFSA and ECDC, 2010).

The *Listeria* spp. genus includes 16 species (Euzéby and Parte, 2018), where *L. monocytogenes, L. ivanovii,* and *L. seeligeri* are pathogenic (Zhang et al., 2007; Guillet et al., 2010; Rocourt et al., 1986).

However, there are reports of human infection with species which are considered as non-pathogenic. A fatal case of a 62-year-old patient after septicemia caused by *L. innocua* against cholangitis is reported (Perrin, Bemer and Delamare, 2003).

The cases *L. welshimeri* (Andre and Genicot, 1987) and *L. grayi* (Rapose, Lick and Ismail, 2008; Salimnia et al., 2010) isolated in adult patients are described in literature. *Listeria* spp. are sensitive to penicillin, macrolides (except azithromycin and spiramycin), fluoroquinolones, aminoglycosides, tetracycline, and vancomycin (EFSA and ECDC, 2010; Doganay, 2003; Volokhov et al., 2007; Johnson et al., 2004; Clayton et al., 2014).

However, to sensitivity of *L. monocytogenes* species to antibiotics is most extensively studied (EFSA and ECDC, 2010; Zhang et al., 2007; Volokhov et al., 2007; Johnson et al., 2004). Therefore, the issue of species sensitivity within the genus is constantly studied and remains relevant for today.

The aim of the study was to determine the sensitivity of *Listeria* spp. (*L. welshimeri*, *L. grayi*, *L. murrayi*, *L. innocua*) to following antibiotic groups: penicillins, cephalosporins, carbapenems, aminoglycosides, macrolides, lincosamides, tetracyclines, quinolones, nitrofurans, chloramphenicol, and vancomycin.

Materials and methods. Sensitivity of 14 isolates of *Listeria* spp. (4 isolates of *L. welshimeri*, 5 isolates of *L. grayi*, 3 isolates of *L. murrayi*, and 2 isolates of *L. innocua* isolated from rodent feces, soil samples, and plant material) and reference cultures of *L. welshimeri* 1, *L. grayi* LMG 16490, *L. murrayi* LMG 16491 (Belgian Coordinated Collections of Microorganisms), *L. innocua* ATCC 33090 to antibiotics was determined.

Studies were conducted using nutrient media and disks with minimal concentrations of active ingredient produced by 'HiMedia'.

Determination of antibiotic sensitivity by diffusion method and evaluation of obtained results was carried out according to EUCAST prescriptions (EUCAST, 2018) and 'Determination of the Sensitivity of Microorganisms to Antibacterial Drugs' (MHU, 2007).

Results. Sensitivity of *L. welshimeri* 1, *L. grayi* LMG 16490, *L. murrayi* LMG 16491, and *L. innocua* ATCC 33090, as well as field cultures of *Listeria* spp. to

penicillins (benzylpenicillin, piperacillin, and ampicillin) has been determined. *L. welshimeri* 1 reference strain and *L. welshimeri* isolates #21–24 were highly sensitive to piperacillin and ampicillin (Table 1).

As for cephalosporins' group, the sensitivity of *Listeria* spp. was determined to cefazolin (I), cefalexin (I), cefuroxime (II), cefaclor (II), cefamandole (II), ceftazidime (III), cefotaxime (III), ceftriaxone (III), cefoperazone (III), and cefepime (IV). As a result of our research, it has been found that studied cultures were non-sensitive, but with some exceptions: strains *L. welshimeri* 1 and #21–24 are sensitive to cefazolin (I) and ceftriaxone (III), and *L. grayi* LMG 16490 strain is sensitive to cefuroxime (II) (Table 1).

L. welshimeri 1 and *L. murrayi* LMG 16491 reference strains, as well as *L. welshimeri* field cultures #21–24 were sensitive to carbapenems (imipenem, meropenem). *L. grayi* LMG 16490 reference strain showed sensitivity to imipenem and was not sensitive to meropenem. *L. murrayi* isolates 12/0811, 14/0811, 22 and *L. grayi* 2/09, 5/09, 6/09, 10/0811, 11/0811 are non-sensitive. *L. innocua* ATCC 33090 reference strain and *L. innocua* isolates #22 and #23 were insensitive to imipenem and resistant to meropenem (Table 1).

Table 1 — Sensitivity of *Listeria* spp. to the antibiotics (penicillins, cephalosporins, carbapenems)

Name of antibiotic / Content of antimicrobial substance, μg (ED)	Diameters of inhibition of cultural growth, mm																	
	L. welshimeri					*L. grayi*						*L. murrayi*				*L. innocua*		
	1	21	22	23	24	LMG 16490	2/09	5/09	6/09	10/0811	11/0811	LMG 16491	12/0811	14/0811	22	ATCC 33090	22	23
Penicillins																		
Benzylpenicillin, 10	19	22	23	20	24	20	17*	17*	18	18*	16*	28+	17*	22	21	25+*	26+*	24+*
Piperacillin, 100	40	39+*	36+*	39+*	32+*	28	18+*	15+*	19+*	18+*	20+*	29+	21+	18+	19+	24	23	25
Ampicillin, 10	34	37+*	35+*	29+*	37+*	32	25+	26+*	22+	27+*	23+*	30+	27+	22+	23+	34+	33+	35+
Cephalosporins																		
Cefazolin, 30	34	35	33	36	32	25	21+*	17+*	17+*	26+*	18	20+	16*	17	16	22	21	20
Cefalexin, 30	26	27+*	28+*	30+*	29+*	29	20+*	18+*	21+*	17+*	22	27+	20+*	21+*	22+*	20	19	21
Cefuroxime, 30	26	27+*	28+*	29+*	26+*	32	20	20	23	17	26	24	20	18	18	20	22	21
Cefaclor, 30	28	28+*	27+*	27+*	29+*	27	15	17	14	19	21	14+	9	6	8	15	16	16
Cefotaxime, 30	27	29+*	29+*	28+*	27+*	27	18+	19+	22+	16+	17+	19+*	13	14	15	17	15	17
Ceftriaxone, 30	32	33+*	30+*	31+*	34+*	11	0	8	16	0	12	11	8	0	0	12	11	11
Cefoperazone, 75	28	27+*	29+*	27+*	30+*	25	15+	12+	17+	18+	21+	26+	20	18	18	20	21	22
Ceftazidime, 30	12	11	14	12	13	12	8	7	0	9	0	17	12	13	13	10	11	11
Cefepime, 30	20	20+*	20+*	20+*	20+*	18	12	11	10	7	13	16	11	12	14	12	12	11
Cefamandole, 30	26	28+*	27+*	29+*	27+*	27	21	17	21	16	25	23+	17	20	19	23+	22+	24+
Carbapenems																		
Imipenem, 10	44	40	41	43	44	28	25	19	20	22	20+	28	20+	21	20	25+	26+	26+
Meropenem, 10	34	35+*	37+*	36+*	35+*	22	14	17	14	19	17	30	20	22	21	0	6	0

Notes: * — stimulation of culture growth around the zone of inhibition; + — the normal growth of resistant colonies in the zone of inhibition of culture growth.

As for aminoglycosides, sensitivity to streptomycin (I), kanamycin (I), neomycin (I), gentamicin (II), netilmicin (II), tobramycin (II), and amikacin (III) was studied. *L. welshimeri* 1, *L. grayi* LMG 16490, *L. murrayi* LMG 16491, and *L. innocua* ATCC 33090 reference strains are mainly sensitive, and isolates mostly have low sensitivity (Table 2).

From the group of macrolides (erythromycin, azithromycin, and oleandomycin), *L. welshimeri* strains are sensitive to erythromycin, resistant to azithromycin and oleandomycin and *L. grayi* strains are resistant to these antibiotics. *L. murrayi* LMG 16491 strain is sensitive to erythromycin, but resistant to azithromycin and oleandomycin, *L. murrayi* isolates 2/09, 5/09, 6/09, 10/0811, and 11/08/11 are not sensitive to macrolides. *L. innocua* ATCC 33090 reference strain and field cultures are not sensitive to erythromycin, but resistant to azithromycin and oleandomycin (Table 2).

As for quinolones' group which is considered as moderately active against *Listeria* spp., the sensitivity of *Listeria* spp. to nalidixic acid (I), ciprofloxacin (II), norfloxacin (II), pefloxacin (II), ofloxacin (II), and

levofloxacin (III) was studied. All strains of *L. welshimeri*, *L. grayi*, and *L. murrayi* were resistant to nalidixic acid, and reference strains showed sensitivity to quinolones II–III generations in 60% of cases; isolates have low sensitivity. *L. innocua* strains are resistant to nalidixic acid and less sensitive to preparations II–III generations (Table 2).

Referring to drugs of the nitrofurans' group, the sensitivity of *Listeria* spp. to furazidine and furazolidone was determined. *L. welshimeri* cultures 1, 7/10, 22, 23, 24 and *L. murrayi* LMG 16491 were sensitive to furazidine and more resistant to furazolidone. Other cultures were not less sensitive to furazidine and furazolidone (Table 2).

Table 2 — Sensitivity of *Listeria* spp. to the antibiotics (aminoglycosides, macrolides, lincosamides, tetracyclines, fluoroquinolones, nitrofurans, chloramphenicol, vancomycin)

Name of antibiotic / Content of antimicrobial substance, μg (ED)	Diameters of inhibition of cultural growth, mm																	
	L. welshimeri					*L. grayi*						*L. murrayi*				*L. innocua*		
	1	21	22	23	24	LMG 16490	2/09	5/09	6/09	10/0811	11/0811	LMG 16491	12/0811	14/0811	22	ATCC 33090	22	23
Aminoglycosides																		
Streptomycin, 30	40	37+	38+	39+	37+	32	24	22+*	17	26+*	24+*	24+*	14	20+	21+	21	22	23
Kanamycin, 30	14	13+*	14+*	15+*	13+*	27	16	16	17+*	16	20	19	14	15	14	16	16	17
Neomycin, 30	20	21	20	21	22	24	17	19	14+*	20+*	20+*	22+*	18+*	17	16	24+*	23+*	24+*
Gentamicin, 10	26	26+*	27+*	28+*	27+*	26	19	20+*	19	18	17	23	20	18	18	20	22	21
Netilmicin, 30	22	22	23	22	24	28	25+	22+	25+	20+	21+	24+	17+*	18+	19+	25+*	24+*	23+*
Amikacin, 30	26	26+*	27+*	28+*	27+*	26	20	16	18	20	17	27	21	20	21	20	19	21
Tobramycin, 10	26	26+	28+	27+	26+	30	23+-	22	24	25+	26	27	22+*	20	19	30	33	29
Macrolides																		
Erythromycin, 15	30	30+	32+	31+	32+	24	17	19	20	20+	21	28	22+	20+	21+	25+	27+	26+
Azithromycin, 15	14	14	15	13	14	17	13	15	11	9	12	17	15	13	13	17	16	15
Oleandomycin, 15	16	16	15	17	16	14	9	10	14	9	10	13	9	10	9	14	13	15
Lincosamides																		
Lincomycin, 15	20	21+*	23+*	22+*	23+*	0	0	0	0	0	0	0	0	0	7	0	0	7
Clindamycin, 2	14	15	16	14	14	24	16	15	16+-	18+	12	22+	16+	14	15	24+	24+	25+
Tetracyclines																		
Tetracycline, 30	46	45+	46+	44+	43+	23	16	17+-	18	20+-	18	25+	18	21	20	22+	21+	22+
Doxycycline, 30	32	31+	30+	32+	31+	25	18	17	20	18	17	27	21	21	22	25	25	25
Quinolones																		
Nalidixic acid, 30	7	0	0	6	0	9	0	7	7	0	6	0	0	6	7	9	9	9
Ciprofloxacin, 5	30	31+*	30+*	29+*	32+*	17	12	13	10	10	9+-	27+	14+	16	15	17+	16+	17+
Norfloxacin, 10	15	14	15	15	13	28	17	20	21+-	25+-	19+-	29	17	19	18	18	18	19
Pefloxacin, 10	16	16	17	16	15	25	19	18	17	22+-	19+-	25	20+	21	20	19	20	19
Ofloxacin, 5	26	27+	26+	27+	28+	24	20	20	19	21	20+-	26	19	22+	20+	20	21	20
Levofloxacin, 5	30	28+*	28+*	26+*	27+*	15	14	10	12	11	10	20	16	14	15	15	16	14
Nitrofurans																		
Furazidine, 300	24	22	23	22	25	12	9	10+	0	9	7+	23	14	13+	12+	12	11	11
Furazolidone, 300	15	16	14	16	17	13	9	10	8	7	6	18	11	12	11	12+	12+	11+
Chloramphenicol, 30	26	27+*	28+*	27+*	26+*	17	15	10	14	16	14	24+	18+	17	16	17+*	16+*	15+*
Vancomycin, 30	36	36+*	35+*	34+*	33+*	27	22+	20+	21+	22+	20+*	23+*	20	22+*	20+*	20	19	21

Notes: * — stimulation of culture growth around the zone of inhibition; + — the normal growth of resistant colonies in the zone of inhibition of culture growth.

