Morphological Changes in Bird Viscera in Experimental Infection by Highly Pathogenic H5N1 Avian Influenza Virus

L. V. Shestopalova, V. A. Shkurupiy*, T. V. Sharkova, and A. M. Shestopalov**

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Intravenous infection of chicken with H5N1 avian influenza virus (strain A/Gs/Krasnoozerskoye/627/05) causes rapid lethal outcome. Pathomorphological study of bird viscera showed manifestations of disseminated intravascular coagulation syndrome, generalized inflammatory reaction, and wide-scale necrobiotic changes in tissues.

Key Words: *avian influenza; H5N1 subtype; chicken; pathogenesis; pathomorphology*

Avian influenza is a hazardous infectious disease caused by avian influenza A viruses (AIV). Waterfowl are also AIV carriers. Subtype H5 AIV changed in recent years, due to which it overcame the species barrier. Its virulence for not only domestic geese and ducks, but also for its natural hosts (wild waterfowl) increased. Previously rare epizootics caused by highly pathogenic AIV strains are now characterized by involvement of some mammalian species and humans [2,9,12]. Since 2003 highly pathogenic H5N1 AIV caused overall deaths of birds not once [10] and was responsible for human disease with lethal outcomes in more than 50% cases [3,6,7].

Highly pathogenic H5N1 AIV strains were isolated in summer, 2005 during an epizootic in birds in the Novosibirsk region [1]. The pathological changes in organs of dead animals were not studied then. High variability of AIV and the threat of infection for mammals and humans prompt studying the pathological changes in the viscera of domestic chicken as the most probable source of human infection.

We studied morphological changes in chicken viscera after infection with a new highly pathogenic H5N1 AIV strain isolated from birds which fell ill during an epizootic of 2005 in the Novosibirsk region.

MATERIALS AND METHODS

H5N1 viruses were isolated from the lung tissue and cloacal washings from fowl (domestic chicken, ducks, geese) which fell ill or were dying. The isolated strains were used for infection of 10-day chicken embryos, in which allantoic fluid was collected after 36-48 h of egg incubation at 33-34°C. The virus was identified using antibodies to hemagglutinin (gracious gift from Dr. R. Webster, Infectious Disease Department of St. Jude Children's Research Hospital, Memphis, USA). The initial virus titer was $10^{9.2}$ EID_{50}/ml (EID_{50} is embryonic infective dose, at which 50% embryos are infected).

Virological analysis of H5N1 AIV strains isolated from birds dead during the disease outbreak in July, 2005 in Novosibirsk region showed that strain A/Gs/Krasnoozerskoye/627/05 was highly

Department of Physiology, Novosibirsk State University; *Center of Clinical and Experimental Medicine, Siberian Division of Russian Academy of Medical Sciences, Novosibirsk; **Vector State Research Center of Virology and Biotechnology, Novosibirsk Region, Koltsovo, Russia. ***Address for correspondence:*** lv@fen.nsu.ru. L. V. Shestopalova

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pathogenic for chicken [5], due to which it was used in our experiment.

Clinically healthy chicken aged 6 weeks were used. They were infected with a solution containing A/Gs/Krasnoozerskoye/627/05 virus in a concentration corresponding to 1:100 dilution of the suspension with the initial titer of $10^{9.2}$ EID_{50}/ml. The birds were infected into the axillary vein in a dose 0.5 ml per bird.

Material for the analysis was collected after 24-36 h from 10 chicken in a state of agony. Specimens of the lungs, liver, kidneys, spleen, small intestine, and brain were collected.

The specimens were fixed in 10% formalin and dehydrated in ascending alcohols, butanol, xylene, and embedded in paraffin with bee wax. The sections (5-7 μ) were stained with hematoxylin and eosin and examined under an Axioscop 40 microscope fitted with an AxioCam MRc digital photocam (Carl Zeiss).

RESULTS

The first signs of the disease in chicken manifested 6-8 h after infection, which was in line with the previous report [4]. The disease manifested by decreased mobility, lameness, loss of coordination, progressive dyspnea and diarrhea. The birds died after 24-36 h.

The lungs are one of the main targets for AIV [11,14]. Incorporations seen in the alveolar cells were presumably aggregations of viral particles. This hypothesis is based on findings of electron microscopy of the viscera of birds dead from H5N1 AIV [13]. Viral particles in infected chicken were most often detected in the lung alveolocytes, blood vessel endotheliocytes, macrophages, and granular leukocytes [13].

Pulmonary blood capillaries were dilated and hyperemic. Clots including cell detritus and fibrin were often seen in the vascular lumen. Vascular walls were in a state of plasmatic imbibition, endothelial lining was impaired. Alveolar cavities were filled with blood cells; in addition to erythrocytes and leukocytes, platelets were seen. Alveolar and bronchial epitheliocytes were necrotic. Cell necroses and small foci of leukocytic infiltration were detected also in the lung stroma (Fig. 1, *a*).

