The Experimental Infection of Vaccine-like Isolates of Infectious Laryngotracheitis Virus Isolated in Ukraine in 2010–2012

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Abstract. *Infectious laryngotracheitis (ILT) is an important respiratory infection of chickens that can pose a serious threat to poultry. Despite the fact that the infection has been known for a long time and a large number of vaccines have been developed for specific prevention, the disease still occurs quite often worldwide. This is due to the pathogen characteristics and its ability to mutate, resulting in new isolates. Despite the extensive ILT vaccination program used in commercial poultry farms, the circulation of 2 types of virus isolates is registered among poultry. The first type is the field isolates of the ILT virus (ITLV), which are significantly different from the vaccine strains of the ILTV. The second type is vaccine-like isolates, which have differences compared with vaccine strains and can cause clinical disease in birds.*

The paper presents the data on the study of the biological properties of 2 ILTV isolates: A 4-12 and B 2-10. These isolates were isolated from sick hens from commercial and backyard poultry of Ukraine in 2010–2012. Both isolates were identified as vaccine-like viruses by PCR. All isolates were pathogenic for chicken embryos (CE) and caused death and typical postmortem changes. During the experimental infection of susceptible chickens (at age of 60 days) at the laboratory conditions, it was found that the isolates caused typical clinical signs and pathological macroscopic and microscopic changes in internal organs. ILTV isolate B 2-10 caused the death of 80% of the experimental chicken, and isolate A 4-12 caused only clinical manifestations of the disease in chickens, but not death of chickens.

Postmortem studies showed that vaccine-like isolates cause a threat to cells and changes in the tissue of the internal organs (trachea, lungs, spleen, intestines) of infected chickens, which are typical for other ILTV strains. At the same time, the reaction of the immune system of chickens against the virus was observed at the cell level.

Introduction

Infectious laryngotracheitis (ILT) is a highly contagious upper respiratory tract disease of chickens and hens caused by a Gallid herpesvirus 1 (GaHV-1) belonging to the genus *Iltovirus* and subfamily *Alphaherpesvirinae* within *Herpesviridae* family. The disease is characterized by sinusitis, conjunctivitis, oculo-nasal discharge, respiratory distress, bloody mucus, swollen orbital sinuses, high morbidity, considerable mortality and decreased egg production. Co-infections with other respiratory pathogens and environmental factors adversely affect the respiratory system and prolong the course of the disease. Latently infected chickens are the primary source of ILT virus (ILTV) outbreaks irrespective of vaccination (Gowthaman et al., 2020).

The disease is reported in most countries with developed industry poultry farming, but outbreaks occur mostly in small-scale poultry farms (Bagust et al., 2000). Chickens are considered like the primary host of the virus (Bagust, 1986), but natural disease has been reported in peafowls and pheasants (Crawshaw et al., 1982). ILT causes production losses due to increased morbidity, moderate mortality, decreased weight gain, reduced egg production and expenses spent on vaccination, biosecurity measures and therapy to counteract secondary infection by other avian pathogens (Jones, 2010; Saif et al., 2008).

Ukrainian poultry industry is an important sector of the national economy. There are two segments of the poultry farming: developed industrial poultry farming as well as significant backyard poultry farming. Therefore, ILT as a respiratory disease can pose a threat to the poultry industry. Considering the circulation of the ILTV in Ukraine, the significant economic losses from the disease and the need to choose the right vaccination strategy, the constant monitoring of the ILT, isolation and study of new isolates of the virus are important to ensure the epizootic well-being of the poultry industry. The National Scientific Center Institute of Experimental and Clinical Veterinary Medicine (Kharkiv) has been a center for scientific research on poultry infectious diseases, including infectious laryngotracheitis, for almost 100 years. Scientists constantly conduct

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epizootological monitoring of ILTV circulation among industrial and backyard poultry farms in different regions of Ukraine, virus isolation and in-depth studies of their biological properties, as well as their impact on poultry.