Regarding to chloramphenicol, which is active against many types of Gram-positive and Gram-negative bacilli, *L. welshimeri* and *L. murrayi* reference strains are sensitive, as for field strains, they are low sensitive. *L. grayi,* and *L. innocua* reference and field cultures are low sensitive (Table 2).

Reference and field cultures of *L. welshimeri*, *L. grayi*, *L. murrayi*, and *L. innocua* are sensitive to vancomycin (Table 2).

Conclusions. 1. All studied *L. welshimeri*, *L. grayi*, *L. murrayi*, and *L. innocua* cultures showed sensitivity to natural, semi-synthetic penicillins, and vancomycin, but showed resistance to nalidixic acid.

2. Cultures showed selective sensitivity to carbapenems, macrolides, lincosamides, tetracyclines, aminoglycosides, cephalosporins, quinolones II and III generations, nitrofurans, and chloramphenicol.

3. Reference cultures of *Listeria* spp. differed in the increased level of sensitivity to antibiotics in comparison with isolates.

4. Differences in sensitivity of cultures to certain groups of antibiotics have been noted.

5. The question of specific sensitivity characteristics for certain cultures and serotypes to antibiotics needs further study using a wide range of active substances of existing pharmacological groups.

References

Andre, P. and Genicot, A. (1987) 'First isolation of *Listeria welshimeri* from human beings' [Premier isolement de *Listeria welshimeri* chez l'homme], *Zentralblatt für Bakteriologie, Mikrobiologie und Hygiene. Series A: Medical Microbiology, Infectious Diseases, Virology, Parasitology*, 263(4), pp. 605–606. doi: 10.1016/S0176-6724(87)80205-5. [in French].

Clayton, E. M., Daly, K. M., Guinane, C. M., Hill, C., Cotter, P. D. and Ross, P. R. (2014) 'Atypical *Listeria innocua* strains possess an intact LIPI-3', *BMC Microbiology*, 14(1), p. 58. doi: 10.1186/1471-2180-14-58.

Doganay, M. (2003) 'Listeriosis: clinical presentation', *FEMS Immunology and Medical Microbiology*, 35(3), pp. 173–175. doi: 10.1016/S0928-8244(02)00467-4.

EFSA and ECDC (European Food Safety Authority and European Centre for Disease Prevention and Control). (2010) 'The Community Summary Report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in the European Union in 2008', *EFSA Journal*, 8(1), p. 1496. doi: 10.2903/j.efsa.2010.1496.

EUCAST (European Committee on Antimicrobial Susceptibility Testing). (2018) *Breakpoint tables for interpretation of MICs and zone diameters*. Ver. 8.0. Available at: http://www.eucast.org/ast_of_bacteria/previous_versions_of_documents.

Euzéby, J. P. and Parte, A. C. (2018) 'Genus *Listeria*', in *List of Prokaryotic names with Standing in Nomenclature*. Available at: http://www.bacterio.net/*Listeria*.html.

Guillet, C., Join-Lambert, O., Le Monnier, A., Leclercq, A., Mechaï, F., Mamzer-Bruneel, M.-F., Bielecka, M. K., Scortti, M., Disson, O., Berche, P., Vazquez-Boland, J., Lortholary, O. and Lecuit, M. (2010) 'Human listeriosis caused by *Listeria ivanovii*', *Emerging Infectious Diseases*, 16(1), pp. 136–138. doi: 10.3201/eid1601.091155.

Johnson, J., Jinneman, K., Stelma, G., Smith, B. G., Lye, D., Messer, J., Ulaszek, J., Evsen, L., Gendel, S., Bennett, R. W., Swaminathan, B., Pruckler, J., Steigerwalt, A., Kathariou, S., Yildirim, S., Volokhov, D., Rasooly, A., Chizhikov, V., Wiedmann, M., Fortes, E., Duvall, R. E. and Hitchins, A. D. (2004) 'Natural atypical *Listeria innocua* strains with *Listeria monocytogenes* pathogenicity island 1 genes', *Applied and Environmental Microbiology*, 70(7), pp. 4256–4266. doi: 10.1128/AEM.70.7.4256-4266.2004.

MHU (Ministry of Health of Ukraine). (2007) *On approval of the methodological guidelines 'Determination of the Sensitivity of Microorganisms to Antibacterial Drugs' [Pro zatverdzhennia metodychnykh vkazivok 'Vyznachennia chutlyvosti mikroorhanizmiv do antybakterialnykh preparativ'] (decree № 167, 05.04.2007)*. Available at: http://mozdocs.kiev.ua/view.php?id=6958. [in Ukrainian].

Perrin, M., Bemer, M. and Delamare, C. (2003) 'Fatal case of *Listeria innocua* bacteremia', *Journal of Clinical Microbiology*, 41(11), pp. 5308–5309. doi: 10.1128/JCM.41.11.5308-5309.2003.

Rapose, A., Lick, S. D. and Ismail, N. (2008) '*Listeria grayi* bacteremia in a heart transplant recipient', *Transplant Infectious Disease*, 10(6), pp. 434–436. doi: 10.1111/j.1399-3062.2008.00333.x.

Rocourt, J., Hof, H., Schrettenbrunner, A., Malinverni, R. and Bille, J. (1986) 'Acute purulent *Listeria seelingeri* meningitis in an immunocompetent adult' [Méningite purulente aiguë à *Listeria* seeligeri chez un adulte immunocompétent], *Schweizerische Medizinische Wochenschrift*, 116(8), pp. 248–251. PMID: 3082004. [in French].

Salimnia, H., Patel, D., Lephart, P. R., Fairfax, M. R. and Chandrasekar, P. H. (2010) '*Listeria grayi*: vancomycin-resistant, gram-positive rod causing bacteremia in a stem cell transplant recipient', *Transplant Infectious Disease*, 12(6), pp. 526–528. doi: 10.1111/j.1399-3062.2010.00539.x.

Volokhov, D. V., Duperrier, S., Neverov, A. A., George, J., Buchrieser, C. and Hitchins, A. D. (2007) 'The presence of the internalin gene in natural atypically hemolytic *Listeria innocua* strains suggests descent from *L. monocytogenes*', *Applied and Environmental Microbiology*, 73(6), pp. 1928–1939. doi: 10.1128/AEM.01796-06.

Zhang, Y., Yeh, E., Hall, G., Cripe, J., Bhagwat, A. A. and Meng, J. (2007) 'Characterization of *Listeria monocytogenes* isolated from retail foods', *International Journal of Food Microbiology*, 113(1), pp. 47–53. doi: 10.1016/j.ijfoodmicro.2006.07.010.

UDC 619:616.98-078:578.82/.83:57.083.33:636.5

ELISA DIAGNOSTIC OF METAPNEUMOVIRUS AND REOVIRUS INFECTIONS IN POULTRY

Ivleva O. V. [1], Nalyvaiko L. I. [1], Ryabinin S. V. [2]

[1] Luhansk National Agrarian University, Kharkiv, Ukraine, e-mail: sauce1908@gmail.com
[2] State Research Station of Poultry Industry of the National Academy of Agrarian Sciences of Ukraine, Kharkiv, Ukraine, e-mail: ryabinin.sv1979@gmail.com

Summary. The import of high-yielding poultry breeds has caused the proliferation of viral diseases which previously did not occur at Ukrainian poultry enterprises, such as avian metapneumovirus (AMPV) and avian reovirus (ARV) infections. Most pathogens cause immunosuppression in poultry (including ARV infection) and are frequently diagnosed as co-infections with other infectious agents which are capable of causing pathological changes in poultry (especially if aided by stress). AMPV and ARV co-infection cannot be excluded; these diseases are manifested in respiratory and intestinal disorders, their subclinical, latent, and associated forms present a challenge for laboratory diagnostic as well as for the implementation of prophylactic measures. The objective of the present work was to conduct the epizootological survey of AMPV and ARV infections incidence at poultry enterprises of different forms of property and to produce an antigen suitable for the development of an ELISA test-system for the determination of antibodies against AMPV in blood serums of turkeys and hens. The pathological material used in the experiments (heads, tracheas, and lungs from ill or deceased poultry) for the isolation of pneumovirus was collected from 60-day-old turkeys in 2009. The pathological material for virology research was preserved at – 20 °C. Serology and virology research was conducted using standard methods. AMPV and ARV incidence monitoring was conducted at poultry enterprises of Kharkiv region among turkeys of 'Big-6' and 'Big-8' breeds imported from Germany and Hungary (aged 4–480 days), among turkeys of 'Large White' breed (aged 70 days), and hens of 'Birkivska Barvysta' and 'Hisex Brown' (aged 250 and 180 days correspondingly). On average, 78.0% of the examined turkeys with antibody titers 1:3,776–1:27,869, 100% of the 'Birkivska Barvysta" hens with antibody titers 1:4,997–1:10,414 and 0% of the 'Hisex Brown' hens were AMPV-positive. No hens were ARV-positive. At the Luhansk National Agrarian University (Kharkiv) the development of a domestic ELISA test-system for the diagnostic of AMPV in turkeys and hens is carried out.

Keywords: epizootiology, metapneumovirus, reovirus, infection, poultry, purification and concentration, ELISA

Introduction. Some pathogens can cause diseases regardless of the environmental factors; however, the majority of diseases occur under specific conditions. Stress frequently plays the role of such a condition. The pathogens of the first group mentioned cause immunosuppression in poultry, and are frequently diagnosed as co-infections. Avian metapneumovirus (AMPV) and avian reovirus (ARV) co-infections cannot be excluded; these infections cause respiratory and gastric disorders.

AMPV infection is a respiratory disease manifested in nasal discharge, sneezing, labored breathing accompanied by rales, inflammation of the upper airways and infraorbital sinuses. This disease in turkeys is frequently called rhinotracheitis (TRT) and in broiler chickens — 'swollen head syndrome' (SHS). In recent literature, the disease of the upper airways of turkeys and hens is frequently called avian metapneumovirus (AMPV) (Borisova and Starov, 2006).

ARV infection is a highly contagious disease of young poultry and synanthropic birds of all breeds and species, which takes latent and persistent forms and is manifested by respiratory and intestinal disorders. For the first time in Ukraine ARV was registered and described by V. V. Herman in 1972. Immunological properties of more than 10 reovirus strains, isolated from different poultry species have been investigated (Abdil'aziz, 1990; German and German, 2002; Aliev, 2005).

For the first time the viral etiology or rhinotracheitis (TRT) in Ukraine was demonstrated in turkeys at poultry enterprises in Donetsk, Chernivtsi and Kharkiv regions in 2008. Clinical examination of 'Big-6' turkeys aged 30–70 days revealed the following symptoms in diseased individuals: inactivity, drowsiness, swelling of the head, intermaxillary space, and infraorbital sinuses, labored breathing, mucus outflow from the nasal passages. Mortality varied from 5% to 14%. Samples of pathological material were collected from the diseased poultry; the causative agent was identified using PCR and isolated using virology methods, it belongs to the genus *Metapneumovirus*, subtype APV/B (Nalyvaiko et al., 2011).

Latent and subclinical forms of infectious diseases, as well as their ability to cause co-infections present a challenge for laboratory diagnostic, implementation of prophylactic and anti-epizootic measures.

To control the immunity stress in immunized poultry against the infections in question certain laboratories of veterinary medicine used 'IDEXX' (USA) ELISA test-kits, which were registered in Ukraine and cost up to 8–10 thousand UAH. Domestically produced test-kits at that time were absent.