Splenic red pulp sinuses were dilated; hemostasis and erythrocyte hemolysis were seen in the blood vessels. Small hemorrhagic foci, multifocal necroses, separate necrotic cells were seen in these zones (Fig. 1, *b*). Numerous macrophages in necrotic zones contained large and small incorporations. Higher polymorphism of cellular composition in the center of primary nodules indicated the beginning formation of germinative centers, sites of proliferation of B-immunoblasts. However, rapidly progressing destructive processes in vitally important organs led to bird death long before the potential of defense forces could be realized.

The cord structure of the liver was as a rule impaired by hemorrhages. Vascular wall of large

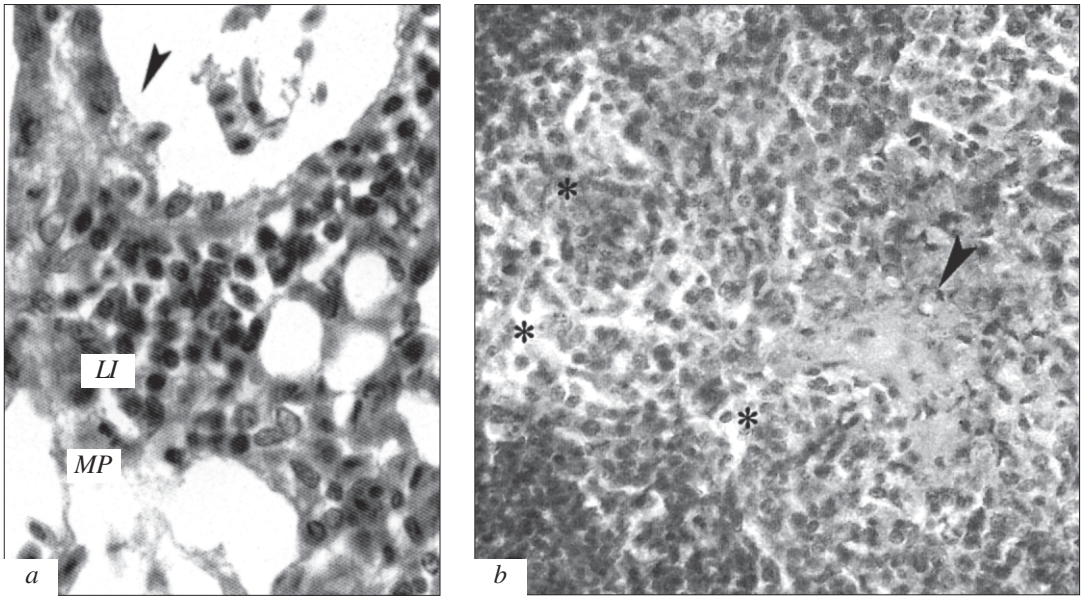

Fig. 1. Fragments of the lung (*a*) and spleen (*b*) of chicken infected by H5N1 AIV. *a*) alveolar cavities filled by blood cells; necrotic epitheliocytes in the alveoles (arrow) and bronchi; necrotic cells and small foci of leukocytic infiltration (LI) in lung stroma; macrophages (MP) with large and small incorporations; *b*) impaired structure of the spleen, large and small vessels are dilated; hemorrhagic foci (arrow) and necroses (asterisk). Here and in Figs. 2, 3: hematoxylin and eosin staining, ×1600.

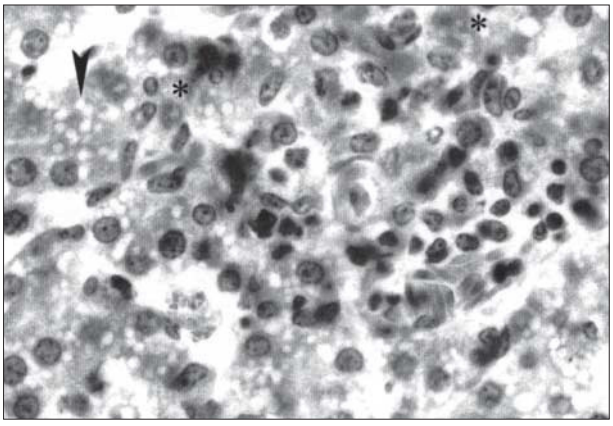

Fig. 2. Liver fragment from a chicken infected with H5N1 AIV. Girder structure is impaired; hemostasis, erythrocyte hemolysis in large vessels; sinusoids are dilated; foci of hemorrhages and necroses (asterisk), leukocytic infiltration; hepatocytes (arrow) in a state of vacuolar degeneration.

hepatic vessels and central veins was edematous, in many cases the endothelial lining was impaired, and erythrocytes were hemolyzed. The sinusoids and Disse spaces were significantly dilated, particularly in the subcapsular zone. Numerous cell elements and macrophages with dark brown incorporations in the cytoplasm were seen in the sinusoids and Disse space. Similar incorporations were seen also in the central vein and sinusoidal endotheliocytes. Centrolobularl leukocytic infiltration was seen. Hepatocytes were in a state of hydropic degeneration (Fig. 2). Hepatocyte necroses were most often seen at the periphery of hepatic lobules, most often near large blood vessels, and apoptotic bodies were seen in the stroma and macrophages.