Over the last 10 years, several cases of ILT have been recorded in Ukraine: in the Kharkiv region in 2010, 2011, and 2012; in the Luhansk region in 2010; in the Autonomous Republic of Crimea in 2012 (Воротилова, 2014); in Donetsk region in 2012 (Музика et al., 2021; Veretsun et al., 2021); and in the Sumy region in 2019 (Veretsun et al., 2021). In addition, due to the results of serological monitoring, 7.1%–42.9% of unvaccinated chickens in industrial poultry farms reveal specific antibodies to the ILTV (in press). Thus, the ILTV is an important pathogen for poultry in Ukraine. It is also important to estimate the pathotype of the isolates which are circulating in the country. Particular attention is paid to the study of the features of vaccine-like ILTV isolates, their ability to cause clinical disease and postmortem changes. The aim of the research was to study the biological properties (infectious activity, ability to cause death of chicken embryos, ability to cause clinical disease in experimental infection) and the postmortem changes in the tissues of experimentally infected chickens of 2 vaccine-like isolates of the ILTV isolated in 2010–2012.

Materials and Methods

Sampling. Samples (trachea, lungs, and spleen) were obtained from sick and dead hens in 2 poultry farms, where the disease cases were registered during 2010–2012.

Farm 1 is located in Kharkiv region. The flock was vaccinated against ILT. Birds were at the age of 180 days. Clinical signs were general oppression, feed refusal, cough. Postmortem changes were tracheitis, conjunctivitis, sinusitis, and catarrhal rhinitis.

Farm 2 is located in Donetsk region. The flock was not vaccinated against ILT. Birds were at the age of 138 days. Clinical signs were a sudden death of birds without significant visible clinical signs. Postmortem changes were hyperemia of the mucous membrane of the lower eyelid, tracheitis, blood shears in the lumen of trachea, pneumonia, aerosacculitus, pericarditis, hydropericarditis, hemorrhages on the heart, enlarged spleen and kidneys, and catarrhal-hemorrhagic enteritis.

Virological studies. The studies were conducted at the Department of Poultry Diseases of the National Scientific Center "Institute of Experimental and Clinical Veterinary Medicine". Virological studies were performed by conventional methods (inoculation of chicken embryos, determination of infectious and lethal titers) (A laboratory manual, 2008). For this purpose, a 10%–20% suspension of pathological material on phosphate buffer (pH 7.2–7.4) was prepared. The suspension was centrifuged at 3000 rpm for 30 min, and then a mixture of antibiotics was added and kept for 30 min at room temperature. The 10–12-day-old CE were infected (inoculation dose was 0.2 mL) on the chorio-allantoic membrane. Infected embryos were incubated for 7 days (A laboratory manual, 2008; OIE, 2008).

Statistical analysis. The definition and evaluation of biological activity (infectious and lethal titer) was carried out according to the common method on chicken embryos by titration. Infectious titer was calculated by the method of Reed and Muench (A laboratory manual, 2008).

Experimental infections. Two experiments on sensitive chickens were conducted. All studies were conducted from observing all necessary requirements of biosafety and biosecurity in conditions that exclude the release of the pathogen. Experimental infection was carried out taking into account the principles of bioethics (the scheme of the experiment was considered and approved at the meeting of the Bioethics Commission of the NSC IECVM).

In the experiments, 60-day-old specific pathogen-free non-vaccinated chickens were used. The absence of specific antibodies to ILT virus was confirmed by ELISA (Infectious Laryngotracheitis Antibody test kit, BioChek, UK)(OIE, 2008).

The first experiment was to determine the pathogenicity of the isolate B 2-10. Experimental infection of chickens was performed by intratracheal application of a 10% suspension of native pathological material (spleen, trachea, and lungs), which contained isolate B 2-10 (dose 0.5 mL per bird). The infected birds were monitored for 15 days. In the first experiment, 15 chicks were used.

The second experiment was to determine the pathogenicity of the isolate A 04-12. Experimental infection of chickens was performed by intratracheal application of a 10% suspension of native pathological material (spleen, trachea, and lungs), which contained isolate A 04-12, using a dose of 0.5 mL per bird (group 1), and extraembryonic fluid of infected CE passage I, using a dose of 0.5 mL (2.15 lg EID_{50}) per bird (group 2). The infected birds were monitored for 15 days. In the second experiment, 2 chickens in each group were used.