However, in 2012 at the Poultry Research Institute of the National Academy of Agrarian Sciences of Ukraine (PRI NAAS), using a domestic virus strain isolated from broiler chickens, an ELISA test system was developed (Riabinin, Nikolaenko and Nalyvaiko, 2012).

Considering the advanced nature of this method, the development of a domestic ELISA test system for the determination of antibodies against AMPV in hen and turkey blood serums is urgent.

The rapidity of the reaction, reproducibility of the results and their automated recording, a potential for standardization make ELISA the most effective, easy to use, and economically sound method for surveying the incidence of the diseases in question at poultry enterprises.

The aim of the work was to conduct an epizootological monitoring of AMPV and ARV infections at some poultry enterprises, to obtain an antigen for the development of an ELISA test system for the determination of antibodies against AMPV in turkey and hen blood serums.

Material and methods. The pathological material used in the experiments (heads, tracheas, and lungs from ill or deceased poultry) for the isolation of pneumovirus was collected from 60-day-old turkeys in 2009 at poultry enterprises in Chernivtsi region. The pathological material for virology research was preserved at – 20 °C.

Indication and identification of the causative agent in the pathologic material were conducted at the Laboratory of the diagnostic of poultry viral diseases FGBI 'ARRIAH' (Vladimir, Russia).

For the determination and quantification of antibodies against AMPV in blood serum 'IDEXX' test-kits (USA) were used. For the determination of ARV infection, a domestic ELISA test-kit developed by the PRI NAAS was used.

Incubatory eggs and chickens: intact hen and turkey embryos; chickens obtained from hens without antibodies against AMPV infection.

Cell cultures. A primary cell culture of chick embryo fibroblasts (CEF).

Nutrient mediums, serums, and solutions: MEM (Minimum Essential Medium) or DMEM (Dulbecco's Modified Eagle's Medium) liquid growth medium with L-glutamine; liquid growth medium No. 199 with L-glutamine; liquid growth medium DMEM/F12 with HEPES; cow embryos blood serum; 0.25% trypsin solution 'USBIO'; 0.02% Versen solution; Hank's solution 'Hyclone', produced by 'Biolot'.

Virology research. Reproduction and the main biological properties of the isolated pneumovirus were studies using quail, hen, and turkey embryos: changes in the embryos and in the chorioallantoic membrane were recorded. The infectious titer of the virus was determined according to Reed and Muench (1938) and expressed in lg $TCID_{50}$ in 1.0 cm^3 (tissue culture infectious dose).

Purification and concentration of the virus. The virus was concentrated using PC-6 and MSE centrifuges.

Results. In 2016–2017 epizootological monitoring of the incidence of AMPV and ARV was conducted at poultry enterprises of Kharkov region among turkeys of 'Big-6' and 'Big-8' breeds imported from Germany and Hungary (aged 4–480 days), among turkeys of 'Large White' breed aged 70 days, parent flocks of 'Birkivska Barvysta' and 'Hisex brown' hens aged 250 and 189 days respectively. 206 samples of hen and turkey blood serums from 5 poultry enterprises were examined using serologic assays. On average, 78.0% of the examined turkeys with antibody titers 1:3,776–1:27,869, 100% of the 'Birkivska barvysta' hens with antibody titers 1:4,997–1:10,414 and 0% of the 'Hisex Brown' hens were AMPV-positive (IDEXX). No hens were positive towards ARV PRI NAAS) (Table 1).

In order to create a domestic ELISA test system for the determination of antibodies against AMPV in hen and turkey blood serums, first, its main components were produced.

Table 1 — The results of the serology research conducted using ELISA (n = 206)

Poultry breed	Number of samples	Age, days	ELISA antibody titers (M ± m) towards:	
			AMPV	ARV
Hyperimmune chicken serum (control)	1	1	1:4,963	1:6,400
Negative serum (control)	1	1	0	0
Turkeys				
'Big-6' (Enterprise 1)	40	4	1:3,776–1:14,404 (1:7,505 ± 698)	—
		22–25	1:68–1:594 (1:363 ± 70)	—
'Big-8' (Enterprise 2)	17	25–30	1:78–1:740 (1:394 ± 69)	—
	20	480	1:4,865–1:27,869 (1:16,564 ± 1,599)	—
'Large White' (Enterprise 3)	80	70	1:0–1:9,547 (1:1,298 ± 192)	—
Hens				
'Birkivska Barvysta' (Enterprise 4)	24	250	1:4,997–1:10,414 (1:7,162 ± 633)	1:1,402 ± 83
'Hisex Brown' (Enterprise 5)	25	189	1:87–1:958 (1:453 ± 54)	1:127 ± 190

Production of the antigen. The accumulation of AMPV was accomplished by reproduction in CEF cell culture. The development of characteristic syncytium took place after 3 passages, in 48–72 h after inoculation. The infectious titer of PVT-09/B strain grown in CEF cell culture amounted to 4.33 lg $TCID_{50}/cm^3$.

Purification and concentration of the antigen. 1,200 ml of the obtained antigen were purified and concentrated using an original method: PVT-09/B AMPV strain seed of an established infectious titer was frozen to – 20 °C with subsequent thawing to + 4 °C twice.

1st step. Preliminary virus purification — the cellular debris was removed from the culture liquid by centrifugation at 1,000 g and + 4 °C, the supernatant was collected.

2nd step. Virus concentration — the purified virus seed was mixed with PEG 6000 (polyethyleneglycol) (6% by volume) and incubated at + 4 °C, and then centrifugated at 12,000 g and + 4 °C. The obtained sediments were resuspended in TSE buffer (Tris/Saline/EDTA buffer pH 7.6).

3rd step. Final virus purification — the samples were centrifugated through 20–30% sucrose solution at 76,000 g and + 4 °C. The obtained sediment was resuspended in TSE buffer (10 ml). The obtained antigen was used to produce hyperimmune chicken blood serums and to sensitize ELISA test plates.

Negative and positive serums production. Hyperimmunization of chickens and turkey with TRT antigen yielded positive chicken blood serums with antibody titers 1:3,200, turkey 1:6,400 (IDEXX).

Negative serums were obtained from healthy 30-day-old chickens grown in a sterile environment. The antibodies in the negative serums were absent.

Conclusions. According to the results of the immunosurvey, conducted using ELISA test systems, at poultry enterprises in eastern regions of Ukraine AMPV infection is prevalent among turkeys and hens of different breeds and age. Meanwhile, ARV infection, in its latent and persistent forms (not manifested in specific symptoms) is prevalent in domestic populations of hens, with antibody titers 1:1,402 ± 83. The most susceptible turkey breed towards AMPV is 'Big-6' (an imported breed), and the least — 'Large White' (36%). 100% of the examined 'Birkivska Barvysta' hens were virus carriers, with antibody titers 1:10,414 (as determined by ELISA). Technological parameters for the purification and concentration of AMPV antigen were developed; positive chicken blood serums with antibody titers 1:3,200, turkey 1:6,400 (as determined by ELISA) were obtained.

References

Abdil'aziz, F. (1990) 'Study of the biological properties of reoviruses isolated from chickens and turkeys' [Izuchenie biologicheskikh svoystv reovirusov, vydelennykh ot kur i indeek], *Veterinary Medicine [Veterinariya: respublikanskiy mezhvedomstvennyy tematicheskiy nauchnyy sbornik]*, 65, pp. 104–107. [in Russian].

Aliev, A. S. (2005) 'Avian reovirus infection' [Reovirusnaya infektsiya ptits], *Livestock Veterinary Medicine [Veterinariya sel'skokhozyaystvennykh zhivotnykh]*, 12, pp. 28–32. [in Russian].

Borisova, I. A. and Starov, S. K. (2006) 'Avian pneumovirus infection' [Pnevmovirusnaya infektsiya ptits]. *Proceedings of the Federal Centre for Animal Health [Trudy Federal'nogo tsentra okhrany zdorov'ya zhivotnykh]*, 4, pp. 281–296. Available at: https://elibrary.ru/item.asp?id=14454020. [in Russian].

German, I. V. and German, V. V. (2002) 'The study of the virulent properties of avireoviruses isolated from laying hens, broilers and goslings' [Vyvchennia virulentnykh vlastyvostei avireovirusiv, izolovanykh vid kurei-nesuchok, broileriv ta huseniat], *Veterinary Medicine of Ukraine [Veterynarna medytsyna Ukrainy]*, 7, pp. 37–38. [in Ukrainian].

Nalyvaiko, L. I., Bezrukava, I. Yu., Nikolaenko, Yu. Yu., Bondarenko, A. L. and Riabeka, D. A. (2011) 'Epizootiological monitoring of avian metapneumovirus infection in poultry farms in Ukraine' [Epizootolohichnyi monitorynh metapnevmovirusnoi infektsii u ptakhivnychykh hospodarstvakh Ukrainy], *Veterinary Medicine of Ukraine [Veterynarna medytsyna Ukrainy]*, 1, pp. 9–11. [in Ukrainian].

Reed, L. J. and Muench, H. (1938) 'A simple method of estimating fifty per cent endpoints', *American Journal of Hygiene*, 27(3), pp. 493–497. Available at: http://aje.oxfordjournals.org/content/27/3/493.full.pdf.

Riabinin, S. V., Nikolaenko, Yu. Yu. and Nalyvaiko, L. I. (2012) 'Tenosynovitis of hens: diagnostic and prevention' [Tenosynovit kurei: diahnostyka ta profilaktyka], *Veterinary Medicine of Ukraine [Veterynarna medytsyna Ukrainy]*, 10, pp. 17–20. [in Ukrainian].

Part 2. Biology and biotechnology

UDC 619:612.111:636.7:57.087:613.168

CORRECTION OF THE FUNCTIONAL STATE OF THE BLOOD OXYGENATION SYSTEM IN DOGS BY BIORESONANCE METHOD

Bobrytska O. M. [1], Karpovskyi V. I. [2], Yuhai K. D. [1], Vodopianova L. A. [1]

[1] Kharkiv State Zooveterinary Academy, Kharkiv, Ukraine, e-mail: olga.bobritskaya2410@gmail.com
[2] National University of Life and Environmental Sciences of Ukraine, Kyiv, Ukraine

Summary. The aim of the research was to justification of the peculiarities of the functioning and correction of the system of oxygenation of blood of dog organism by bioresonance method using the 'PARKES-L' device. The experiment was conducted on 20 dogs of different breeds aged 3 to 5 years and with a weight of 9–30 kg. For the experiment, four groups of animals (control and three experimental) were formed. Animals of the control and 1st experimental groups were characterized by physiological indicators of the oxygen-transport function of the blood. In animals of the 2nd and 3rd experimental groups, laboratory tests have shown a decrease in the abovementioned blood function. Physical therapy complex 'PARKES-L' was used to correct the functional state of the blood oxygenation system in dogs of the 1st and 3rd experimental groups, the working range of electromagnetic radiation frequencies ranging from 0.1 Hz to 30 Hz. Materials for research were blood samples of dogs obtained from the surface vein of the forearm, before correction, and two and five days after the beginning of the correction of the functional state of the oxygen-transport function of the blood. The effectiveness of the bioresonance method of correction of the functional state of the blood oxygenation system in dogs has been established. In particular, in dogs with a low functional state an increase in the number of erythrocytes, hemoglobin content and the average content of hemoglobin in erythrocyte as compared to the animals in the control group, which allows using this method to improve the system of oxygenation of blood in dogs.

Keywords: dogs, blood oxygenation system, bioresonance, 'PARKES-L'

Introduction. In modern biology, the human and animal organisms are considered as a complex self-regulating biological systems in which all organs and systems are closely interrelated and their activities are aimed at supporting physiological homeostasis.

Nervous and humoral regulation in the body are generally recognized and have a well-studied morphological basis, which cannot be said about the energy information system (EIS), which according to the latest data includes the following structures: energy shell, biologically active points (BAP), energy centers, in which energy is transformed and transmitted by meridians (channels) to cells, tissues, and organs (Pavlusenko, 2013; Sadykova, 2008).

