

**EPIZOOTOLOGICAL STUDY CONCERNING ASSOCIATIVE
(MIXED) METAPNEUMOVIRAL INFECTION WITH VIRAL-BACTERIAL
MICROFLORA IN PRIVATE POULTRY FARMS**

Ivleva O. V.¹, Nalyvaiko L. I.²

Luhansk National Agrarian University, Kharkiv, Ukraine,
e-mail: ¹ sause1908@gmail.com, ² vet-doctor@ukr.net

Summary. The first reports of respiratory diseases with swollen head syndrome SHS in laying hens were made in South Africa in 1978. The etiological factor of this disease was finally proved by the scientists only in 1987. It turned out to be a virus belonging to Paramyxoviridae family, the *Pneumovirus* (*Metapneumovirus*) genus. Chickens and turkeys of different ages suffer from *Metapneumovirus* infection (MPVI). The infection might reach 100% of the susceptible livestock population on poultry farm, with a mortality rate of 2–5%. Clinical features of MPVI are not pathognomonic. The cases of asymptomatic course of the disease in wild birds and poultry were described. According to literature data, retroviral and metapneumoviral infections, mycoplasmosis, colibacteriosis, both independently and in association, are particularly dangerous for the poultry, which complicates the clinical onset of the disease and the epizootic situation in certain regions. As for Ukraine, such studies have not been conducted. The aim of our research has been to make the epizootic monitoring on poultry farms of different forms of ownership to study MPVI flow on the background of viral and bacterial microflora, using the modern research methods. 9-day-old chicken embryos, free from specific antibodies to MPVI and *Reovirus* and 6-day-old quail embryos served as the materials for the research. Molecular-genetic (PCR) and serological (indirect hemagglutination test and ELISA), virological and bacteriological research have been performed in accordance to generally accepted methods. The article presented the data of epizootological monitoring that has been carried out among the turkeys at 170-day-old of 'Big-8' cross and geese at the age of 180–210 days of large grey breed on two private farms of Kharkiv region. Serological studies have established the associative flow of MPVI and *Reovirus* infection on the background of colibacillosis, pseudomonosis and staphylococcosis at the investigated species of poultry. Existence of *Reovirus* and *Pneumovirus* in diseased poultry has been confirmed by virological studies. The conclusion is that 100% of positive-reflex to *Metapneumovirus* and *Reovirus* infection have been found on private poultry households among the turkeys of 'Big-8' cross and geese of large grey breeds at 170–210 days age, indicating their associated flow on the background of colibacillosis, pseudomonosis and staphylococcosis. The sensitivity of isolated culture to medicinal products is determined. Pathogenic disease — *Reovirus* have been isolated and identified from the seropositive reacted to the *Reovirus* infection.

Keywords: turkeys, geese, epizootological and clinical features, serological and virological investigations, *Metapneumovirus*, *Reovirus*, bacterial infections

Introduction. The disease is accompanied by respiratory symptoms and chicken heads swelling (swollen head syndrome, SHS), was first described in South Africa in 1978 (Buys, du Preez and Els, 1989).

In 1985, similar disease was observed in turkeys in the United Kingdom (Norfolk) and Wales, which was spread rapidly to other countries of the world (Anon., 1985). Nowadays, the disease is registered in Israel, the USA, Canada, England, Northern Ireland, Spain, Australia, Brazil, Morocco, and Russia (Borisova and Starov, 2006; Vinokhodov, 2003; Volkova et al., 2003; Irza et al., 2003; Bennett et al., 2002). In 1987, scientists finally proved that the etiological factor of this disease is a virus, and according to its biological characteristics, it labels to family Paramyxoviridae, genus *Pneumovirus* (German, 2007). Chickens and turkeys of different ages are sensitive to metapneumoviral infection. Clinical features of MPVI are non-pathognomonic and characterized by respiratory disturbances: sneezing, tracheal rales, nasal secretions, and conjunctivitis, swelling of infraorbital sinuses. Asymptomatic form of the disease is described in feral

and domestic poultry (Alexander, 1988; Bennett et al., 2002).

Metapneumovirus (MPV) was first isolated in 2001 in the Netherlands from children samples (Van den Hoogen et al., 2001). This poultry demonstrated the respiratory tract acute disease symptoms. About 25% 6–12-month-old chicken had antibodies of this virus, and 5 to 10 years age poultry had antibodies in 100% cases (Human metapneumovirus, 2017).

Metapneumoviral infection (MPVI) is capable to cover up to 100% of the susceptible poultry farm population in a short time, but the mortality rate is usually not more than 2–5%. The acute of clinical disease features depends on the sanitary maintenance conditions and the presence of bacterial pathogens and other viral diseases. According to literature data, especially dangerous for birds are *Reovirus* and *Metapneumovirus* mixt-infection, associations with mycoplasmosis, colibacteriosis, which complicates the clinical features of the disease and epizootic situation in some regions (Apatenko, 2002).

The diagnosis of poultry MPVI is relied on laboratory tests based on epizootological data, clinical signs, serological examination, and pathologic-anatomical changes.

The aim of the research was to make epizootic monitoring on poultry farms of different forms of ownership to study MPVI flow on the background of viral and bacterial microflora using modern research methods.

Materials and methods. To isolate the virus 9-day-old chicken embryos and 6-day-old quail embryos free from specific antibodies to *Metapneumovirus* and *Reovirus* were used.

Virology and bacteriology studies were carried out according to generally accepted methods using MPB, MPA, Salt Agar, Endo, Muller, Hoatinger Agar (Syurin et al., 1984; Birger, 1982).