Molecular genetic studies. Nucleic acid extraction was performed using the AmpliSens® DNA-sorb-B kit (Russian Federation). Detection of the ILTV DNA was conducted using conventional PCR according to the typical PCR protocol (Kirkpatrick et al., 2006). In brief, a pair of forward (5'-CTGGGC-TAA-ATC-ATC-CAA-GAC-ATC-A-3') and reverse (5'-GCT-CTC-TCG-AGT-AAGAAT-GAG-TAC-A-3') primers was used for the amplification of 2.24 kbp region of the ILTV thymidine kinase gene following the amplicons separation by electrophoresis through 0.8% agarose gels, stained with an appropriate nucleic acid stain and exposed to UV light for visualisation.

Field type or vaccine isolates of the ILTV were determined in a commercial laboratory Royal GD (Deventer, The Netherlends) by PCR in a combination of the restriction analysis method in accordance with the OIE recommendations (OIE, 2008) and local SOPs.

Histopathological studies. Internal organs of chickens were routinely processed, embedded in paraffin wax, and histological sections were stained by hematoxylin-eosin. Organ samples were washed under running water for 12 hours to remove the formalin solution. Dehydration of organ samples was carried out in alcohols of increasing concentration: 60, 70, 80, 90, 96 for 12 hours. Further organ slices were treated with a solution of alcohol-chloroform for 30 min, chloroform for 1 hour, chloroform-paraffin for 1 hour and alternately moved into paraffin 1, paraffin 2, and paraffin 3 for 60 min. The sections were prepared using rotary microtome, 3–5 microns thick. Sections were stained with hematoxylin-eosin according to the accepted procedure (Luna, 1968).

Results

Isolate B 2-10 was isolated from pathological material of affected and dead hens with clinical signs typical for ILT from farm 1 (Kharkiv region). Hens from this farm were routinely vaccinated against ILT. Isolate A 04-12 was isolated from pathological material of infected chickens without clinical signs, but with pathological changes of respiratory organs from farm 2 (Donetsk region), where vaccination against ILT was not carried out.

As a result of molecular biological studies (PCR), the samples contained the genetic material of the ILTV. Restriction analysis and PCR studies conducted in Royal GD showed that both isolates (B 2-10 and A 04-12) belonged to a vaccine-like ILTV.

Also, we tested the biological activity (infection and lethal titer) of the isolates B 2-10 and A 04-12. It was found that the infection titer of ILTV isolate B 2-10 was 6.5 lg EID_{50}/1 mL, and that of isolate A 04-12 was 5.3 lg EID_{50}/1 mL.

The next stage of our research was to study the pathogenicity of these isolates in the laboratory conditions. In order to study the pathogenicity of the isolates, infection of 60-day-old chickens, free from antibodies to ILT virus, was performed. The infected birds were monitored for 15 days. The results of experimental inoculation of chickens with isolate B 2-10 (number of chickens with clinical signs and the number of fatalities) are shown in Table 1.

The results in Table 1 show that the clinical signs of the disease (general depression, diarrhea, respiratory noises, labored breathing) were observed from day 10. The incidence was 100% of the experimentally infected population. Specific deaths of chicken were observed from day 13 (for isolate B 2-10). All infected chickens died at the end of the experiment as a result of ChP 96-10 isolate. Dead bird autopsy showed pathological changes typical for ILT.

For the second isolate A 04-12, experimental infection of chickens was performed by intratracheal application of a 10% suspension of native pathological material (group 1) and intratracheal application of extraembryonic fluid from infected CE of passage I (group 2). The number of affected and dead chickens was recorded in Table 2.

Obtained results showed that in both groups

Table 1. Dynamics of the clinical signs and death of the experimentally infected chickens with ILTV isolate B 2-10 (application of 10% suspension of native pathological material).

Clinical state of poultry	Day after virus inoculation														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
	Amount of chickens														
Healthy	15	15	15	15	15	15	15	15	15	–	–	–	–	–	–
Affected	–	–	–	–	–	–	–	–	–	15	15	15	9	3	–
Fatalities	–	–	–	–	–	–	–	–	–	–	–	–	6	6	3

Table 2. Dynamics of clinical signs and death of chickens experimentally infected with ILTV isolate A 04-12.