Numerous studies have found that each cell, organ, system of organs, as well as the whole organism is a source of low-frequency electromagnetic radiation, parameters of which depend on the functional state of cells of organs and systems of the body (Trukhachev, 2009). In this case, physiologically normal organs and tissues generate electromagnetic radiation, differing in their parameters from cells, tissues and organs with a changed functional state (Bobritska, 2017; Kazeev, 2000).

Supply of tissues and organs depends on the degree of blood oxygenation. Oxygen provides a higher level of energy generation and energy use and it is an indicator of the intensity of metabolism. The system of oxygenation of blood depends on the content of red blood cells and hemoglobin in the body (Sadykova, 2008).

The purpose of the work was to justification of the peculiarities of the functioning and correction of the system of oxygenation of blood of dog organism by bioresonance method using the 'PARKES-L' device.

Materials and methods. The experiment was conducted in the conditions of the clinic 'Druzhochok' (Kharkiv, Ukraine) on 20 dogs of different breeds aged 3 to 5 years and with a weight of 9–30 kg. For the experiment, four groups of animals (control and three experimental) were formed. Animals of the control and 1st experimental groups were characterized by physiological indicators of the oxygen-transport function of the blood. In animals of the 2nd and 3rd experimental groups, laboratory tests have shown a decrease in the abovementioned blood function.

Physical therapy complex 'PARKES-L' was used to correct the functional state of the system of oxygenation of blood, the working range of electromagnetic radiation frequencies ranging from 0.1 Hz to 30 Hz. The effect of the device is achieved by radiation of electromagnetic pulses with infrared LEDs located on the rear and front sides of the device. This allows to apply the device by placing it on the body of dogs, as well as remotely, placing the device at

a distance not more than 50 cm from the body of animals. The 'PARKES-L' complex has a program for correction of the functional state of the blood oxygenation system for dogs.

For dogs of experimental groups I and III there was performed correction of the functional state of the oxygen transport system according to the following scheme: twice a day the program of correction of oxygen-transfer function of blood in the following automatic mode was used: 27 min — mode of operation, 10 min — interruption, 27 min — mode of operation, after which the device automatically switches off. The program was used twice a day for 5 days in animals of the experimental groups I and III.

Materials for research were blood samples of dogs obtained from the surface vein of the forearm, before correction, and two and five days after the beginning of the correction of the functional state of the oxygen-transport function of the blood.

In whole blood there was performed: counting the number of red blood cells on a grid of Goryaev counting chamber; the hemoglobin content was determined by the cyanmethemoglobin method; hematocrit value — by centrifugation.

The data obtained in the experiments were processed statistically, determining the arithmetic mean (M), the mean square error (m) and the probability of the differences (p) between the investigated parameters. The probability of differences in average values was established according to the Student's *t*-test.

In addition, one-factor dispersion analysis was performed to determine the degree and probability of exposure.

Results. The conducted researches showed that in dogs of the control group the amount of erythrocytes and hemoglobin content in the blood were 6.64 ± 0.13 T/l and 152.2 ± 3.6 g/l respectively, which determines the adequate supply of tissues and cells of the animal body with oxygen.

It should be noted that throughout the study period, the number of erythrocytes, hemoglobin content, and hematocrit index of the dog blood in the control group does not significantly change and fluctuates within 0.2–2.6% (Table 1).

Table 1 — Indicators of blood of dogs with the correction of blood oxygenation system (M ± m, n = 10)

Indicators	Animal groups			
	Control	Experimental		
		I	II	III
Before correction				
Erythrocytes, T/l	6.64 ± 0.13	6.5 ± 0.16	4.76 ± 0.07***	4.80 ± 0.15***
Hemoglobin, g/l	152.2 ± 3.6	151.3 ± 4.1	103.0 ± 1.4***	103.4 ± 2.8***
Hematocrit, l/l	48.8 ± 0.7	46.0 ± 1.7	33.4 ± 2.0***	34.6 ± 0.9***
In 2 days				
Erythrocytes, T/l	6.47 ± 0.15	6.63 ± 0.21	4.85 ± 0.06***	4.94 ± 0.14***
Hemoglobin, g/l	151.7 ± 3.9	156.4 ± 5.8	101.6 ± 1.5***	112.0 ± 4.3***
Hematocrit, l/l	48.7 ± 0.6	48.2 ± 1.9	33.2 ± 2.0***	36.3 ± 1.1***
In 5 days				
Erythrocytes, T/l	6.51 ± 0.13	6.69 ± 0.15	4.8 ± 0.1***	6.09 ± 0.18
Hemoglobin, g/l	149.9 ± 3.3	166.7 ± 7.2***	101.8 ± 2.2***	137.7 ± 6.5
Hematocrit, l/l	47.6 ± 0.8	50.0 ± 1.7	34.2 ± 1.0***	44.7 ± 1.8

Note. Reliable difference from the control group: * — $p < 0.05$; ** — $p < 0.01$; *** — $p < 0.001$.

Indicators of dog blood in the experimental group I before the correction of the functional state of the oxygenation system did not differ significantly from those in the control animals. Two days after the start of bioresonance correction in the blood of dogs in the experimental group I, the number of erythrocytes, hemoglobin content and hematocrit index showed only a tendency to increase (within the range of 2.0–4.8%), at that these indicators are not significantly different from those in animals of the control group at this stage of research. From the second to the fifth day after the beginning of the correction of the blood oxygenation system in animals in the experimental group I, the hemoglobin content increased by 6.6%, the hematocrit index — by 3.7%, and the number of erythrocytes — only 0.9% (although within the trend).

The low functional state of the blood oxygenation system in dogs (experimental group II) is characterized by a smaller number of erythrocytes in the blood, by 25–28% ($p < 0.001$), hemoglobin content — by 32–33% ($p < 0.001$) and a hematocrit index — by 28–32% ($p < 0.001$) compared with the control group dogs for the entire period of the research.

Before the correction of the functional state of the system of blood oxygenation in dogs of the third experimental group, the number of erythrocytes,

hemoglobin content, and hematocrit index were not significantly different from those in animals of the second experimental group.

Two days after the start of bioresonance correction in animals of the third experimental group, the number of erythrocytes, hemoglobin content, and hematocrit index increased by 2.9%, 8.3% ($p < 0.05$) and 4.9% respectively. Therefore, the hemoglobin content in blood of these dogs increases by 10.2% ($p < 0.05$), and the hematocrit index by 9.3% ($p < 0.05$) compared with the animals of the experimental group II, although it is less by 25.5–26.2% ($p < 0.001$) compared with the control animals. From the 2nd to the 5th day after the beginning of the correction of the functional state of the system of oxygenation of dog blood by the bioresonance method in animals of the experimental group III, the number of erythrocytes increased by 23.3% ($p < 0.001$), the hemoglobin content — by 22.9% ($p < 0.001$), and the hematocrit index — by 23.1% ($p < 0.001$).

Moreover, these indicators are no longer different from the indexes of dogs of the control group (although they are lower within the trend by 6.1–8.1%). It should be noted that in 5 days after the beginning of the correction of the functional state of the oxygen transport system, the number of erythrocytes in the blood of dogs of the 3rd experimental group was higher by 26.9% ($p < 0.001$), the hemoglobin content — by 35.3% ($p < 0.001$), and the hematocrit index — by 30.7% ($p < 0.001$) compared with the values in animals of the second experimental group at this stage of the research.

Low frequency electromagnetic radiation under the correction of the functional state of the blood oxygenation system in dogs of the experimental group I has a significant effect on the hemoglobin content in the blood of dogs only in 5 days after the start of correction — $\eta^2_x = 0.41$ ($p < 0.05$).

While as for the number of erythrocytes and the hematocrit index in the blood of these dogs, the reliable influence of electromagnetic radiation during the entire period of research has not been established ($\eta^2_x = 0.00$–0.07).

In animals with a low functional state of the blood oxygen transport system (experimental group III), the bioresonance method of correction is significantly more effective than in animals in of the experimental group I. Thus, just in two days after the beginning of the correction, the reliable influence of electromagnetic radiation on the hemoglobin content in the blood of dogs was established — $\eta^2_x = 0.45$ ($p < 0.05$). Five days after the start of the correction, the effect on the hemoglobin content only increases ($\eta^2_x = 0.81$; $p < 0.001$) and there is a significant effect of ultra low frequency electromagnetic radiation on the number of erythrocytes ($\eta^2_x = 0.86$; $p < 0.001$) and the hematocrit index ($\eta^2_x = 0.80$; $p < 0.001$) in the blood of dogs of the third experimental group (Fig. 1).

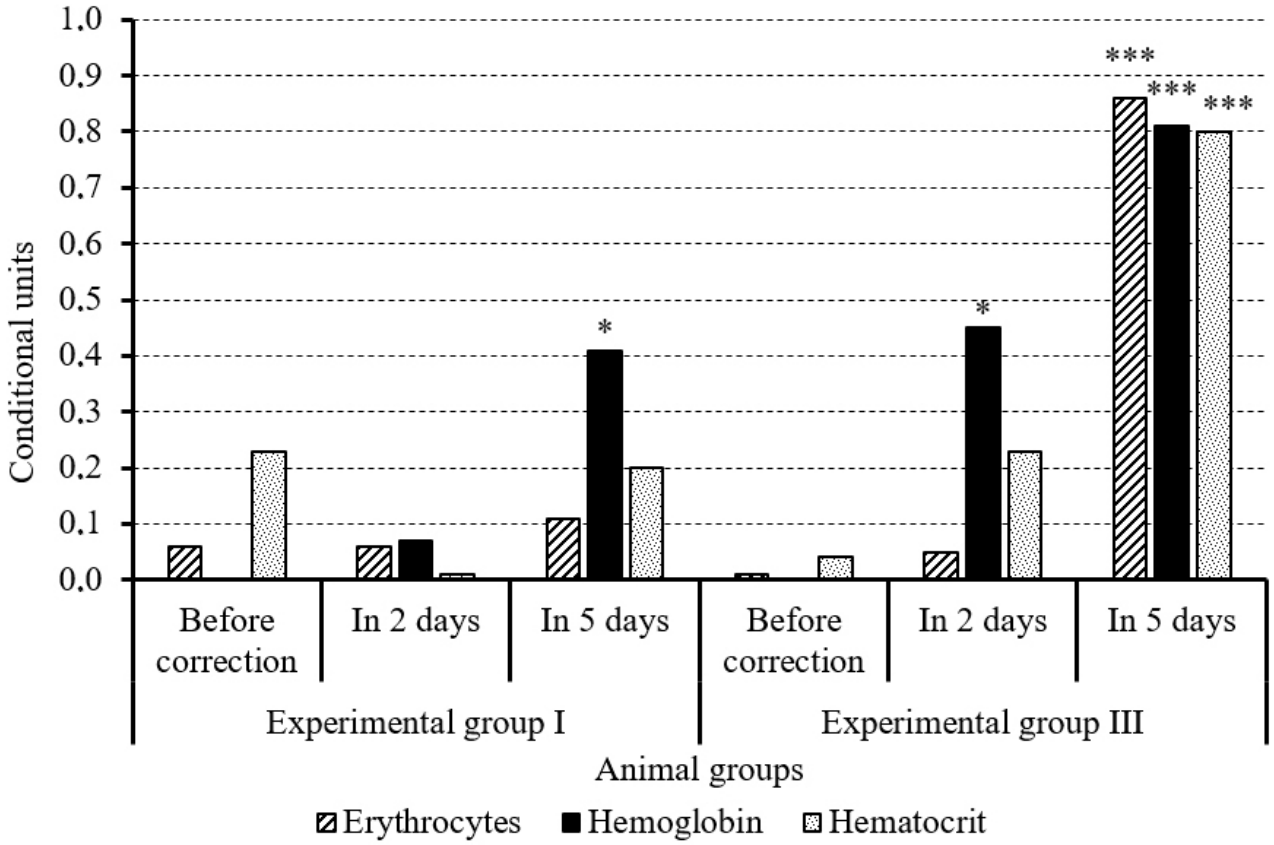


Figure 1. Influence of low-frequency electromagnetic radiation on blood indexes of dogs (η^2_x; n = 10; indicators are reliable at: * — $p < 0.05$; ** — $p < 0.01$; *** — $p < 0.001$)

Before the correction of the functional state of the blood oxygenation system in dogs of the 3rd experimental group, the number of indices of red blood did not significantly differ from those of animals of the 2nd experimental group. Two days after the start of bioresonance correction in animals of the 3rd group, the average content and average concentration of hemoglobin in the erythrocyte increased by 2.0–3.2%, the average amount of erythrocytes — by 5.4% ($p < 0.05$) and the value of color index — by 5.8% ($p < 0.05$).

