

DETECTION OF INFLUENZA A VIRUSES BY SENTINEL DOMESTIC DUCKS IN AN ECOLOGICAL SURVEY

H. SINNECKER, R. SINNECKER, E. ZILSKE

Epidemiological Centre of the State Hygiene Inspection of the German Democratic Republic,
DDR-1500 Potsdam, G.D.R.

Received June 2, 1981

Summary. — In a 7-month field experiment domestic ducks were used as sentinels to detect influenza A virus in a seabird-breeding island in the Baltic sea. Twenty influenza A virus isolates representing 3 subtypes (Hav7Neq2, Hav6N2 and Hav4Nav1) were recovered from tracheal and cloacal swabs from 10 sentinel ducks. The ducks responded to natural infection by a short rise in antibodies; there was a correlation between virus isolation and appearance of antibodies.

Key words: influenza A virus; virus detection; sentinel ducks

Introduction

The surveillance of birds and other animals for influenza A viruses has been aimed at elucidating the ecology of these viruses. Over the past few years, numerous influenza A viruses have been isolated especially from ducks, which suggests that the duck is highly susceptible and may play an important role in the ecology of influenza A viruses (Laver and Webster, 1979; Hinshaw *et al.*, 1979).

We are presenting the results of a field experiment designed to determine whether domestic ducks could be used as sentinels at a seabird breeding island to detect the circulation of influenza A viruses and also the inter-species virus transmission. The antibody response in the naturally infected sentinel ducks was recorded over a 7-month period.

Materials and Methods

The exposure of sentinel ducks lasted from May to December, 1980. The 50-days-old white Peking ducks were previously held in special quarters for serological and virological examinations to ascertain that they were free from influenza A viruses. The ducks were exposed on a seabird-breeding island (gulls, terns, ducks) in the Baltic sea, where we previously found influenza A viruses in gulls and terns. The sentinel ducks were kept in a fenced-in part of a breeding site of gulls on the island, with grassland and a waterpool, until death or for the preestablished 7-month period. Inspectors taking care of the ducks visited them weekly and added food as needed.

Virus isolation and serological tests. Tracheal and cloacal swabs were collected about twice monthly. Each swab was placed into 1 ml of transport medium consisting of phosphate-buffered saline (pH 7.2) containing 50% glycerol and penicillin, streptomycin and moronal. Swabs were

Table 1. Isolation of influenza A virus from, and detection of HI and NI antibodies in, sentinel ducks

Date and test*	Sentinel duck No.									
	1	2	4	7	9	11	12	13	21	22
7 May V,H,N	0	0	0	0	0	0	0	0	0	0
22 May V,N	0	0	0	0	0	0	0	0	0	0
H	H0	0	0	0	H0	0	0	0	0	0
30 May V	0	0	0	0	0	0	0	0	0	0
18 June V,N	0	0	0	0	0	0	0	0	0	0
H	0	0	0	0	0	0	0	0	H0	0
3 July V	0	0	0	0	0	0	0	0	0	0
19 July V,H	0	0	0	0	0	0	0	0	0	0
N	0	0	0	0	0	N2	0	0	0	0
4 Aug. V	0	0	0	0	0	0	0	0	0	0
12 Aug. V	0	0	0	0	0	0	0	0	Hav7Neq2 ³	
										Hav7Neq2 ¹
H,N	0	0	0	0	0	0	0	0	0	0
29 Aug. V	0	0	0	0	0	0	0	0	0	0
11 Sept. V	0	0	0	0	0	0	0	0	0	0
H	0	0	0	0	0	0	0	0	H3	0
N	Neq2	0	0	Neq2	Nav5	0	Neq2	0	Neq2	0
28 Sept. V	0	0	0	0	0	0	0	0	0	0
9 Oct. V	Hav6N2 ²		Hav6N2 ³		Hav6N2 ³		Hav6N2 ¹		Hav6N2 ³	Hav6N2 ³
H	0	0	0	death	?		0	0	0	0
N	0	0	0				0	0	0	Neq ²
23 Oct. V	0	0	0				Hav4Nav1 ²	0	Hav4Nav1 ²	
										Hav4 ² Nav1 ³
5 Nov. V	0	0	0		0		0	0	0	0
H	0	Heq1	Hav6		0		Hav6	Hav6	0	0
N	0	0	0		Nav5,N2		N2	0	0	0
3 Dec. V,H	0	0	0		death		0	0	0	0
N	0	0	0				0	0	0	N2
10 Dec. V,H	0	0	0				0	0	0	0
N	Nav1	0	0				0	0	0	N2
No. of subtypes isolated	1	1	1	0	1	0	2	1	3	3