Clinical state of poultry		Day after virus inoculation														
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
		Amount of chickens														
Group 1	Healthy	2	2	2	2	2	2	2	–	–	–	–	–	–	–	–
	Affected	–	–	–	–	–	–	–	2	2	2	2	2	2	2	2
	Fatalities	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Group 2	Healthy	2	2	2	2	2	2	2	–	–	–	–	–	–	–	–
	Affected	–	–	–	–	–	–	–	2	2	2	2	2	2	2	2
	Fatalities	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–

after virus inoculation the clinical signs of the disease (general depression, diarrhea, respiratory noises, labored breathing) appeared from day 8 of the experiment. No dead chickens were registered throughout the observation period.

On day 15, a forced slaughter of the experimental poultry was conducted. At autopsy, pathological changes characteristic of the ILTV were revealed (catarrhal tracheitis, hyperplasia and hyperemia of the spleen).

For the estimation of tissue changes in the internal organs of experimentally infected chickens with a vaccine-like isolate of the ILTV, postmortem studies were performed. For this purpose, internal organs (trachea, lungs, spleen, intestinal) were obtained from infected chickens with A 04-12 isolate.

As a result of histological examination of the trachea, thickening of the own lamina of the mucous membrane due to infiltration by small and medium lymphocytes, macrophages, and eosinophiles, was determined (Fig. 1). Tracheal epithelial cells have signs of mucoid swelling. The submucosal base of the trachea has signs of edema.

The number of goblet cells was reduced. In some areas of the mucous membrane, there was a desquamation of epithelial cells with exposure of the submucosal base (Fig. 3). There was a small amount of exudate in the lumen.

In chickens of group 2, the changes were less pronounced, but the mucosa was also infiltrated with lymphoid cells, the glands had a larger number of goblet cells, and the capillaries were enlarged. Epithelial cells of the trachea have signs of mucoid swelling, and the lumen contains a small amount of exudate.

In the study of the lungs, it was found that the alveoli wall was formed by a single-layer flat epithelium, a thin layer of connective tissue and blood vessels, mainly capillaries, and lined endothelium, which formed a plexus around each alveolus. Most of the surface of the alveoli was lined by highly flat epithelial cells – respiratory epitheliocytes. Alveolar macrophagocytes were found in the walls of the alveoli. The walls of the bronchioles were lined with a single-layer prismatic ciliated epithelium. The lamina propria was formed by a thin layer of the loose fibrous connective tissue. The muscle plate consisted of smooth muscle cells. In the study of lungs of chickens of group 1, the thickening of the wall of the bronchioles was established (Fig. 4). Lymphoid cells accumulated around them. Capillaries were enlarged, overflowing with blood cells. Actually, the mucous membrane was infiltrated by lymphoid cells. In group 2 chickens, histomorphological changes in the lungs were less pronounced. Infiltration of lymphoid cells, pseudo-eosinophils, and macrophages around the blood vessels and in the lamina propria of some bronchioles was observed (Fig. 5).

As a result of histomorphological examination, it was established that the spleen was surrounded by a connective tissue capsule with elastic fibers and smooth muscle cells. The connective tissue stroma of the organ was poorly developed. In the course of large vessels, there was a small amount of connective tissue. The basis of the parenchyma of the spleen was

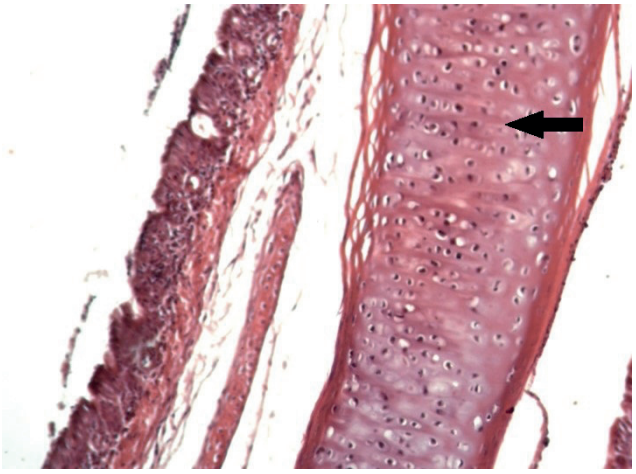

Fig. 1. Trachea of a chicken infected with the ILTV (group 1, isolate A4-12). The lamina of the mucous membrane infiltrated with lymphoid-histiocytic cells. × 100, H+E

