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CLINICAL AND PATHOLOGICAL STUDIES OF DUCKLINGS EXPERIMENTALLY INFECTED WITH AN ISOLATE OF AVIAN INFLUENZA A VIRUS SUBTYPE H6N2

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ABSTRACT

Clinical and pathoanatomical studies were carried out on thirty 28-day old *Mullary* ducklings infected intravenously, tracheally and orally with an isolate of avian influenza A virus H6N2 obtained from wild ducks *Anas plathyrynchos*. With the objective to elucidate the pathogenesis of the isolate for this species, we determined and studied all the possible clinical findings and pathoanatomical lesions in ducklings under conditions of experimental viremia. The avian influenza A virus H6N2 isolate caused disturbances in body development and feathering, well demarcated pathoanatomical changes in the thymus and heart without a fatal terminal episode. Get together these results and the references from experiments with ducks inoculated with various isolates of type A influenza show that the different localization of lesions corresponds to the tissue tropism of the specific viral strain.

Key words: avian influenza A virus, ducklings, experiment, clinical findings, pathoanatomical findings

INTRODUCTION

As early as 1961, when the first avian influenza A virus (AIV) isolates were obtained from ostriches, it was found out that another different species is not infected (1). This fact triggered studies of AIV in different avian species, including ducks; mostly 2–4-week old and sometimes one or more days old ducklings infected intravenously (2), orally and tracheally (3, 4).

Most of the spontaneous and experimental cases are in a sub-clinical form with no clinical signs of a disease (4). In experiments with strain H5N7 (5) and H11N1, H6N2 and H3N1 (3) the same results have been obtained. Clinical signs have been observed with HPAIV isolate H5N1 in ducklings (6) – respiratory disease, depression, diarrhea, loss of appetite and increase in death rate up to 12%. In other experiments loss of weight has been detected in ducklings infected intravenously (2).

In experimental ducks with the absence of clinical signs in the most cases pathoanatomical changes are found. Splenomegalia on the 4^{th} – 10^{th} day in 56% and hyperplasia of the air sacs on the 4^{th} – 7^{th} day in 11% of the cases were observed (7). In spontaneous clinical cases were detect necrosis in the pancreas, splenomegalia, hepatomegalia and enlarged kidneys (8).

This paper describes the experimental infection in ducklings with isolate AIV subtype H6N2 and possible clinical signs and pathoanatomical lesions.

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MATRIALS AND METHODS

1. Virus. The fourth passage with a titre of $10^{5,0}$ ELD_{50}/0,1 ml of an isolate of avian influenza A virus subtype H6N2 obtained from wild ducks (*Anas plathyrynchos*) was

applied (9).

2. Experimental birds. Thirty 28-day old ducklings a cross between the Pekin and the dumb Turkish Mallard breeds (Mullary) were used. The birds were kept in two separate isolated 2 x 2 m rooms at 13-hour regimen of day-light, temperature of 20 ^{0}C, humidity of 70% and feeding and water front of 0,9 m (regulation No 13/25.04.2002). The birds were fed the same quantity and kind of feed.

3. Infection. Intravenous, tracheal and oral infections were carried out (8 birds each route) with 0,1 ml allantoic fluid from virus cultures in chicken embryos (CE); 6 birds were inoculated with allantoic fluid from intact CE and used for control.

4. Clinical examination. The ducklings were monitored for clinical signs daily throughout the experiment. Live body weight measurements were determined with electronic balance (ATEW-15, division:lg, capacity:15 kg, S/N:1412274001) the day before the infection and on the 3rd, 5th, 7th, 10th, 14th, 21st, and 28th day.

5. Pathoanatomical examinations. Pathoanatomical examinations (all visceral organs and brain) of some of the ducklings were carried out on the 8th, 14th, 21st, and 28th day post infection, following the humanity provisions of regulation No 25/10.06.2003 (article 20. 1, appendix 4, point B) and permit No 3/27.06.2006 issued by the Bulgarian Ministry of Agriculture and Forests.

6. Statistic processing of the weight data. The obtained absolute statistic values of the ducklings live body weights were processed with STATISTICA version 6,0 (StatSift, Tulsa, OK, USA) and the average arithmetic values (X), the average arithmetic errors (SX) and the standard deviations (Sd) were determined.