V = virus isolation; H = HI test; N = NI test.

1, 2, 3 - Virus isolated from tracheal (1), cloacal (2) or both tracheal and cloacal (3) swabs.

? - Duck escaped?

kept at 4 °C for 3-5 hr and then at -80 °C. Blood samples were collected at about monthly intervals by peripheral venepuncture and the serum was stored at -20 °C until tested.

For virus isolation, the samples were processed in a laboratory in a house used only for processing field specimens. The samples were thawed and 0.1 ml volumes were inoculated into the allantoic cavities of 11-day-old chick embryos; 3 eggs were used for each sample over 1-3 passages. The eggs were incubated at 36 °C for 48 hr and tested individually for haemagglutinin activity with chicken red cells. Haemagglutination inhibition (HI) and neuraminidase inhibition (NI) tests were carried out as described by Palmer *et al.* (1975). Antisera to haemagglutinin and neuraminidase antigens of reference strains, checked with a set of antisera obtained from Dr. R. Webster, were prepared in rabbits, ferrets and chickens.

Results

A total of 20 influenza A viruses belonging to 3 subtypes were isolated from 8 of 10 sentinel ducks on the island during the study period (Table 1). These 20 strains represented 13 infections in the sentinel ducks. Seven isolations were positive from both tracheal and cloacal swabs, three only from tracheal swabs, and another three only from cloacal swabs. The isolates were obtained after the first (11), second (1) or third (1) passage.

All 10 sentinel ducks possessed antibodies with HI titres of ≥ 20 and NI titres of ≥ 15 (Table 1). Low-level and short-term positive reactions were observed against the following virus subtypes: H0 (3 ducks), Hav6 (3 ducks), H3 (1 duck), Heq 1 (1 duck), Neq2 (5 ducks), N2 (4 ducks), Nav1 (1 duck) and Nav5 (1 duck).

Discussion

The efficiency of field exposure of domestic ducks in detecting influenza A viruses was amply demonstrated by the present results. The fact that during a 7-month period a total of 20 influenza A viruses were isolated from the sentinel ducks, while only 4 were isolated over a 4-year period from captured wild birds, shows that the sentinel-duck technique was highly successful in detecting influenza A viruses in the seabird-breeding island.

Serological studies on the sentinel ducks revealed a correlation between virus isolation and the appearance of antibodies. The sentinel ducks responded to natural influenza A virus infection with a rise in antibodies but antibody titres declined within 1 to 2 months.

The appearance of Neq2 antibodies also in ducks Nos. 1, 7 and 12 one month after isolation of influenza A virus (Hav7Neq2) from sentinel ducks Nos 21 and 22 indicates that there was an Hav7Neq2 outbreak in August similar to the Hav6N2 outbreak which had occurred in October.

References

- Hinshaw, V. S., Webster, R. G., and Rodriguez, R. J. (1979): Influenza A viruses — combinations of haemagglutinin and neuraminidase subtypes isolated from animals and other sources. *Arch. Virol.* **62**, 281—290.
- Laver, W. G., and Webster, R. G. (1979): Ecology of influenza A viruses in lower mammals and birds. *Brit. med. Bull.* **35**, 29—33.
- Palmer, D. F., Coleman, M. T., Dorodle, W. R., and Schild, G. C. (1975): *Advanced laboratory techniques for influenza diagnosis*, U.S. Dept. Hlth Educ. Welfare, Atlanta.