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5) SEROLOGICAL EVIDENCES SHOWING THE INVOLVEMENT OF FREE-LIVING PHEASANTS IN THE INFLUENZA ECOLOGY (NORTHERN ITALY, 1995-2002)

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ABSTRACT From 1995 to 2002, 219 sera were collected in Northern Italy from wild pheasants. A serological survey for avian influenza viruses (AIVs) was carried out by ELISA test in order to detect type A influenza antibodies. The overall seroprevalence was 12.3%, with yearly values ranging from 0% to 42.5%. No antibodies against either H5 or H7 AIV subtypes were found by hemagglutination-inhibition test. Data from 16 recaptured birds, among 113 animals banded for individual identification, showed seroconversions in 2 pheasants. Our results indicate AIV circulation in free-living pheasants; the involvement of this land-based bird species in influenza ecology is discussed.

KEY WORDS: Pheasant, Avian influenza, Influenza ecology, Serological survey

INTRODUCTION

Land-based birds belonging to the *Galliformes* Order include species, such as turkey, chicken, and quail, that are highly susceptible to avian influenza viruses (AIVs) primarily harboured in wild aquatic birds (Webster et al., 1992). In addition to heavy economic losses due to influenza epidemics in poultry, important public health implications could arise from AIV circulation in land-based birds, recently indicated as a potential source of pandemic strains (Perez et al., 2003).

AIV infections have been described in Italy in reared pheasants (*Phasianus colchicus*) both as limited outbreaks (Rinaldi et al., 1967) or associated to severe poultry epidemics (Capua et al., 2003). Although sporadic isolations of AIVs have been reported in free-living pheasants (Romváry et al., 1976), the epidemiological role played by wild populations of this *Galliformes* species is not well understood, to date.

Aims of this serological survey, carried out on wild pheasants trapped in northern Italy, were: i) to establish the occurrence of type A influenza infection; ii) possibly, to detect the circulation of H5 and H7 AIV subtypes.

MATERIALS AND METHODS

Sampling - Free-living pheasants were monitored on an estate (about 35 hectares) located in a protected lowland area (Bologna province, Emilia Romagna region). The number of birds occupying the study area ranged from 150 to 40 in autumn and spring, respectively. From 1995 to 2002 a total of 196 pheasants were trapped; 113 of them were banded for individual identification then released into the wild. Overall, 219 sera were collected and examined (Table 1) including 23 recapture samples (Table 2). Bird sex and age were recorded whenever possible.

Serological test - Sera were assayed for antibodies against influenza A virus nucleoprotein using an ELISA test (NP-ELISA) performed as described (De Marco et al., 2003b).

Available NP-ELISA positive sera were assayed as described (De Marco et al., 2004) by hemagglutination-inhibition (HI) test, in order to detect antibodies against 5 different Italian strains belonging to both H5 and H7 subtypes of AIVs (Table 1).

Recapture data (Table 2) were analysed in order to evidence a significant increase in antibody titres (De Marco et al., 2003b).

Statistical analysis – Chi-square test was performed in order to test non-random associations between the overall seroprevalences and: i) pheasant age; ii) pheasant sex. The significance level was set at a $P < 0.05$.

RESULTS AND DISCUSSION

As shown in Table 1, the overall NP-ELISA antibody frequency to avian influenza viruses (AIVs), including data from 23 recaptures, was 12.3% (27/219). Pheasants seropositive for influenza A viruses were found in 4 of the 5 sampling periods, and the prevalence of sera found positive ranged from 0% (in 1995) to 42.5% (in 2001). No sex-related differences were found whereas the age-related NP-ELISA seroprevalences resulted significantly higher in the juvenile birds, compared to the adult ones, thus suggesting a higher susceptibility of juvenile pheasants that congregate in post-breeding periods to AIV transmission.

No H5 and H7 positive sera were found by the HI assay (Table 1).

Among 16 birds captured more than once, seroconversion for type A influenza viruses (Table 2, data in bold type) was observed in pheasants n. 3 and n. 5, indicating that AIVs circulated in the study area during the winter 2000.

CONCLUSIONS

Our findings indicate the occurrence of avian influenza virus (AIV) infection in free-living pheasants. In general wild birds are potentially exposed to AIVs perpetuated by natural reservoirs, in particular the pheasants examined in the present study drank from small ponds located in the study area and occasionally used by migrating ducks. Faecal contamination of waters could represent an ecological interface between primary hosts of AIVs and other susceptible bird species (Webster et al., 1992).