Due to this increase, the average amount of erythrocytes in these dogs' blood is increased by 7.6%, and the average content of hemoglobin in erythrocyte is more by 8.2–8.9% ($p < 0.01$–0.001) compared with the indices of animals of the experimental group II.

From the 2nd to the 5th day after the beginning of the correction of the functional state of the respiratory function of the blood of dogs by the bioresonance method, in animals of the experimental group III, the erythrocytic indices of the dog blood do not significantly change and do not significantly differ from the animals in the control group.

It should be noted that in 5 days after the beginning of the correction of the functional state of the oxygen transport system, the average hemoglobin content in the erythrocyte of the dogs of the experimental group III was 6.6% higher ($p < 0.05$), and the value of the color index was 5.8% higher than indicators of animals of the experimental group II at this stage of research.

In animals with a low functional state of the system of blood oxygenation (III experimental group), the bioresonance method of correction is accompanied by the formation after two days of reliable influence of electromagnetic radiation on the average content of hemoglobin in erythrocyte — $\eta^2_x = 0.82$ ($p < 0.001$).

Conclusions. Thus, the effectiveness of the bioresonance method of correction of the functional state of the blood oxygenation system in dogs has been established. In particular, in dogs with a low functional state an increase in the number of erythrocytes, hemoglobin content and the average content of hemoglobin in erythrocyte as compared to the animals in the control group, which allows using this method to improve the system of oxygenation of blood in dogs.

References

Bobritska, O. M. (2017) 'The physiological basis for the use of the bioresonance method for the determination and correction of the functional state of organs and systems of the body' [Fiziolohichne obgruntuvannia vykorystannia biorezonansnoho metodu dlia vyznachennia ta korektsii funktsionalnoho stanu orhaniv ta system], *Problems of Zooengineering and Veterinary Medicine [Problemy zooinzhenerii ta veterynarnoi medytsyny]*, 34(2), pp. 19–23. Available at: http://nbuv.gov.ua/UJRN/pzvm_2017_34(2)__4. [in Ukrainian].

Kazeev, G. V. (2000) *Veterinary acupuncture [Veterinarnaya akupunktura]*. Moscow: RIO RGAZU. ISBN: 5901240014. [in Russian].

Pavlusenko, I. I. (2013) Physiotherapy equipment Parkes-L [Fizioterapevticheskaya apparatura Parkes-L], *Modern methods of bioresonance diagnostics and electromagnetic therapy: proceedings of the scientific and practical conference with international participation (Kyiv, 6–7 April, 2013) [Suchasni metody biorezonansnoi diahnostyky ta elektromahnitna terapiia: materialy naukovo-praktychnoi konferentsii z mizhnarodnoiu uchastiu (Kyiv, 6–7 kvitnia 2013 r.)]*. Kyiv, pp. 71–73. Available at: http://docplayer.ru/43832256-Suchasni-metodi-biorezonansnoyi-diagnostiki-ta-elektromagnitna-terapiya.html. [in Russian].

Sadykova, Yu. R. (2008) *Morphofunctional state of the blood and urinary system of dogs of service breeds, depending on the conditions of welfare and usage [Morfofunktsional'noe sostoyanie krovi i mochevydelitel'noy sistemy sobak sluzhebnykh porod v zavisimosti ot usloviy soderzhaniya i ekspluatatsii]*. The dissertation thesis for the scientific degree of the candidate of biological sciences. Kazan: Bauman Kazan State Academy of Veterinary Medicine. [in Russian].

Trukhachev, G. Yu. (2009) *The use of electromagnetic radiation of extremely high millimeter-wave frequency for the correction of placental insufficiency in dogs [Primenenie elektromagnitnogo izlucheniya krayne vysokoy chastoty millimetrovogo diapazona dlya korrektsii fetoplatsentarnoy nedostatochnosti u sobak]*. The dissertation thesis for the scientific degree of the candidate of veterinary sciences. Saratov: Vavilov Saratov State Agrarian University. [in Russian].

Part 3. Biosafety

UDC 619:579:615.331

STUDY OF THE EFFECT OF THE PREPARATION BASED ON THE BACTERIOCIN NISIN ON PATHOGENIC BACTERIA

Kucheruk M. D., Zasiekin D. A., Vygovska L. M., Ushkalov V. O.

National University of Life and Environmental Sciences of Ukraine, Kyiv, Ukraine, e-mail: lnvygovska@gmail.com

Summary. The bactericidal effect of the nisin-based preparation on the bacterial cultures (*Escherichia coli* ATCC 25922 (F 50), *Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* ATCC 25923, *Listeria ivanovii* ATCC 19119, *Yersinia enterocolitica* ATCC 23715) was studied *in vitro*. Two postbiotic concentrations were tested. Based on *in vitro* studies, the expressed antimicrobial effect of the test drug on *Escherichia coli* ATCC 25922 (F 50), *Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* ATCC 25923 was established. In addition, postbiotic variant 1 was active in relation to *Listeria ivanovii* ATCC 19119, variant 2 — in relation to *Yersinia enterocolitica* ATCC 23715. The obtained results point to the prospect of further study of the postbiotics effect *in vivo* for the purpose of their application in the treatment of infections caused by pathogenic bacteria with acquired resistance to antibiotics.

Keywords: postbiotics, drug, strains, *in vitro*, sensitivity

Introduction. Postbiotics are bacterial metabolites and breakdown products of probiotic bacteria (mostly gram-positive). Postbiotics include organic acids and biologically active substances — bacteriocins, which effectively counteract pathogenic microorganisms, even in acidic media. The range of antimicrobial postbiotics is ambiguous. It can be both as very narrow as extremely wide. Most of them are produced by gram-positive bacteria (Sorg et al., 2016; Abee, 1995; Collier-Hyams and Neish, 2005; Morowitz et al., 2010; Mack and Lebel, 2004).

Bacteriocin nisin has a broader antimicrobial spectrum than most other bacteriocins. It is non-toxic, and used in the food industry as a natural preservative (E234). Researchers believe that postbiotics affect the inner wall of the colon, but the mechanisms of health influence are not sufficiently clear yet (Neish et al., 2000; Abee, Krockel and Hill, 1995; Aguilar-Toalá et al., 2018).

However, postbiotics are like acid oxidants, because the effective digestion of nutrients requires adequate acidity in the stomach. It has been proved that acidic media (with low pH values) cannot withstand most pathogenic bacteria, but it is favorable for growth and reproduction of lactic acid bacteria. Pepsinogen is converted into a proteolytic enzyme pepsin only in a very acidic medium (pH 2–4). Consequently, when the high-nutritious diet ingested in the stomach, the splitting of proteins is disturbed, which contributes to protein fermentation with the formation of toxic biogenic amines (Amalaradjou and Bhunia, 2013; Amaretti et al., 2013; Anvari, Khayati and Rostami, 2014; Kucheruk and Zasiekin, 2013).

The aim of the study was to determine the bactericidal effect of the nisin-based preparation on the bacterial cultures (*Escherichia coli* ATCC 25922 (F 50), *Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* ATCC 25923, *Listeria ivanovii* ATCC 19119, *Yersinia enterocolitica* ATCC 23715) *in vitro*.

Materials and methods. Tests of the postbiotic drugs were carried out by the of the drug diffusion in agar solutions in accordance with the methodological recommendations (ISO, 2014; Holovko et al., 2013; MHU, 2017).

Two postbiotic concentrations were tested:

Variant 1 — postbiotic formulation (0.05 g of nisin, 10 ml of 40% lactic acid, 89.95 ml of distilled water);

Variant 2 — postbiotic formulation (0.10 g of nisin, 10 ml 40% lactic acid, 89.90 ml distilled water).

Reference strains of microorganisms were used as test cultures: *Escherichia coli* ATCC 25922 (F 50), *Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* ATCC 25923, *Listeria ivanovii* ATCC 19119, *Yersinia enterocolitica* ATCC 23715.

Results. The effectiveness of the two drug variants (with different concentrations of the active substance) was tested in relation to the microorganisms of different groups: gram-negative sticks, representatives of the family Enterobacteriaceae — *Escherichia coli* ATCC 25922 (F 50) (in concentrations 4.3×10^9 CFU/cm^3, 4.3×10^8 CFU/cm^3, 4.3×10^7 CFU/cm^3), *Yersinia enterocolitica* ATCC 23715 (in concentration 9.0×10^8 CFU/cm^3), gram-positive bacillus asporogenous — *Listeria ivanovii* ATCC 19119 (in concentration 5.0×10^8 CFU/cm^3), gram-positive cocci — *Staphylococcus aureus* ATCC 25923 (in concentrations 5.5×10^9 CFU/cm^3, 5.5×10^8 CFU/cm^3, 5.5×10^7 CFU/cm^3), gram-positive spore-forming bacillus — *Bacillus subtilis*

ATCC 6633 (in concentrations 3.5×10^9 CFU/cm^3, 3.5×10^8 CFU/cm^3, 3.5×10^7 CFU/cm^3).

The efficacy of the drug test variants was determined in the native state and in successive ten-time dilutions from 1:100 to 1:1,000,000 (Tables 1 and 2).

The results showed the effectiveness of the drug variant 1 native solution in relation to the test cultures; the dilution of the inhibitory agent in relation to the test cultures was not observed (Table 1).

There was a slight dependence of the *Escherichia coli* ATCC 25922 (F 50) test culture concentration on the efficacy of the test drug: for the ten times test culture concentration gradual reduction, the growth inhibition diameters of the crops increased around the application of the drug. Diameters of growth inhibition of *Escherichia coli* ATCC 25922 (F 50) test culture in concentration 4.3×10^9 CFU/cm^3 around the drug variant 1 application area constituted 8 mm; for 4.3×10^8 CFU/cm^3 — 9–15 mm; for 4.3×10^7 CFU/cm^3 — 15 mm. In the test cultures *Bacillus subtilis* ATCC 6633 and *Staphylococcus aureus* ATCC 25923 the correlation of the test culture concentration on the drug effect was not observed. The diameters of the inhibition of the *Bacillus subtilis* ATCC 6633 test culture growth for concentrations 3.5×10^9 CFU/cm^3, 3.5×10^8 CFU/cm^3, 3.5×10^7 CFU/cm^3 around the zones of the application of the drug variant 1 was 15 mm. The diameters of the inhibition of *Staphylococcus aureus* ATCC 25923 test culture growth for concentrations 5.5×10^9 CFU/cm^3, 5.5×10^8 CFU/cm^3, 5.5×10^7 CFU/cm^3 around the application area of the drug variant 1 was 18 mm. The *Listeria ivanovii* ATCC 19119 culture was tested in concentration 5.0×10^8 CFU/cm^3, zones of drug oppression were 36 mm; the *Yersinia enterocolitica* ATCC 23715 culture tested in the concentration 9.0×10^8 CFU/cm^3, the zones of inhibition were 13 mm.