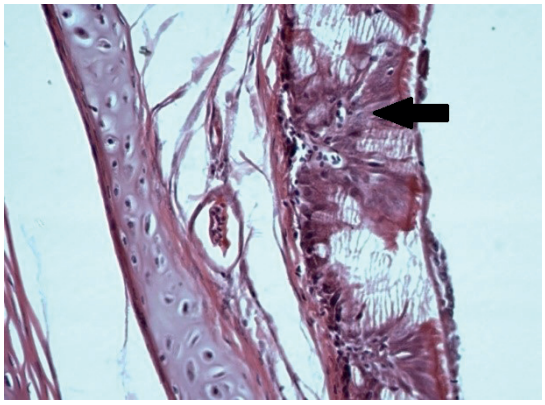

Fig. 2. Trachea of a chicken infected with the ILTV (group 1, isolate A4-12). Epithelial cells with signs of mucoid swelling. × 200, H+E

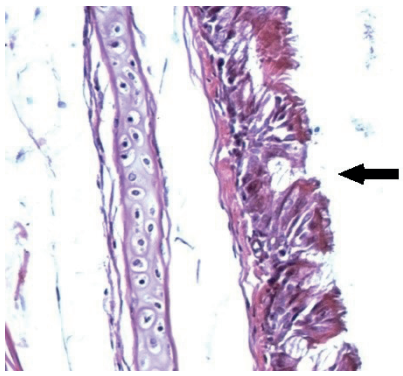

Fig. 3. Trachea of a chicken infected with the ILTV (group 1, isolate A4-12). Desquamation of epithelial cells. × 100, H+E

the reticular tissue. Numerous reticulo-endothelial clutches were seen in the slice plane, having the shape of elongated, elliptical clusters of reticuloendothelial cells around the terminal arterial vessels. Around the blood vessels, lymphoid clutches were visible that were not clearly delimited and had the appearance of homogeneous clusters of lymphoid cells. It was also possible to distinguish germinal follicles that were clearly delimited by the membrane and consisted of blasts, lymphocytes and, to a lesser extent, plasma cells. Germination follicles were bound with small and medium sized arteries and veins. The arterial germinal follicles adjoined the surface of the vessel. And the venous germinative follicles were immersed deeply in the lumen of the veins, lay inside them and changed the shape of the lumen. Histological examination of the spleen of chickens in groups 1 and 2 revealed that the capsule of the organ was unchanged. The organ was hematopoietic, and the white pulp occupied a large portion of the slice area (Fig. 6). The germinal follicles were single, and the periarterial lymphoid clutches were wide. Also, we detected an increasing number of pseudo-eosinophils. Reticuloendothelial clutches were not enlarged in size.

Histological examination of the large intestine and cecal tonsils revealed that the *mucous lamina propria* contained lymphoid tissue with numerous lymphoid follicles and diffuse lymphoid tissue, represented by small and medium lymphocytes, macrophages, plasma cells, and pseudo-eosinophils. The muscular membrane of the mucosa was represented by smooth muscle cells. The mucous membrane was lined by a single-layer prismatic epithelium containing many goblet cells. The muscular membrane consisted of two layers. In chickens of group 2, cecal tonsils contained a small number of lymph nodes that were not densely filled with lymphoid cells (Fig. 7). Signs of catarrhal inflammation were observed. The epithelial layer was thickened, and some of the cells were desquamated. In chickens of group 1, hemorrhages located mainly on the tips of the villi were observed.

The villi were thickened and deformed. In some areas, there was a desquamation of cells and, in others thickening of the epithelial layer was observed.