Renal lesions were not so deep as in the lungs, spleen, and liver. Despite signs of hemodynamic disorders, the organ structure was in general retained. The most significant changes were as follows: small foci of leukocytic infiltration, mainly near large vessels, hemorrhages and fibrin depositions presenting as compact vitreous pink mass in renal bodies, floccular contents in the lumen of proximal and distal tubules of the nephron. Tubular epithelium was edematous, some cells in a state of hydropic and balloon degeneration and necrosis. Epithelial cells in collecting tubules were hydropic and often destroyed (Fig. 3, *a*).

Brain tissue was edematous, blood vessels dilated with signs of hemostasis. Hemorrhagic foci of different size and location were seen. Foci of necrosis and neurons with signs of degeneration, topographically often not linked with blood vessels, were seen everywhere (Fig. 3, *b*). Chromatolysis was observed in many cerebral neurons. Heteromorphic basophilic accumulations (a result of overall neuronal death) were often seen in the tissue; presumably these were nonphagocytosed cell structures. The count of microglial cells significantly increased, particularly in the perivascular zone. Structural lesions of the brain resulted in serious motor disorders.

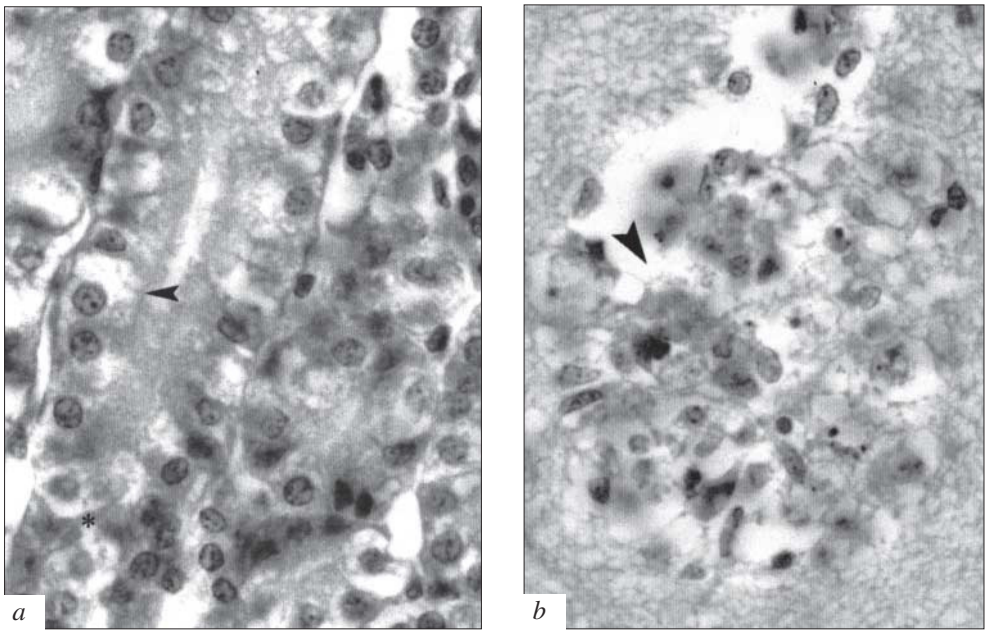

Fig. 3. Fragments of the kidney (*a*) and brain (*b*) of chicken infected by H5N1 AIV. *a*) hemostasis in renal vessels; small foci of leukocytic infiltration; proximal tubules are stenosed, with floccular contents in the lumen; edematous tubular epithelium (arrow); part of cells in a state of hydropic and balloon degeneration, necrosis (asterisk); *b*) edematous brain tissue, dilated blood vessels with signs of hemostasis, foci of hemorrhages of different size and location, necrotic foci (arrow) and neurons with signs of degeneration, chromatolysis everywhere.

Edema of the mucosal and submucosal lamina propria was noted in the small intestine; epitheliocytes with signs of necrosis were rare. On the whole, the organ looked little changed.

Histological study showed pantropism of the virus strain used for infection. Virtually all examined chicken viscera had pronounced destructive changes leading to suppression of their functional activity. Hemostasis, erythrocyte hemolysis and fibrinoid swelling of vascularl walls, plasmatic imbibition of the stroma, leukocytic infiltration, numerous large and small hemorrhagic foci, degenerative changes and necrosis of parenchymatous structures, activated macrophages with signs of phagocytosis were characteristic of the majority of organs of infected birds. Similar changes were observed in birds infected by other highly pathogenic H5N1 AIV strains [7,13]. Japanese scientists detected a gene expressing a tissue factor (the main initiator of coagulopathy development) in chicken infected by AIV [8]. Presumably, the development of coagulopathy and disseminated intravascular coagulation, signs of which were noted in tissues of the majority of examined organs, result in hemodynamic and tissue homeostasis disorders and development of deep destructive lesions. This results in suppression of functional activity of the main body systems and eventuates in death.

Hence, rapid death of birds after intravenous infection with highly pathogenic H5N1 strain A/Gs/Krasnoozerskoye/627/05 is a result of the development of deep morphofunctional disorders in the majority of organs.

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