Table 1 — Test of the variant 1 (0.05 g of nisin, 10 ml of 40% lactic acid, 89.95 ml of distilled water) postbiotic drug effectiveness

Test cultures	Concentration, CFU/cm^3	Diameters of the culture growth inhibition (mm) according to dilutions					
		native	1:100	1:1,000	1:10,000	1:100,000	1:1,000,000
Escherichia coli ATCC 25922 (F 50)	4.3×10^9	8	—	—	—	—	—
	4.3×10^8	9–15	—	—	—	—	—
	4.3×10^7	15	—	—	—	—	—
Bacillus subtilis ATCC 6633	3.5×10^9	15	—	—	—	—	—
	3.5×10^8	15	—	—	—	—	—
	3.5×10^7	15	—	—	—	—	—
Staphylococcus aureus ATCC 25923	5.5×10^9	18	—	—	—	—	—
	5.5×10^8	18	—	—	—	—	—
	5.5×10^7	18	—	—	—	—	—
Listeria ivanovii ATCC 19119	5.0×10^8	36	—	—	—	—	—
Yersinia enterocolitica ATCC 23715	9.0×10^8	13	—	—	—	—	—

For the variant 2 application of the drug in relation to the test cultures, similar results were recorded: the native sample of the drug was effective, its ten-times dilution of inhibitory activity in relation to the test cultures was not shown (Table 2). In addition, there was a certain correlation between the concentration of test cultures *Escherichia coli* ATCC 25922 (F 50), *Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* ATCC 25923 and the efficacy of the test sample preparation: for a gradual reduction of the ten-times concentration of the cultures, the growth inhibition diameters of the cultures around the application of the drug increased. Diameters of the growth inhibition of test culture *Escherichia coli* ATCC 25922 (F 50) for the concentration 4.3×10^9 CFU/cm^3 in the area of application of the drug variant 2 were < 5 mm; for 4.3×10^8 CFU/cm^3 — 15 mm; for 4.3×10^7 CFU/cm^3 — > 15 mm. The diameters of inhibition of growth of test culture *Bacillus subtilis* ATCC 6633 for the concentration 3.5×10^9 CFU/cm^3 in the area of application of the drug variant 2 were 13 mm; for 3.5×10^8 CFU/cm^3 — 14–20 mm; for 3.5×10^7 CFU/cm^3 — 20 mm. The diameters of the growth inhibition of test culture *Staphylococcus aureus* ATCC 25923 for the concentration 5.5×10^9 CFU/cm^3 in the area of application of the drug variant 2 were 12 mm; for 5.5×10^8 CFU/cm^3 — 15 mm; for 5.5×10^7 CFU/cm^3 — 17 mm. The *Listeria ivanovii* ATCC 19119 culture was tested in concentration 5.0×10^8 CFU/cm^3, zones of drug oppression were 13 mm; the *Yersinia enterocolitica* ATCC 23715 culture tested in the concentration 9.0×10^8 CFU/cm^3, the zones of inhibition were 22 mm.

Table 2 — Test of the variant 2 (0.10 g of nisin, 10 ml of 40% lactic acid, 89.90 ml of distilled water) postbiotic drug effectiveness

Test cultures	Concentration, CFU/cm^3	Diameters of the culture growth inhibition (mm) according to dilutions					
		native	1:100	1:1,000	1:10,000	1:100,000	1:1,000,000
Escherichia coli ATCC 25922 (F 50)	4.3×10^9	< 5	—	—	—	—	—
	4.3×10^8	15	—	—	—	—	—
	4.3×10^7	> 15	—	—	—	—	—
Bacillus subtilis ATCC 6633	3.5×10^9	13	—	—	—	—	—
	3.5×10^8	14–20	—	—	—	—	—
	3.5×10^7	20	—	—	—	—	—
Staphylococcus aureus ATCC 25923	5.5×10^9	12	—	—	—	—	—
	5.5×10^8	15	—	—	—	—	—
	5.5×10^7	17	—	—	—	—	—
Listeria ivanovii ATCC 19119	5.0×10^8	13	—	—	—	—	—
Yersinia enterocolitica ATCC 23715	9.0×10^8	22	—	—	—	—	—

Comparing the effect of variants 1 and 2 of the test drug, it should be noted that in relation to the test cultures *Escherichia coli* ATCC 25922 (F 50), *Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* ATCC 25923 the effectiveness of the options manifested itself at approximately the same level.

In relation to the test culture *Listeria ivanovii* ATCC 19119 variant 1 of the drug was much more effective; in relation to the test culture *Yersinia enterocolitica* ATCC 23715 — variant 2.

Conclusions. 1. It was established that the tested variants 1 and 2 of postbiotics containing 0.05 g and 0.10 g of nisin respectively exhibited pronounced inhibitory effect *in vitro* on the growth of test cultures *Escherichia coli* ATCC 25922 (F 50), *Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* ATCC 25923.

2. Postbiotic variant 1 was active in relation to *Listeria ivanovii* ATCC 19119, variant 2 — in relation to *Yersinia enterocolitica* ATCC 23715.

3. The obtained results point to the prospect of further study of the postbiotics effect *in vivo* for the purpose of their application in the treatment of infections caused by pathogenic bacteria with acquired resistance to antibiotics.

References

Abee, T. (1995) 'Pore-forming bacteriocins of gram-positive bacteria and self-protection mechanisms of producer organisms', *FEMS Microbiology Letters*, 129(1), pp. 1–9. doi: 10.1016/0378-1097(95)00137-T.

Abee, T., Krockel, L. and Hill, C. (1995) 'Bacteriocins: modes of action and potentials in food preservation and control of food poisoning', *International Journal of Food Microbiology*, 28(2), pp. 169–185. doi: 10.1016/0168-1605(95)00055-0.

Aguilar-Toalá, J. E., Garcia-Varela, R., Garcia, H. S., Mata-Haro, V., González-Córdova, A. F., Vallejo-Cordoba, B. and Hernández-Mendoza, A. (2018) 'Postbiotics: An evolving term within the functional foods field', *Trends in Food Science and Technology*, 75, pp. 105–114. doi: 10.1016/j.tifs.2018.03.009.

Amalaradjou, M. A. R. and Bhunia, A. K. (2013) 'Bioengineered probiotics, a strategic approach to control enteric infections', *Bioengineered*, 4(6), pp. 379–387. doi: 10.4161/bioe.23574.

Amaretti, A., di Nunzio, M., Pompei, A., Raimondi, S., Rossi, M. and Bordoni, A. (2013) 'Antioxidant properties of potentially probiotic bacteria: in vitro and in vivo activities', *Applied Microbiology and Biotechnology*, 97(2), pp. 809–817. doi: 10.1007/s00253-012-4241-7.

Anvari, M., Khayati, G. and Rostami, S. (2014) 'Optimisation of medium composition for probiotic biomass production using response surface methodology', *Journal of Dairy Research*, 81(1), pp. 59–64. doi: 10.1017/S0022029913000733.

Collier-Hyams, L. S. and Neish, A. S. (2005) 'Intestinal epithelial barrier and mucosal immunity: Innate immune relationship between commensal flora and the mammalian intestinal epithelium', *Cellular and Molecular Life Sciences*, 62(12), pp. 1339–1348. doi: 10.1007/s00018-005-5038-y.

Holovko, A. M., Ushkalov, V. O., Pinchuk, N. H., Kyselova, T. F. and Dmytriieva, H. V. (2013) *Rules for working with reference test strains of microorganisms designed to determine the activity and residual amount of antimicrobial drugs in raw materials and animal products: methodological recommendations [Pravyla roboty z etalonnymy test-shtamamy mikroorhanizmiv, pryznachennymy dlia vyznachennia aktyvnosti ta zalyshkovoi kilkosti protymikrobnykh preparativ v syrovyni ta produktsii tvarynnoho pokhodzhennia: metodychni rekomendatsii]*. Kyiv: State Veterinary and Phytosanitary Service of Ukraine; State Scientific Control Institute of Biotechnology and Strains of Microorganisms. [in Ukrainian].

ISO (International Organization for Standardization). (2014) *ISO 11133:2014: Microbiology of Food, Animal Feed and Water — Preparation, Production, Storage and Performance Testing of Culture Media*. Geneva: ISO. Available at: https://www.iso.org/standard/53610.html.

Kucheruk, M. D. and Zasiekin, D. A. (2013) *Microendoecology of the intestines of animals. Nutraceutics [Mikroendoekolohiia kyshechnyka tvaryn. Nutrytsevtyky]*. Kyiv: Interservice. ISBN9789662465773. [in Ukrainian].

Mack, D. R. and Lebel, S. (2004) 'Role of probiotics in the modulation of intestinal infections and inflammation', *Current Opinion in Gastroenterology*, 20(1), pp. 22–26. doi: 10.1097/0000 1574-200401000-00006.

MHU (Ministry of Health of Ukraine). (2007) *On approval of the methodological guidelines 'Determination of the Sensitivity of Microorganisms to Antibacterial Drugs' [Pro zatverdzhennia metodychnykh vkazivok 'Vyznachennia chutlyvosti mikroorhanizmiv do antybakterialnykh preparativ'] (decree № 167, 05.04.2007)*. Available at: http://mozdocs.kiev.ua/view.php?id=6958. [in Ukrainian].

Morowitz, M. J., Poroyko, V., Caplan, M., Alverdy, J. and Liu, D. C. (2010) 'Redefining the role of intestinal microbes in the pathogenesis of necrotizing enterocolitis', *Pediatrics*, 125(4), pp. 777–785. doi: 10.1542/peds.2009-3149.

Neish, A. S., Gewirtz, A. T., Zeng, H., Young, A. N., Hobert, M. E., Karmali, V., Rao, A. S. and Madara, J. L. (2000) 'Prokaryotic regulation of epithelial responses by inhibition of IkappaB-alpha ubiquitination', *Science*, 289(5484), pp. 1560–1563. doi: 10.1126/science.289.5484.1560.

Sorg, R. A., Lin, L., van Doorn, G. S., Sorg, M., Olson, J., Nizet, V. and Veening, J.-W. (2016) 'Collective resistance in microbial communities by intracellular antibiotic deactivation', *PLoS Biology*, 14(12), p. e2000631. doi: 10.1371/journal.pbio. 2000631.

UDC 619:616-036.22:[578.832.1A+578.831.11]:598.2(477.6/.7)

VIROLOGICAL MONITORING OF ESPECIALLY DANGEROUS AVIAN PATHOGENS IN SOUTHERN UKRAINE IN 2017 DURING AFTER-EPIZOOTIC PERIOD

Pishchanskyi O. V. [2], Stegniy B. T. [1], Tkachenko S. V. [1], Rula O. M. [1], Muzyka D. V. [1]

[1] National Scientific Center 'Institute of Experimental and Clinical Veterinary Medicine', Kharkiv, Ukraine, e-mail: dmuzyka77@gmail.com
[2] State Scientific and Research Institute of Laboratory Diagnostics and Veterinary and Sanitary Expertise, Kyiv, Ukraine

Summary. Constant monitoring of the circulation of particularly dangerous avian diseases' causative agents (influenza and Newcastle disease) in natural reservoir is an important component of global surveillance and control of these diseases. The main objective of the research is to control the circulation of influenza viruses, Newcastle disease and other avulaviruses in poultry in Ukraine, isolation of influenza virus isolates from H5 and H7 subtypes for further study. Classical virological methods of viruses' isolation using chicken embryos, serological tests using referent blood sera as well as identification of viruses via real-time PCR were used for research. Ten influenza viruses of different subtypes (including one influenza virus of H5 subtype and two influenza viruses of H7 subtype), as well as various avulaviruses of different serotypes (including AvV-1 — Newcastle disease virus) were isolated from 1,619 wild waterfowl and water-related birds of 28 species during autumn migration in 2017 in Kherson, Odesa, and Zaporizhia regions. All the viruses are isolated from wild ducks, geese and gulls. Hemagglutinating viruses from wild birds of such ecological groups as waders, herons and land birds have not been isolated. The level of infection of wild waterfowl (wild ducks) during the autumn migration in 2017 was 1.44–1.58%, and waterfowl (gulls) — 0.42%. The data we obtained is very important for predicting the epizootic situation in Ukraine, understanding the ecological features of low-pathogenic variants of viruses, which cause particularly dangerous avian viral diseases. In addition, conducting regular virological monitoring in a natural reservoir allows us to receive new strains for improvement of laboratory diagnostics.

Keywords: H5 and H7 subtypes of avian influenza, Newcastle disease, wild birds, surveillance, Ukraine

Introduction. Avian influenza (AI) and Newcastle disease (ND) are two major diseases that are particularly dangerous for birds. They have a huge impact on the poultry industry and might lead to huge economic losses: the extinction of poultry, the imposition of quarantine and other restrictive measures, the ban on trade, additional veterinary and sanitary measures). In addition, avian influenza is one of the few infections, which poses a serious threat to humans. Influenza occupies a special place as a pathogen which is dangerous to human health and which may cause a serious pandemic. Newcastle disease does not have such an epidemic potential and does not pose a serious threat to human health (Saif, 2008).

Influenza viruses belong to the Orthomyxoviridae family (Capua and Alexander, 2009; Swayne, 2016). Wild waterfowl and water-related birds are the main natural reservoir of all influenza viruses and play a major role in the circulation of this pathogen (Swayne, 2016). With regard to Newcastle disease, this pathogen belongs to avian avulaviruses (Knipe and Howley, 2013). The main role in the natural circulation of this pathogen also belongs to wild waterfowl and water-related birds (Camenisch, Bandli and Hoop, 2008; Saif, 2008).