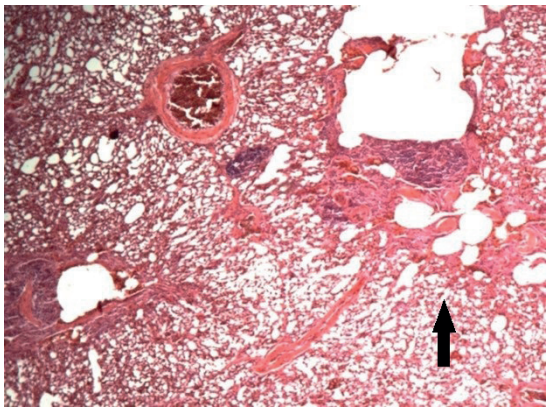

Fig. 4. Lungs of a chicken infected with the ILTV (group 1, isolate A4-12). Thickness of the walls of the bronchioles. × 100, H+E

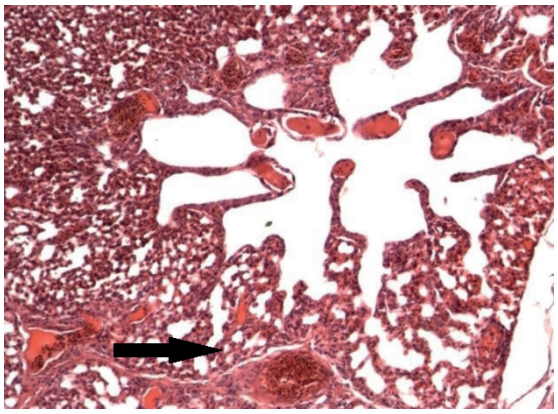

Fig. 5. Lungs of a chicken infected with the ILTV (group 2, isolate A4-12). Lymphoid-histiocytic cell cluster. × 100, H+E

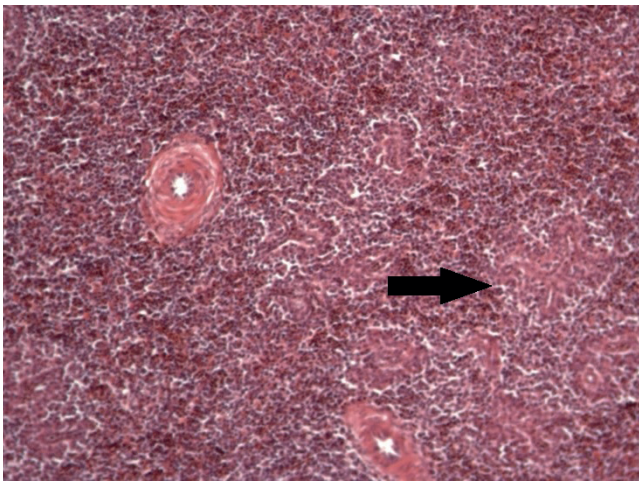

Fig. 6. Spleen of a chicken infected with the ILTV (group 1, isolate A4-12). The white pulp occupies a large portion of the slice area. × 50, H+E

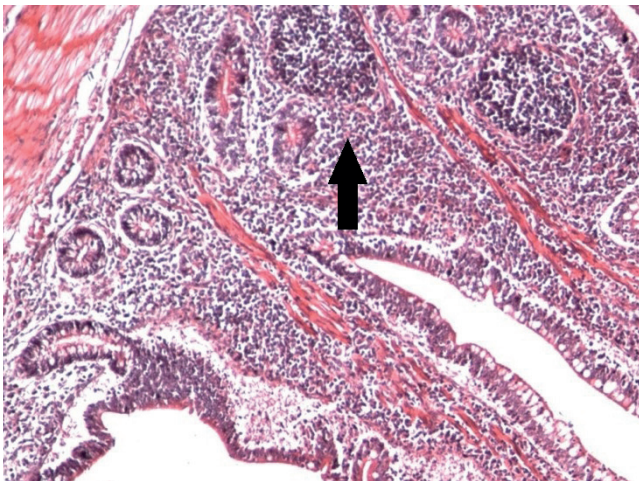

Fig. 7. The cecum of a chicken infected with the ILTV (group 2, isolate A4-12). The lymph nodes are few, loosely filled with lymphoid cells. × 100, H + E

Discussion and Conclusions

Nowadays, one of the biggest problems of industrial poultry farming in the world is infectious diseases. Despite much research in the study of the biological properties of pathogens, the development of modern tools for early diagnosis and specific prevention, outbreaks of infectious viral and bacterial diseases are registered almost constantly in different countries. Respiratory viral diseases such as infectious laryngotracheitis remain an urgent problem and are often complicated by bacterial infections, which are significantly aggravating the course of the disease.