The high population density characterising the study area could facilitate the virus circulation; indeed the data we observed are in contrast with seronegative results obtained from pheasants living in protected sites characterised by a lower population density (De Marco et al., 2003a).

Fortunately, our results indicate that neither H5 nor H7 subtypes of AIV, recently involved in Italian poultry epidemics (Capua et al., 2003; Campitelli et al., 2004), circulated within the examined bird population. Further surveillance studies will enable us to acquire information to better understand the dynamics of influenza infection in pheasants, a land-based bird species potentially implicated in the interspecies transmission of AIVs harboured in natural reservoirs (Wood et al., 1985; Perez et al., 2003).

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Table 1 – Serological results for antibodies against avian influenza viruses in 196 free-living pheasants trapped in a protected area of the Emilia Romagna region (Northern Italy). Seroprevalences were calculated on overall sera sampled, including 23 sera obtained from 16 recaptured bird.

Sampling period	NP-ELISA prevalence % (positive/tested sera)	HI antibody frequencies (positive/tested sera) calculated on NP-ELISA positive sera against the following AIV subtypes ^(*) :				
		H5N2	H5N3	H5N9	H7N1	H7N3
Mar. 1995	0 (0/34)	n.d.	n.d.	n.d.	n.d.	n.d.
Jan. 1999/Feb. 1999	3.8 (2/53)	0 (0/2)	0 (0/2)	0 (0/2)	0 (0/2)	0 (0/2)
Feb. 2000/Mar. 2000	5.6 (3/54)	0 (0/2)	0 (0/2)	0 (0/2)	0 (0/2)	0 (0/2)
Jan. 2001/Mar. 2001	42.5 (17/40)	0 (0/15)	0 (0/16)	0 (0/16)	0 (0/15)	0 (0/16)
Jan. 2002	13.2 (5/38)	0 (0/4)	0 (0/5)	0 (0/5)	0 (0/4)	0 (0/5)
Total 1995/2002	12.3 (27/219)	0 (0/23)	0 (0/25)	0 (0/25)	0 (0/23)	0 (0/25)

^(*) Low-pathogenic AIV strains used: A/mallard/Italy/80/93(H5N2); A/mallard/Italy/208/00(H5N3); A/chicken/Italy/9097/97(H5N9); A/turkey/Italy/6423-1/99(H7N1); A/mallard/Italy/33/01(H7N3); n.d. not done.

Table 2 – Serological data for antibodies against influenza A viruses obtained from 16 free-living pheasants trapped between 1995 and 2002 in the study area, and recaptured once or twice.

Bird No.	Bird age [^]	1 st capture time (NP-ELISA result)	1st recapture time (NP-ELISA result)	2 nd recapture time (NP-ELISA result)
1	Ad	14/03/95 (-)	02/02/00 (-)	n.d.
2	Ad	03/02/99 (-)	22/02/00 (-)	02/03/00 (-)
3	Ad	04/02/99 (-)	22/02/00 (-)	29/02/00 (+)^{^^}
4	Ad	09/02/00 (-)	18/02/00 (-)	24/02/00 (-)
5	Ad	09/02/00 (-)	14/02/00 (-)	29/02/00 (+)^{^^}
6	Juv	11/02/00 (-)	15/02/00 (-)	n.d.
7	Un	11/02/00 (-)	22/02/00 (-)	01/03/00
8	Ad	11/02/00 (-)	28/01/02 (-)	n.d.
9	Ad	22/02/00 (-)	24/02/00 (-)	29/02/00 (-)
10	Ad	23/02/00 (-)	29/02/00 (-)	n.d.
11	Ad	23/02/00 (-)	08/02/01 (-)	n.d.
12	Juv	23/02/00 (-)	01/03/00 (-)	n.d.
13	Juv	24/02/00 (-)	29/01/02 (-)	n.d.
14	Ad	29/02/00 (-)	06/02/01 (-)	28/01/02 (-)
15	Ad	13/02/01 (-)	27/02/01 (-)	n.d.
16	Un	01/03/01 (+) ^{^^}	06/03/01 (+) ^{^^}	n.d.

Ad adult; Juv juvenile; Un unknown; (+) positive; (-) negative; n.d. not done; [^] determined during the 1st capture; ^{^^} positive antibody titre = 8.