Due to the extraordinary importance of these two diseases, the need to control the circulation of pathogens in natural reservoir worldwide, birds are constantly monitored. These activities have been carried out in Ukraine since 2005. Research results obtained at NSC 'IECVM' have proven a widespread natural circulation of influenza virus and avulaviruses (the former name — avian paramyxoviruses) among wild birds in Ukraine (Muzyka et al., 2012, 2016a, 2016b). Ninety-five AI viruses, belonging to 15 out of 16 known HA subtypes and up to 7 out of 9 known NA subtypes, have been isolated by the beginning of 2017. However, monitoring studies should be conducted on an ongoing basis, enabling valuable information on new variants of the virus and carry out preparatory work for the next outbreaks, improve laboratory diagnostics.

Therefore, the **main objective of our research** in 2017 was to conduct virological monitoring of wild birds in places of their mass accumulation in the southern region of Ukraine.

Materials and methods. Wild bird surveillance was conducted during the autumn migration 2017 in south regions of Ukraine (Kherson, Zaporizhia, and Odesa regions). In 2017 samples of biological material (fecal samples) were collected from 1,619 wild birds of 28 species of wild waterfowl birds, shore birds and some birds of other ecological groups and environmental samples (Table 1).

Virus isolation. Virus isolation was conducted in accordance with the OIE procedures (OIE, 2012). Fecal samples were inoculated into the allantoic cavity of 9–10-day-old chicken embryonated eggs. Every sample was passaged three times.

Table 1 — Number of samples isolated from different species of wild birds in south regions of Ukraine in 2017

Species	Samples
Anseriformes	
Mallard	442
Greylag goose	123
Mute swan	23
Ruddy shelduck	30
Shelduck	135
Shoveler	2
Garganey	18
Red-breasted goose	1
Charadriiformes	
Black-headed gull	238
Gull spp.	10
Mediterranean gull	169
Ruff	1
Slender-billed gull	16
Spoonbill	7
White-winged black tern	20
Little tern	5
Yellow-legged gull	104
Dunlin	10
Little gull	5
Podicipediformes	
Great crested grebe	1
Gruiformes	
Crane	11
Demoiselle crane	12
Ciconiiformes	
Grey heron	29
Great white egret	15
Little egret	5
Pelecaniformes	
Cormorant	101
Dalmatian pelican	15
White pelican	26
Environmental	45
Total	16,919

The presence of hemagglutinating viruses in allantoic fluid was determined by the HA test with a 1% suspension of chicken red blood cells (OIE, 2012).

Virus identification. The hemagglutinin (HA) virus subtype was determined by HI tests as previously described (Capua and Alexander, 2009; OIE, 2012; Spackman, 2014).

For these studies, the following antisera were used: H1N1, H2N3, H3N8, H4N6, H5N1, H6N8, H7N1, H8N4, H9N2, H10N7, H10N9, H11N6, H12N5, H13N6, H14N6, H15N9, H16N3, AvV-1, AvV-2, AvV-3, AvV-4, AvV-6, AvV-7, AvV-8, AvV-9 produced by Veterinary Laboratories Agency (Weybridge, UK); and the antisera H1N1, H2N3, H3N8, H4N8, H5N3, H6N2, H7N3, H8N4, H9N7, H10N1, H11N9, H12N5, H13N6, H14N5, H15N9, H16N3 produced by the Instituto Zooprofilattio Sperimentale delle Venezie (Padova, Italy).

Results. According to the results of virological studies using chicken embryos, sixteen hemagglutinating isolates with activity from 1:32 to 1:2048 were obtained from wild birds' fecal samples. These isolates were obtained from the mallards, shelducks, gray geese, gulls as well as from environmental samples in Kherson and Odesa Regions. No positive specimen was found in the Zaporizhia Region. After isolation, all hemagglutinating isolates were identified using HI test with reference sera. The results of virological research and identification are shown in Table 2.

Table 2 — Results of virological studies of wild birds' fecal samples in 2017

Place	Species	Total amount	Result of virological study			HI identification
			Negative	Positive	Name of isolates	
Kherson Region	Mallard	250	242	8	Mallard/Drugelubovka-3/6-10/6-08/17	H4
					Mallard/Drugelubovka-3/1-5/6-08/17	H3
					Mallard/Drugelubovka/1-3/5-09/17	AvV-4
					Mallard/Syvashivka/1-4/4-09/17	H5/AvV-6
					Mallard/Khorly/16-20/29-11/17	H7/H9
					Mallard/Chongar/13-16/3-09/17	H5
					Mallard/Mytrofanivka/1-4/4-09/17	AvV-1
					Mallard/Oleksiivka-2/33-36/5-09/17	H4
	Black-headed gull	48	47	1	B.h.Gull/Drugelubovka-3/1-3/6-08/17	H11/H13
	Greylag goose	21	20	1	Greylag Goose/Mytrofanivka/1-4/4-09/17	AvV-1
	Shelduck	46	45	1	Shelduck/Sergiivka/11-15/6-02/17	AvV-1
	Environmental	45	44	1	Envir./Novodmytrivka/41-45/7-08/17	AvV-6
Odesa Region	Mallard	192	190	2	Mallard/Zhovtyi Yar/1-5/10-08/17	H4
					Mallard/Ermakov/41-50/17	H3
	Shelduck	89	87	2	Shelduck/Zhovtyi Yar /1-5/27-10/17	H7/H3
					Shelduck/Bazivka/13-16/28-10/17	H2/H5/H7

Thus, 10 influenza viruses of different subtypes (H2, H3, H4, H5, H7, H9, H11, H13), as well as five avulaviruses and one mix of isolates were obtained during the autumn migration from wild waterfowl. Based on the results of virological studies, we calculated the infection level of wild birds. It has been found that 1.44–1.58% of wild waterfowl (wild ducks) during the autumn migration in 2017 were infected with the influenza virus, while wild shore birds (gulls) were only 0.42% infected. According to the results of serological identification, some viral isolates showed cross-reactions with referent sera to different subtypes of hemagglutinin H7/H9, H11/H13, H2/H5/H7. In this regard, they were studied in real-time PCR.

According to the results of real-time PCR, it was found that all viral isolates that had cross-reactions were A virus viruses, the Mallard/Chongar/13-16/3-09/17 viral isolate belongs to the influenza virus of the subtype H5 and 3 viral isolates (Shelduck/Bazivka/13-16/28-10/17, Shelduck/Zhovtyi Yar/1-5/27-10/17 and Mallard/Khorly/16-20/29-11/17) belong to the H7 subtype influenza virus. Thus, the circulation of influenza viruses has been confirmed to be the most dangerous for poultry wildlife subtypes in 2017.

Discussion. It should be noted that the study of wild birds in 2017 was carried out in places where outbreaks of H5N1, H5N8 subtypes of highly pathogenic avian influenza were recorded in 2006, 2016–2017. In addition, in previous years, for the first time in Ukraine, the circulation of low-pathogenic influenza viruses in these regions was detected in 15 of 16 known hemagglutinin subtypes and 7 of the 9 known neuraminidase subtypes. There is also a large genetic diversity of these viruses, and their connection with other geographic regions (Europe, Southeast Asia, Western Siberia) (Muzyka et al., 2012, 2016a, 2016b). That is to say that this territory is the place of traditional circulation of influenza viruses. The results we obtained confirm this assertion. These days, the circulation of avian influenza viruses of different subtypes continues in this region. According to many scientists, today a large global circulation of low-pathogenic influenza viruses in a natural reservoir has been proved, and our data coincides with these studies. It is also important to note that in the post-epizootic period (after the outbreaks of highly pathogenic H5N8 avian influenza in Ukraine in 2017) in wild birds, we obtained field isolates of H5 and H7 influenza virus. This may also indicate an independent circulation of highly pathogenic and low pathogenic avian influenza viruses in wild birds. As in our previous studies, all influenza viruses were isolated only from waterfowl and water-related birds, mainly from ducks.

Concerning avulaviruses, including Newcastle disease virus, we can also confirm that the circulation of these pathogens in wild birds continues, and this should be taken into account when planning of preventive measures in poultry farming.

Conclusion. The data we obtained is very important for predicting the epizootic situation in Ukraine, understanding the ecological features of low-pathogenic variants of pathogens, which cause particularly dangerous viral diseases of poultry. In addition, conducting regular virological monitoring in a natural reservoir allows us to receive new strains to improve laboratory diagnostics.

References

Camenisch, G., Bandli, R. and Hoop, R. (2008) 'Monitoring of wild birds for Newcastle disease virus in Switzerland using real time RT-PCR', *Journal of Wildlife Diseases*, 44(3), pp. 772–776. doi: 10.7589/0090-3558-44.3.772.

Capua, I. and Alexander, D. J. (eds.) (2009) *Avian influenza and Newcastle disease: A field and laboratory manual*. Milan: Springer. doi: 10.1007/978-88-470-0826-7.

Knipe, D. M. and Howley, P. M. (2013) *Fields virology*. 6th ed. Philadelphia, PA: Lippincott Williams & Wilkins. ISBN 9781451105636.

Muzyka, D., Pantin-Jackwood, M., Spackman, E., Stegniy, B., Rula, O. and Shutchenko, P. (2012) 'Avian influenza virus wild bird surveillance in the Azov and Black Sea Regions of Ukraine (2010–2011)', *Avian Diseases*, 56(4s1), pp. 1010–1016. doi: 10.1637/10157-040912-ResNote.1.

Muzyka, D., Pantin-Jackwood, M., Starick, E. and Fereidouni, S. (2016a) 'Evidence for genetic variation of Eurasian avian influenza viruses of subtype H15: the first report of an H15N7 virus', *Archives of Virology*, 161(3), pp. 605–612. doi: 10.1007/s00705-015-2629-2.

Muzyka, D., Pantin-Jackwood, M., Spackman, E., Smith, D., Rula, O., Muzyka, N. and Stegniy, B. (2016b) 'Isolation and genetic characterization of Avian influenza viruses isolated from wild birds in the Azov-Black Sea Region of Ukraine (2001–2012)', *Avian Diseases*, 60(1s), pp. 365–377. doi: 10.1637/11114-050115-Reg.

OIE (World Organisation for Animal Health) (2012) *Manual of diagnostic tests and vaccines for terrestrial animals (mammals, birds and bees)*. 7th ed. Vol. 1–2. Paris: OIE. ISBN 9789290448785.

Saif, Y. M. (ed.) (2008) *Diseases of poultry*. 12th ed. Ames, IA: Blackwell Publishing. ISBN 9780813807188.

Spackman, E. (ed.) (2014) *Animal influenza virus*. New York, NY: Springer (Methods in Molecular Biology, 1161). doi: 10.1007/978-1-4939-0758-8.

Swayne, D. E. (ed.) (2016) *Animal influenza*. 2nd ed. Hoboken, NJ: John Wiley & Sons, Inc. doi: 10.1002/9781118924341.

Part 4. Brief communications

UDC 619:616.98:578.832.1A:598.2(574)

CIRCULATION OF INFLUENZA A VIRUS AMONG WILD BIRDS IN KAZAKHSTAN

Sultankulova K. T., Akylbayeva K. K., Kerimbayev A., Burashev E. D., Orynbayev M. B.

Research Institute for Biological Safety Problems of the Science Committee
of the Ministry of Education and Science of the Republic of Kazakhstan,
Gvardeyskiy, Korday District, Jambyl Region, Republic of Kazakhstan, e-mail: sultankul70@mail.ru

Introduction. Because of the incessant ill situation of avian influenza worldwide the circulation of influenza A virus among wild birds is constantly monitored on the territory of Kazakhstan. Wild birds are a natural reservoir of all known variants, including subtypes, of the human and animal influenza agent that have caused all pandemics and large-scaled epizooties in the past.

The possible emergence of new modified potentially dangerous variants of the virus stipulates the need of complex monitoring of influenza in populations of wild birds, particularly at the key points such as habitation areas and major migration routes.