Ukraine has a developed industrial poultry farming (Асоціація «Союз птахівників України», 2014) and the problem of infectious laryngotracheitis has been known since the 70s of the last century. At that time, this disease was registered quite often and led to significant economic losses (Бабкин, 1975; Бабкин, 1986; Бабкин et al., 1997). Nowadays, ILT remains an important viral disease for poultry. Active international trade between Ukraine and other countries, export and import of poultry products, genetic material, veterinary drugs and veterinary technology contribute to the circulation of the pathogen. In addition, the properties of the ILTV and the ability of latent infection lead to the emergence of new isolates. In the last 10 years, the circulation of ILTV isolates in both industrial and backyard poultry farms has been detected in Ukraine [Музика et al., 2021; Veretsun et al., 2021]. Several ILTV strains have been isolated from infected birds in different regions of Ukraine, but at the same time their pathotype and affiliation to field or vaccine-like isolates remains unknown.

The results presented in this work show the first data regarding the vaccine-like strain circulating in Ukraine. For the first time in Ukraine, we found that the ILTV isolates from industrial poultry farms were characterized as vaccine strains. It should also be noted that isolate B2-10 was obtained from sick vaccinated birds, while isolate A 04-12 was isolated from sick non-vaccinated chickens. This information suggests that vaccine strains are able to spread between poultry farms and more detailed investigation is needed.

In our studies, vaccine-like isolates of the ILTV (A 04-12 and B2-10), which were isolated in different regions of Ukraine in 2010 and 2012, showed high reproducibility in CE and were pathogenic for them. They caused typical changes in the chorioallantoic membrane of CE. Similar changes were caused by other isolates of the ILTV (Saif, 2008). During experimental infection, both isolates were pathogenic for 60-day-old chickens. According to our data, the incubation period of vaccine-like ILTV infection in chickens varied from 7 to 9 days, which is consistent with other authors (Saif, 2008). Ukrainian isolates ILTV (A4-12 and B2-10) caused not only typical clinical signs in chickens, but also death of chickens (B2-10) within 12 days after infection. These results confirm the ability of ILTV vaccine-like isolates to cause disease in susceptible chickens (Hughes et al., 1987; Hughes et al., 1989; Saif et al., 2008).

Concerning postmortem studies, the changes we found in the tissues of internal organs, especially the respiratory system, indicate that vaccine-like isolates of the ILTV can cause significant damage to organs at the cellular level. At the same time, we also found the reaction of the immune system of chickens to infection (Fletcher et al., 2008).

Based on the obtained data, we can conclude that vaccine-like isolates of the ILTV can be pathogenic to birds, cause them clinical manifestations of the disease and death of infected chicken. Thus, there is no effective method regarding differentiating between vaccine-like and field isolates of the virus without conducting in-depth molecular genetic studies and sequencing (A laboratory manual, 2008; Creelan et al., 2006, Saif et al., 2008). The pathogenicity level of the vaccine-like isolates can be different, and the key factors in the deterioration of the disease in poultry will be external factors (veterinary and sanitary conditions of poultry farms, feed quality, and other infections, especially bacterial). In conclusion, it is necessary to note the importance of the further research: regular epidemiological monitoring, isolation of pathogens and study of their biological and molecular genetic properties, which will allow timely control of the emergence of new variants of viruses, improvement of diagnostics and specific prevention. These studies are extremely important for poultry farming in Ukraine, because it remains unknown which field ILTV isolates circulate in Ukraine and their origin.