RESULTS

Clinical findings. Clinical changes were not observed in the respiratory, digestive, secretory and nervous systems. However, in a long-term aspect disturbances were observed in the body development and feathering rate in the control intact and intravenously infected ducklings, and to a smaller extent in the tracheally infected ducklings (Figure 1).

In 28 days live weight increased with 208.2% in the control, with 203.4% in the orally infected and with 188,3% in the tracheally infected group. The slowest gain (174.3%) and extensive feathering disturbances were observed in the intravenously infected

Figure 1. *Ducklings. Underdevelopment and disturbances of feathering in intravenously infected ducklings. A – control, B – infected (21 days after infection, 46-day old ducklings)*

ducklings. In the tracheally and intravenously infected ducklings retarded body development was detected as early as the 3rd – 5th day, followed by an accelerated body development on the 10th day. In different periods after the intravenous and tracheal infection significant weight differences were obtained. Among the intravenously infected ducklings compared to the control significant weight differences ($P<0.001$) were obtained on the 10th, 14th, 21st and 28th day; differences were obtained also on the 5th ($P<0.01$) and the 7th day ($P<0.05$). Among the tracheally infected ducklings significant weight differences ($P<0.05$) compared to the control were observed on the 3rd, 5th, 10th, 14th and 21st day.

Pathoanatomical changes. Character and severity of lesions varied from high after the intravenous infection to low after the tracheal and oral infection. Lesions prevailed in the period between the 8th – 14th days after exposure to the virus.

The gross lesions were mainly of viremic or necrobiotic character. Petechial, ecchymose or vibic hemorrhages in the epicardium, myocardium and endocardium were observed in 50% of the ducklings, independently on the length of exposure (Figure 2). Hemorrhages, not clearly demarcated, were localized in the left and right coronary walls, both in the atrium and the ventricle areas.

Numerous petechial hemorrhages were found out bilaterally in the thymus glands in 50% of the ducklings on the 8th – 14th day after infection (Figure 3). Persistent hyperemia of the parenchymal organs (liver, spleen, kidneys and lungs) was observed throughout the experiment.

No lesions were detected in the control group.

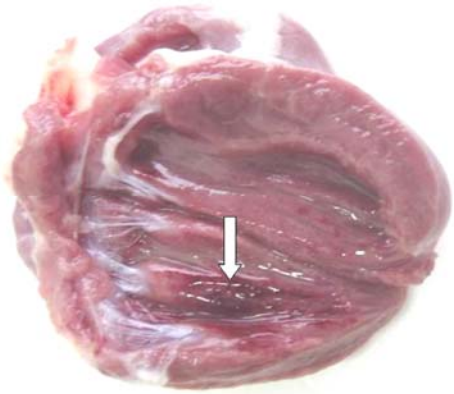

Figure 2. *Heart; duckling. Ecchymose and vibic haemorrhages in the mio- and endocardium of the left ventricle, 28 days after tracheal infection (arrow).*

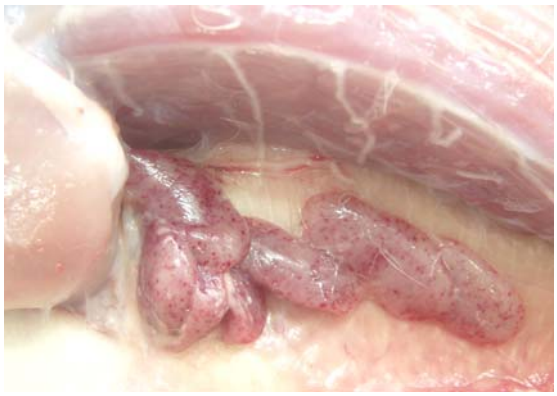

Figure 3. *Thymus; duckling. Numerous petechial haemorrhages in all parts of the gland, 8 days after tracheal infection.*

DISCUSSION

Experimental infection of ducklings with an isolate of avian influenza A virus strain H6N2 resulted in delayed development and pathoanatomical changes in the thymus and the heart but did not cause death.

