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EXCRETION OF AVIAN INFLUENZA A VIRUS SUBTYPE H6N2 IN INTRAVENOUSLY INFECTED DUCKLINGS AND CHICKENS POULTS

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ABSTRACT

Experimentally infected with avian influenza A virus of H6N2 subtype chickens (n=9) and ducklings (n=7) and control uninfected birds (n=11) were used. Virus was isolated from all the ducklings (100%) and from 33% of the chickens for various periods of time (21 and 5 days, respectively); the numbers of isolates being higher in the beginning of the infection. Positive cloacal samples prevailed compared to the oropharengeal (42.9% vs. 16.3% in the ducklings and 6.3% vs. 1.6% in the chickens) for a shorter period of isolation from the oropharynx.

Keywords: chickens, ducklings, avian influenza virus, excretion

INTRODUCTION

Wild waterfowl, in particular A*nas plathyrynchos*, are natural hosts and a reservoir of the influenza A virus infection mainly poultry *Gallus domesticus* (1). Infection with the low-pathogenic avian influenza A virus (LPAIV) depends on the avian species and the duration of re-isolation of the virus from the oropharynx and the cloaca varies widely (2, 3, 4). From ducklings virus has been isolated from 8.3% (5) to 45.7% (2) of the birds, virus excretion lasting from 30 days (6) to 4 – 7 days (2). In chickens virus isolates have been obtained from 0 (2) and 4% (7) to 43% (4) and 95.2% (8) of the birds. Virus excretion continued for 5 – 7 days (3, 4, 9, 10) or 14 days (11), in most cases from the oropharynx lasted longer than from the cloacae (4, 8), in other cases for the same (10) or shorter (3) time.

The present study with experimentally infected ducklings and chickens with LPAIV H6N2 subtype was aimed at determining the specific number of isolates, duration and site of virus excretion.

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MATERIAL AND METHODS

Virus. Low-pathogenic avian influenza A virus of the H6N2 subtype obtained from a wild duck *Anas plathyrynchos* was used at a titer of 105 ELD50 /0.1 ml.

Birds. Chickens of the Decalp breed (n=15) and *Anas plathyrynchos* ducklings (n=9), all 30 days old, were used. Part of them (9 chickens and 7 ducklings) was intravenously infected each with 0.1 ml allantoic fluid from infected chicken embryos (CE), the rest (uninfected control group) obtained 0.1 ml allantoic fluid from intact CE.

Procedures. Birds were kept separately in 4 x 4 m rooms at 1.8 m feeding and watering front, 20 0C and 70% humidity. Cloacal and oropharyngeal swabs from all the infected and uninfected birds were collected on days 0 (before infecting), 3, 5, 7, 10, 14, 21 and 28 post infection (P.I.).

A 10% suspension of the samples (prepared in MEM) were inoculated into the allantoic sac of three 9-day old CE. The infected embryos were incubated at 36 0C for 120 hours, then the dead and living CE were cooled at 4 0C for other 2 hours and allantoic fluid collected. Presence of hemagglutinating virus and titre of viral hemagglutinins were determined by hemagglutination inhibition (HI) as described Annonimus, 2005 (12).

RESULTS

Avian influenza A virus was isolated from all the ducklings (100%) and from 33% of the chickens (Table 1). The number of isolates varied with time: from 29% on day 21 – 86% on day 7 for the ducklings and 11% on day 3 – 22% on day 5 for the chickens. The results obtained for the specific species, site and period are given in Table 2. The percent of positive samples was highest in the ducklings (29.6%), followed by the chickens (4.0%). The positive cloacal samples prevailed at 42.9% in the ducklings and 6.3% in the chickens compared to the positive oropharyngeal samples whose number was 2.5 times less in the ducklings (16.3%) and 4 times less in the chickens (1.6%). Positive oropharyngeal samples were isolated until the 10th day P.I. in the ducklings and the 3rd day P.I. in the chickens.

Table 1. *Number of birds from which influenza A virus H6N2 subtype is isolated after intravenous infection.*

Day	Ducklings	Chickens
3	5*/71**	1/11
5	5/71	2/22
7	6/86	0/0
10	3/43	0/0
14	3/43	0/0
21	2/29	0/0
28	0/0	0/0
Total	7/100	3/33

*Legend: *- number, **- percent*

Table 2. *Number of positive birds from chicken infected intravenously with influenza A virus H6N2 subtype*

Day	Ducklings			Chickens		
	Total n=98	Cloacae n=49	Oroph. n=49	Total n=126	Cloacae n=63	Oroph. n=63
3	10/9.8	5*/10.2**	5/10.2	4/3.2	3/4.8	1/1.6
5	6/6.1	4/8.2	2/4.1	1/0.8	1/1.6	0/0
7	4/4.1	4/8.2	0/0	0/0	0/0	0/0
10	4/4.1	3/6.1	1/2.0	0/0	0/0	0/0
14	3/3.1	3/6.1	0/0	0/0	0/0	0/0
21	2/2.0	2/4.1	0/0	0/0	0/0	0/0
28	0/0	0/0	0/0	0/0	0/0	0/0
Total	29/29.6	21/42.9	8/16.3	5/4.0	4/6.3	1/1.6