Molecular-genetic (PCR) and serological studies were carried out by indirect hemagglutination test and ELISA method ('Bio-Check', the Netherlands) in the Quality and Veterinary Welfare Department of the State Research Poultry Station of the National Academy of Agrarian Sciences of Ukraine (Birky, Kharkiv region) and in the National Scientific Center 'Institute of Experimental and Clinical Veterinary Medicine' (Kharkiv) (Ushkalov, 2013).

Results. The clinical examination of two poultry species: 170-day-old turkeys ('Big-8' cross) and 170–180-days-old geese (Large grey breed) was conducted in 2016–2017 on a private poultry farm in Kharkiv region.

The poultry with clinical manifestation of the disease was detected among turkeys. The clinical signs included depressions, drowsiness, swelling of the head, inter jaw space and infraorbital sinuses, difficult breathing and mucus leakage from the beak during the tribal season in 2016. The number of death cases was 1.7% of the total number of 600 heads. Among geese, there were growth and development inhibition, lameness.

At the autopsy of dead turkeys, there has been established periorbital sinusitis, fibrin accumulation on the conjunctiva and mucous trachea, increase, blood filling and flaccid of liver, spleen and kidneys, bilateral pneumonia with the fibrin presence, hemorrhagic enteritis.

Visible pathologic-anatomical changes were not detected at forcedly killed geese.

The heart, trachea, lungs, liver, spleen, kidneys were taken from clinically diseased turkeys and geese for virological studies.

Pathogenic serotype of *E. coli* (ser.O115) that was detected from the blood of the lungs and hearts of the turkeys and geese by bacteriological studies was not insensitive to the tested antibiotics (gentamicin, oxytetracycline, floron, fluorophenicol, colistin, enrofloxacin, ciprofloxacin, norfloxacin, doxycycline).

In 2017, by serological studies of 150 blood samples were revealed 100% positive turkeys reflex on MPVI with antibody titers from 2,483 to 20,851 (ELISA) and *Reovirus* infection as in turkeys and as geese with antibody titers of 1:8 (3lg) to 1:128 (7lg) (indirect haemagglutination). At the end of lay eggs period, the antibodies titers were not beyond 1:4.

Over a period of two years, on the same farm, there were deaths of 1, 11, 18, 26, 56-day-old turkeys (up to 2%) and 5-days age goslings (1.8%), obtained from seropositive reflex parents to metapneumoviral and reoviral infections.

Pathogenic for poultry serotype *E. coli* (ser.O26), *Staphylococcus*, sensitive to gentamicin (14 mm), enrofloxacin (13 mm), ciprofloxacin (16 mm), and fluorophenicol (16 mm), *Pseudomonas aeruginosa*—gentamicin (15 mm) of fluorophenicol (18 mm), and chloramphenicol (20 mm) are isolated from the turkeys of different ages by bacteriological studies carried out in 2016–2017 (Table 1).

Table 1 — Determined sensibilities of isolates as to the medical preparations

Preparation	Lytic zone, mm
<i>E. coli</i> (ser. O26), <i>Staphylococcus</i>	
Tiamulin	0
Tilozin	0
Tilmicozin	0
Lincomycin	0
Gentamicin	14
Oxytetracycline	0
Floron	0
Colistin	0
Fluorophenicol	16
Enrofloxacin	13
Ciprofloxacin	16
Doxycycline	0
Amoxicillin	0
Spectinomycinum	0
<i>Pseudomonas aeruginosa</i>	
Gentamicin	15
Fluorophenicol	18
Chloramphenicol	20

Isolation of *Reovirus* from turkey and geese pathological material from, which were seropositive to *Metapneumovirus* and *Reovirus* as well, was carried out in 9–10-day-old embryos. These embryos were free from maternal yolk antibodies to these pathogens. The suspension of clinical materials (organs) was inoculated onto chorion-alantoic membrane of embryos according to accepted method. Three passages of each material were carried out.

Pathognomonic changes concerning reoviral infection (increased blood flow and yellow fate right of liver) were observed while postmortem autopsy at the infected embryos by suspension of organs taken from sick geese. Identification of isolated virus carried out in indirect hemagglutination test with using of positive and negative blood serum, that are included in the ELISA panel for the diagnosis of *Reovirus* infection. *Reovirus*, isolated from turkeys, was used in indirect hemagglutination diagnostic test as the antigen for red blood cells marking. In the study of field blood serums of geese and turkeys by this red blood cells virus contained antigen, the specific antibodies to *Reovirus* were detected in 6–7 log₂ titers.

100% seropositive to *Reovirus* and *Metapneumovirus* as well in titers 1:16–1:64 was detected by indirect hemagglutination in the 210-day-old geese of large grey breed in other courtyard.

Conclusions. 1. On private poultry farms among the turkeys of 'Big-8' cross and geese of large grey breeds at 170–210 days age were detected 100% positive- reflex on *Metapneumovirus* and *Reovirus* infection with titers from 2,483 to 20,851 (ELISA) and from 3 to 7 lg (indirect hemagglutination test), that indicates their associated course.

2. Pathogenic disease — *Reovirus* has been isolated and identified from the seropositive reacted to the *Reovirus* infection.

3. Pathogenic serotype of *E. coli* (ser. O115), that is not sensitive to antibiotics, has been isolated from clinically diseased by MPV and *Reovirus* turkeys and geese (2016). In turkeys (2017), MPVI was marked by colibacillosis, staphylococcosis, and pseudomonosis, the causative agents of which were sensitive to gentamicin, enrofloxacin, ciprofloxacin, fluorophenicol, and chloramphenicol.

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