We observed severe clinical disturbances in growth of higher value than Laudert et al. (1993) accompanied with feathering disturbances (no references found), both resulting in serious economic losses, which can be explained with the immunosuppressive effect of the isolates due to atrophy of the periphery immune organs (bursa of Fabricius, thymus, and spleen). These results are similar to those, described in cases of thymus hypofunction and reduced secretion of thymus hormones including slow growth, cachexia, retarded development and loss of feathers (Carpenter, 2004). These prolonged effects contribute to the pathoanatomical changes after the 21st day, such as hypotrophy of liver, atrophy of the bursa of Fabricius (Perkins and Swayne, 2002) and atrophy of spleen.

Significant differences are described related to the type of strain and the pathoanatomical changes. Laudert et al. (1993) demonstrate the role of the strain type by experiments with 11 strains from different sources (aquatic birds, pheasants, and turkeys). The same authors detect changes induced by the most of the applied strains in the spleen (86%), in the liver and the bursa of Fabricius (57%), kidneys (43%) , lungs (28.5%), none in the thymus and the heart (observed in the described experiment) and none in the pancreas and intestines. Comparing the reference data with our pathoanatomical results from ducklings experimentally inoculated with different isolates of type A influenza, we concluded that the different localization of lesions corresponds to the tissue tropism of the specific virus strain (Laudert et al., 1993). Perkins and Swayne (2002) in experiments with HPAIV strain H5N1 and Cooley et al. (1989) with other H5 strain detect major changes mainly in the respiratory tract (rhinitis, laryngitis, interstitial pneumonia, aerosacculitis) and in the spleen and no lesions in other organs. In contrast, the respiratory lesions did not prevail in our cases.

REFERENCES

1. Becker, W.B. Experimental infection of common terns with tern virus. *Journal of Hygiene,* 65: 61-65, 1966.
2. Laudert, E., Halvorson, D., Sivanandan, V., Shaus, D. Comparative evaluation of tissue tropism characteristics in turkeys and mallard ducks after intravenous inoculation of type A influenza viruses. *Avian Diseases,* 37: 773-780, 1993.
3. Sandhu, T., Honshaw, V.S. Influenza A virus infection in domestic ducks. In R.A.Bankowski (Ed.). Proceedings of the *First International Symposium on Avian Influenza*. Carter Printing Co. Lib. Cong. Cat Card No 86-051243, April 22-24. Beltsville, USA, 93-99, 1981.
4. Cooley, A.J., Campen, van H., Philpott, M.S., Easterday, B.C., Hinshaw, V.S. Pathological lesions in the lungs of ducks Infected with Influenza A viruses. *Veterinary Pathology,* 26: 1-5, 1989.
5. Wood, J.M., Webster, R.C., Nettles, V.F. Host range of A /chicken/

Pennsylvania /83/H5N2/ influenza virus. *Avian Diseases,* 29: 198-207, 1985

6. Webster, R.G., Peiris, M., Chen, H., Guan, Yi. H5N1 Outbreaks of Enzootic Influenza. *http://www.cdc.gov/ncidod/eid/, 12*, № 01/05-1024.htm, 2005.
7. Perkins, L.E. and Swayne, D.E. Pathogenicity of a Hong Kong – origin H5N1 highly pathogenic avian influenza virus for emus, geese, ducks, and pigeons. *Avian Diseases,* 46, 1: 53-63, 2002.
8. Yong-Kuk Kwon, J. S-J., Kim, M-Ch., Sung, H-W., Lee, Y-J., Choi, J-G., Lee, E-K., Kim, J. H. Highly pathogenic avian influenza (H5N1) in the commercial domestic ducks of South Korea. *Avian Pathology, 34*, 4: 367-370, 2005.
9. Zarkov, I.S., I. Bochev, R. Manvell, W. Shell. The First Isolation of Avian Influenza Virus in Bulgaria. *Journal of Veterinary Record, 158*, 3: 106-107, 2006.
10. Carpenter, S.H. Avian Immune System. Holistic bird newsletter. *(http://www.holistic birds.com/hbn04/spring 04/immunesystem.htm.)*, 4, 1: 1-6, 2004