*Legend: *- number, **- percent, Oroph.= Oropharynx*

DISCUSSION

In the present study virus was isolated from all species: *Anas plathyrynchos* ducklings and chickens, infected intravenously with LPAIV H6N2 subtype, in contrast to earlier negative results obtained with chickens (2). Replication of the virus occurred in the digestive and respiratory tracts.. This and other LPAIV strains are locate in sites with the presence of tripsin-like ferments - the respiratory and digestive tracts.

Ducklings (the natural host) are much more sensitive to the virus than chickens (100%, 33%, respectively). The present results show that if the virus is obtained from one species and experimentally inoculated into other species the course of infection changed (supporting the results of 2, 4) which was manifested by a longer period of virus isolation from the original host (21 days) compared to the other species (5 days). Unlike LAUDERT *et al.* (2) we isolated the virus from more birds: 100% vs. 45.7% in the ducklings, 33% vs. 0 in the chickens. Excration of the virus by the natural host in this experiment lasted 21 days vs. 30 days reported by WEBSTER *et al.* (6). In the chickens in this case the virus was shed for 5 days as the results obtained by LAUDERT *et*

al. (2), OTSUKI *et al.* (3), TUMPEY *et al.* (4)and MO *et al.* (9).

Virus isolates from the respiratory (compared to the digestive tract) vary in number. We, like OTSUKI *et al.* (3), obtained more isolates and for a longer period from the cloacae than from the oropharynx. Other researchers obtain similar to our results (10) or a longer period of oropharyngeal isolates (4, 8). The longer persistence of virus in the cloacae in our study could be explained, on the one side, with the fact that the intravenous inoculation results in a rapid and wide distribution of the virus in the whole body including the kidneys and, on the other side, with the specific avian anatomy in which the fore intestines and the kidneys via the urethra are connected to the cloacae. These data are indirectly supported by SLEMONS and SWAYNE (8) who isolated virus both from the cloacae (95.2%) and the kidneys (61.9%).

REFERENSES

1. Alexander, D. J., A review of avian influenza in different bird species. *Veterinary Microbiology*, 74 :3-13, 2000.
2. Laudert, E., Halvorson, D., V. Sivanandan, D. Shaus, 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. Otsuki, K., Kawaoka, Y., Nakamura, T., Tsubokura, M., Pathogenicity for chickens of avian influenza viruses inoculated from whistling swans and a black-tailed gull in Japan. *Avian Diseases*, 26: 314-320, 1982.
4. Tumpey, T., Kapczynski, D., Swayne, D., Comparative Susceptibility of chickens and turkeys to avian influenza A H7N2 virus infection and protective efficacy of a commercial avian influenza H7N2 virus vaccine. *Avian Diseases*, 48: 167-176, 2004.
5. Wood, J., Webster, R., Nettles, V., Host range of A/chicken /Pennsylvania/83/ H5N2/ influenza virus. *Avian Disease*, 29: 198-207, 1985.
6. Webster, R., Yakhno, M., Hinshaw, V., Bean, W., Murti, J., Intestinal influenza: replication and characterization of influenza viruses in ducks. *Virology*, 84: 268-278, 1978.
7. Swayne, D .and Halvorson, D., Influenza. In: Disease of poultry, 11th ed. Y.M.Saif, H. J. Barnes, A. M. Fadly, J. R. Glisson, IA., USA, 135-160, 2003.
8. Slemons, R. and Swayne, D., Replication of a Water fowl-origin influenza virus in the kidney and intestine of chickens. *Avian Diseases*, 34: 277-284, 1990.
9. Mo, L., Brugh, M., Fletcher, O., Rowland, G., Swayne, D., Comparative pathology of chickens experimentally inoculated with avian influenza viruses of low and high pathogenicity. *Avian Diseases*, 41: 125-136, 1997.
10. Swayne, D. and Beck, J., Experimental study to determine of low-pathogenicity and high pathogenicity avian influenza viruses can be present in chicken breast and thigh meat following intranasal virus inoculation. *Avian Diseases*, 49: 81-85, 2005.
11. Lu, H.and Castro, A., Evaluation of the infectivity, Length of infection, and Immune Response of a Low-Pathogenicity H7N2 Avian Influenza Virus in Specific-Pathogen-Free Chickens. *Avian Disease*, 48: 263-270, 2004.
12. Anonimmous, Office International des Epizooties /OIE/. Avian Influenza, Manual of diagnostic procedures. Version adapted, 05, Chapter 2.7.12, 1-28, 2005.