Materials and methods. ***Field samples.*** The biological material (cloacal swabs) from near-water and terrestrial wild birds, that was delivered from the ornithological station 'Shakpak' (Jambyl Region) and from Sorbulak lake system (Almaty Region) in 2018, was used in the work.

Viral RNA extraction. RNA of the influenza virus was extracted with use of the TRI Reagent, Sigma, following the manufacture's instructions.

PCR amplification. Real time RT-PCR was performed with the help of specific primers and a matrix gene probe (M+25, M-124 and probe M+64) and hemagglutinins (H5+1456, H5-1685 and probe H5+1637) with 'OneStep RT-PCR Kit' of Qiagen Company. The amplification was carried out in 'Light Cycler 2.0' of Roche Company following the manufacture's instructions.

Results and discussion. In 2018 in the course of monitoring expeditions samples were taken from terrestrial and near-aquatic wild birds (Fringillidae, Passeridae, Accipitridae, Coraciidae, Hirundinidae, Columbidae, Muscicapidae, Strigidae, Cuculidae, Emberizidae, Motacillidae, Turdidae, Sylviidae, Acrocephalidae, Phylloscopidae, and Panuridae families) on the ornithological station 'Shakpak' (Jambyl Region) and in Sorbulak lake system (Almaty Region).

Influenza A virus was identified in 16.6% of samples, including the virus of subtype H5 in 2.8% of samples from the Blyth's reed warblers (*Acrocephalus dumetorum*, Acrocephalidae) that inhabit Sorbulak lake system (Almaty Region). In addition, the influenza A virus was identified in single samples (1.3%) from Spanish sparrows (*Passer hispaniolensis*, Passeridae) inhabiting the ornithological station 'Shakpak' (Almaty Region).

Conclusion. The influenza A virus was detected in 17.9% of samples taken from wild birds thus confirming their role in influenza A existence on the territory of Kazakhstan.

Acknowledgments. The work was implemented under the grant project 'Molecular and epizootological monitoring of avian influenza in Kazakhstan' (2018–2020, AP05132659).

Keywords: monitoring, wild birds, influenza A virus, Kazakhstan

UDC 602.6:615.371:619:616.98:578.842.2:636.4

EFFORTS TOWARDS DEVELOPING AN AFRICAN SWINE FEVER VACCINE

Sunwoo S.-Y. [1], Pérez-Núñez D. [2], Morozov I. [1], Sánchez E. G. [2], Gaudreault N. N. [1], Madden D. [1], Trujillo J. [1], Urbaniak K. [1], Kim I. J. [1], Revilla Y. [2], Richt J. A. [1]

[1] Department of Diagnostic Medicine and Pathobiology, College of Veterinary Medicine, Kansas State University, Manhattan, KS, USA, e-mail: jricht@vet.k-state.edu

[2] Centro de Biología Molecular Severo Ochoa, Consejo Superior de Investigaciones Científicas, Universidad Autónoma de Madrid, Madrid, Spain

The African swine fever virus (ASFV) causes high morbidity and mortality in swine of the species *Sus scrofa,* for which there is currently no commercially available vaccine. Recent outbreaks reported in Trans-Caucasus countries, Eastern Europe and China highlight the urgent need to develop effective vaccines against ASFV. We developed an approach based on prime-boost vaccination, combining ASFV antigens encoded by DNA plasmids and recombinant ASFV proteins with the aim to activate both humoral and cellular immunity and tested vaccinated pigs against virulent ASFV challenge. In parallel, gene-deleted ASF viruses to be used as modified live virus vaccines have been developed using CRISP-Cas9 knock-out approaches.

The results of this work will provide insights towards the development of rationally designed, safe, efficacious vaccines for ASF.

Keywords: African swine fever, vaccine, DNA, plasmids, recombinant proteins

News

INTERNATIONAL SCIENTIFIC-AND-PRACTICAL CONFERENCE 'PROBLEMS OF BIOLOGICAL SAFETY AND CONTROL OF TRANSBOUNDARY EMERGENT INFECTIOUS DISEASES (AFRICAN SWINE FEVER, LUMPY SKIN DISEASE, FOOT-AND-MOUTH DISEASE, BRUCELLOSIS, HIGHLY PATHOGENIC AVIAN INFLUENZA)'

In Kharkiv, on September 17–19, the National Scientific Center 'Institute of Experimental and Clinical Veterinary Medicine' held an International Scientific-and-Practical Conference 'Problems of Biological Safety and Control of Transboundary Emergent Infectious Diseases (African Swine Fever, Lumpy Skin Disease, Foot-and-Mouth Disease, Brucellosis, Highly Pathogenic Avian Influenza)' dedicated to four important dates for the development of agrarian science in Ukraine: the 100th anniversary of the National Academy of Agrarian Sciences of Ukraine; the 90th anniversary of the birth of academician of the National Academy of Agrarian Sciences of Ukraine G. A. Krasnikov; the 80th birthday of academician of the National Academy of Agrarian Sciences of Ukraine, Honored Worker of Science and Technology of Ukraine M. V. Zubets; as well as the 95th anniversary of the founding of the National Scientific Center 'Institute of Experimental and Clinical Veterinary Medicine'.

The conference was co-organized with the National Academy of Agrarian Sciences of Ukraine, the State Service of Ukraine for Food Safety and Consumer Protection, and the North-Eastern Scientific Center of the National Academy of Sciences of Ukraine and the Ministry of Education and Science of Ukraine.

The conference was organized and held with the active support of the US Biological Threat Reduction Program, whose leaders are participating in the conference. In general, its participants are 225 scientists and practitioners from the USA, Germany, Poland, France, Spain, Sweden, Switzerland, Turkey, Belarus, Georgia, Armenia, and Ukraine. Among them are representatives of scientific institutions, universities, and the Main Administrations and 15 regional laboratories of veterinary medicine of the State Service of Ukraine for Food Safety and Consumer Protection.

There was also an exhibition of manufacturers of veterinary drugs and laboratory equipment, at which 11 companies represented their achievements.

The First Vice-President of the NAAS, academician of the NAAS, M. V. Gladiy complimentary addressed to the participants of solemn plenary meeting and presented certificates of appreciation and gratitude of the Presidium of NAAS of Ukraine to the best scientists of the NSC 'IECVM'. Congratulations and remarks were made by the Institute's employees on behalf of the Kharkiv Regional State Administration, the Kharkiv Oblast Council, the Kharkiv City Council, and the State Service of Ukraine for Food Safety and Consumer Protection.

In his informative report devoted to important historical dates of the NAAS and the 95th anniversary of the NSC 'IECVM', the director of the Institute, academician of the NAAS of Ukraine B. T. Stegniy outlined the history of creation, development and future directions of the institution.

Speaking with a greeting, the Consul General of the Federal Republic of Germany in Ukraine, Dr. Wolfgang Messinger emphasized the importance of cooperation on topical issues of biosafety and biosecurity within the framework of the German bioprotection program. The presentation of the Ukraine-German Biosafety Initiative and for control the risks of zoonotic diseases on the EU borders was made by an employee of the Bundeswehr Institute of Microbiology Dr. H. von Buttlar.

Of considerable interest were the scientific reports of Dr. J. A. Richt (USA) on the development of African swine fever vaccine, Director of the State Scientific and Research Institute of Laboratory Diagnostics and Veterinary and Sanitary Expertise O. V. Pishchanskiy about epizootic situation on highly pathogenic avian influenza, Dr. M. Polak (Poland) on atypical spongiform encephalopathy, Prof. H. Ilmaz (Turkey) on the spread of lumpy skin disease, and many more. 15 reports were heard and discussed at the plenary meeting.

Significant interest of the participants was aroused by the reports at the sectional sessions, which were devoted to a wide range of topical problems of veterinary medicine, including transboundary infectious diseases, brucellosis, anthrax, rabies, tuberculosis and paratuberculosis, zoonotic hepatitis A, tularemia, resistant of infection diseases' agents to antibiotics, issues of disinfection, control of toxic feed contaminants and the immune-metabolic status of productive animals, and safety using of nanoparticles in poultry.

Within the framework of the conference, a Scientific-and-Practical Seminar 'Actual aspects of the control of African swine fever' was held with the participation of scientists from the USA, Belarus, Ukraine, as well as specialists from the State Service of Ukraine for Food Safety and Consumer Protection.

The final plenary session summed up the conference, discussed and adopted a resolution, which noted, in particular, the need to intensify scientific researches including through international cooperation on emergent and economically significant infectious diseases, ensuring epidemic and veterinary and sanitary welfare, and the quality and safety of animal products.

SCIENTIFIC-AND-PRACTICAL SEMINAR 'AFRICAN SWINE FEVER — TRANSBOUNDARY DISEASE. THE IMPORTANCE OF BIOSAFETY TO PREVENT THE INFECTION OF PIG FARMS'

The National Scientific Center 'Institute of Experimental and Clinical Veterinary Medicine' (Kharkiv) in cooperation with the United States Department of Agriculture (USDA), Purdue University (USA), and the Association 'Pig Breeders of Ukraine' on September 20 held the Scientific-and-Practical Seminar 'African Swine Fever — Transboundary Disease. The Importance of Biosafety to Prevent the Infection of Pig Farms'.

The seminar was opened by the President of the Association 'Pig Breeders of Ukraine' Artur Loza and by Director of the NSC 'IECVM', in which this international forum took place, Academician of the National Academy of Agrarian Sciences of Ukraine, Prof. Boris Stegniy.

The speakers of the seminar were the leading scientists involved in studying the risks of spread and the development of measures to prevent of ASF from leading scientific centers in the world and Ukraine. 9 reports were heard. Director of the WHO ASF Reference Laboratory (Spain), DVM, Prof. José Manuel Sánchez-Vizcaíno Rodríguez revealed the peculiarities of ASF as a transboundary disease and the importance of biological safety in pig farms. In his speech, a national expert on the problems of biosafety and emergent animal diseases, Director of the NSC 'IECVM', Academician of the NAAS, Dr. Sc., Prof. Borys Stegniy suggested a number of urgent measures to prevent the introduction and spread of ASF virus in pig farms and the direction of scientific support for the problem. Epizootological aspects, risk assessment and molecular tools for the disease diagnostics were presented in the speech of the Deputy Director for Scientific Work and Head of the Department of Molecular Diagnostics and Epizootology of the NSC 'IECVM', Corresponding Member of the NAAS, Dr. Sc., Prof. Anton Gerilovych. The report by Professor of the Department of Diagnostics and Pathobiology of the College of Veterinary Medicine (University of Kansas, USA), DVM, Prof. Bob Rowland was devoted to the risk factors for ASF infection through feed and feed additives.

Representatives of Purdue University (USA), inspector of the PQA+ program, trainer on swine diseases and biosafety, DVM, Dr. Darryl Ragland and virologist, specialist in pig health, DVM, Dr. Roman Pogranichniy, spoke on the most important aspects of biosafety in pig farms, in particular by disinfection. The history, challenges and modern methods of diagnosis and prevention of the disease were reflected in the reports of Academician of the NAAS, Dr. Sc., Prof. Sergiy Melnychuk and Deputy Director for Research of the Institute of Veterinary Medicine of NAAS, Dr. Sc. Mykola Sytiuk, requirements for farms with high biosafety level and compartment status — in the speech of an expert in veterinary medicine Vitaliy Bashinskyi, and the influence of the spread of ASF in household and farm enterprises on the meat market of Ukraine — in the information of the Leading Researcher of the State Scientific Control Institute of Biotechnology and Strains of Microorganisms, Dr. Sc., Prof. Liudmyla Oliynyk.

Among the participants of the seminar were scientists and specialists of the NSC 'IECVM', USDA and DTRA (USA), heads and specialists of structural divisions of the Main Branch of the State Service of Ukraine for Food Safety and Consumer Protection in the Kharkiv Oblast, representatives of the leading pig farms of the North-Eastern and Central regions of Ukraine.

In total, 78 people took part in the seminar. It should be noted that the presentations of the speakers were interesting, informative, interactive, there was an active discussion and exchange of views on the most pressing problems of ensuring the biological safety of pig farms for ASF. This form of communication interested the attending practitioners of veterinary medicine and allowed to more deeply perceive the information provided by scientists.

Contents

Part 1. Veterinary medicine

Part 2. Biology and biotechnology

Part 3. Biosafety

Part 4. Brief communications