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References

1. A laboratory manual for the isolation, identification and characterization of avian pathogens. American Association of Avian Pathologists, Athens, GA. 2008
2. Офіційний сайт Асоціації «Союз птахівників України». Союз птахівників України www.ptaha.kiev.ua. 22-1-2014.

Бабкин, В. Ф. 1986. Инфекционный ларинготрахеит, p. 143-150. *In*: Респираторные болезни сельскохозяйственных животных. Киев.

3. Бабкін, В. Ф. 1975. Інфекційний ларинготрахеїт птиці. Київ.
4. Бабкін, В. Ф., Макогон В, and Герман В. 1997. Вакцинопрофілактика інфекційного ларинготрехеїту та ньюкаслської хвороби птиці в умовах півдня України. Вет. медицина України. 12:20-22.
5. Воротилова Н.Г. 2014.Епізотологічний моніторинг високопатогенного грипу, інфекційного ларинготрахеїту птиці та біологічні властивості збудників, Харків.
6. Bagust, T. J. 1986. Laryngotracheitis (Gallid-1) herpesvirus infection in the chicken. 4. Latency establishment by wild and vaccine strains of ILT virus. Avian Pathol. 15:581-595.
7. Bagust, T. J., R. C. Jones, and J. S. Guy. 2000. Avian infectious laryngotracheitis. Rev.Sci Tech. 19:483-492.
8. Crawshaw, G. J. and B. R. Boycott. 1982. Infectious laryngotracheitis in peafowl and pheasants. Avian Dis. 26:397-401.

9. Creelan, J. L., V. M. Calvert, D. A. Graham, and S. J. McCullough. 2006. Rapid detection and characterization from field cases of infectious laryngotracheitis virus by real-time polymerase chain reaction and restriction fragment length polymorphism. Avian Pathol 35:173-179.
10. Gowthaman, V., S. Kumar, M. Koul, U. Dave, T. R. G. K. Murthy, P. Munuswamy, R. Tiwari, K. Karthik, K. Dhama, I. Michalak, and S. K. Joshi. 2020. Infectious laryngotracheitis: Etiology, epidemiology, pathobiology, and advances in diagnosis and control - a comprehensive review. Vet.Q. 40:140-161
11. Hughes, C. S., R. M. Gaskell, R. C. Jones, J. M. Bradbury, and F. T. Jordan. 1989. Effects of certain stress factors on the re-excretion of infectious laryngotracheitis virus from latently infected carrier birds. Res Vet Sci 46:274-276.
12. Hughes, C. S., R. C. Jones, R. M. Gaskell, F. T. Jordan, and J. M. Bradbury. 1987. Demonstration in live chickens of the carrier state in infectious laryngotracheitis. Res Vet Sci 42:407-410.
13. International Office of Epizootics, Biological Standards Commission, International Office of Epizootics, and International Committee. 2008. Manual of diagnostic tests and vaccines for terrestrial animals mammals, birds and bees. Office international des epizooties, Paris.
14. Музика Д.В., Верецун А.Л., Стегній Б.Т., Рула О.М., and Усова Л.П. 2021. Виділення та ідентифікація епізоотичного ізоляту вірусу інфекційного ларинготрахеїту. Вет. медицина: міжвід.темат.наук.зб. 104:137-142.
15. Jones, R. C. 2010. Viral respiratory diseases (ILT, aMPV infections, IB): are they ever under control? Br.Poult.Sci 51: 1-11.
16. Luna, L. G. 1968. Manual of histologic staining methods of the Armed Forces Institute of Pathology. New York: McGraw Hill Book Co..
17. Oscar Jasper Fletcher and C. Riddell. 2008. Avian histopathology. Jacksonville, Fla. : American Association of Avian Pathologists.
18. Saif, Y. M. and A. M. Fadly. 2008. Diseases of poultry. Blackwell Pub, Ames, Iowa.
19. Veretsun, A., Stegniy, B., Rula, O., Bolotin, V., Stegniy, A., Gerilovych, A., & Muzyka, D. (2021). Molecular and genetic characterization of avian laryngotracheitis virus isolates obtained in Ukraine. *Agricultural Science and Practice*, *8*(1), 37-46. https://doi.org/10.15407/agrisp8.01.032

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