Preliminary results of an influenza surveillance in wild birds, game birds, domestic ducks and geese in North Eastern Italy

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ABSTRACT

Following the avian influenza (AI) epidemics which occurred in Italy between 1997 and 2003, a surveillance program, funded by the Italian Ministry of Health was implemented. Among the tasks of this surveillance program was an investigation on wild and domestic birds to asses circulation of avian influenza viruses in their natural reservoirs. In this study we collected samples from migratory wild birds *(Anseriformes* and *Charadriiformes)*, from national and importated game fowls, and from 7 backyard farms of geese and ducks. Cloacal swabs were screened by means of real-time RT-PCR (RRT-PCR) and/or directly processed for attempted virus isolation in embryonated fowl's SPF eggs and blood samples for presence of antibodies against avian influenza viruses. Avian influenza viruses were only obtained from migratory waterfowls belonging to the family *Anseriformes*, and not from domestic waterfowls or game birds. This study confirms that the risk of introduction of novel influenza viruses in densely populated areas of poultry farms in Veneto is linked to migratory wild birds and in particular from birds belonging to the family *Anseriformes*.

Key Words: Avian influenza, Wild birds, Game birds, Ducks, Geese.

RIASSUNTO

INDAGINE SUL RISCHIO DI INTRODUZIONE NEL POLLAME DOMESTICO DI VIRUS DELL'INFLUENZA AVIARIA DA POSSIBILI RESERVOIR IN VENETO

In questi ultimi anni l'allevamento avicolo veneto è stato gravemente colpito da alcune ondate epidemiche di influenza aviaria (AI) responsabili di ingenti danni economici diretti ed indiretti al patrimonio avicolo nazionale. Lo scopo della presente indagine è stato quello di verificare la presenza, nei principali serbatoi naturali, di Influenzavirus A. La ricerca si è svolta mediante l'analisi di campioni prelevati da uccelli acquatici selvatici (anatidi e limicoli), da selvaggina di allevamento di provenienza nazionale ed estera e da allevamenti rurali di anatre ed oche. I risultati indicano che nel nord-est dell'Italia, come nel resto del mondo, gli anatidi selvatici migratori rivestono un ruolo estremamente importante nell'epidemiologia dell'AI. Nessun virus influenzale è stato isolato dai limicoli, ulteriori indagini potranno chiarire in futuro il reale ruolo di questi uccelli in Italia. Tutti gli allevamenti di selvaggina e di anatidi monitorati sono risultati negativi.

Parole chiave: *Influenza aviaria, Uccelli selvatici, Selvaggina, Anatre, Oche.*

Introduction

In previous years Italy was affected by several waves of avian influenza (AI) caused by type A Influenza viruses, thus determining heavy economic losses for the poultry industry and devastating consequences for the social community (Marangon *et al.*, 2003). The epidemics occurred primarily in the densely populated poultry areas (DPPA) of Veneto and Lombardia regions.

Waterfowls, particularly *Anseriformes* (Alexander *et al.*, 2000) and *Charadriiformes* (Stallknecht *et al.*, 1988) are believed to be natural hosts and reservoirs of influenza A viruses. During recent H5N1 outbreaks in Eastern Asia the presence of H5N1 viruses in dead migratory birds suggests that wild bird populations may be involved in spreading Highly Pathogenic Avian Influenza (HPAI). In fact the timing and distribution of H5N1 infection in poultry in China in 2001 coincides with the general period of winter bird migration from northern to southern part of the country (Li *et al.*, 2004). In Europe, Anseriformes coming from north-eastern region use wetlands located in Veneto as a wintering site. *Charadriiformes* originating from the same areas, but with different habitats, use Italian wetlands as foddering sites before they leave for Africa. Thus, several species congregate in Italian wetlands in autumn, and this represents an ideal ecological situation for the perpetuation of avian influenza viruses (AIV). On the contrary, in winter there are fewer species but the numbers of birds present in the wetlands are more significant than those which can be found in the previous season. In addition, the majority of birds which are present in the wetlands are juveniles, born during the previous spring and theoretically highly susceptible to AIV.

Aim of the present study was to evaluate the circulation of AIV in wild birds, game birds, domestic ducks and geese in Veneto region (north eastern Italy).

Material and methods

During 2003, 742 cloacal swabs and 309 serum samples were collected. Of these, 560 cloacal swabs were collected from 9 different species of migratory wild *Anseriformes* and from one species of *Charadriiformes*, *(Calidris alpina)* present in wetlands area of Veneto region. The remaining swabs were collected in 12 game farms rearing birds of national and imported origin and from 7 backyard farms rearing geese and ducks.

Serum samples were tested by means of the haemagglutination inhibition test (HI) and by agar gel immunodiffusion test (AGID) as described in European Union Directive 92/40/CEE (CEC, 1992). The species and samples tested are illustrated in Table 1.

Cloacal swabs from migratory birds were screened by means of a real-time RT-PCR (RRT-PCR) (Cattoli *et al.*, 2004), and the positive samples were subsequently processed for virus isolation.

Cloacal swabs, diluted in phosphate-buffered saline with antibiotics, were inoculated into the allantoic cavity of 9-day-old embryonating specific pathogen free (SPF) eggs for attempted virus isolation according to EU Directive 92/40. Haemagglutinating isolates were identified by means of the haemagglutination-inhibition test, and if shown to be influenza isolates, were fully characterised by means of the neuraminidase inhibition (NI) test.

Results and discussion

All serum samples resulted negative for antibodies to avian influenza. 18 out 560 cloacal swabs (9 mallards, 1 teal, 4 pintails, 1 wigeons, and 3 shovellers) tested were positive for type A avian influenza in RRT-PCR. Following virus isolation attempts, two isolates were obtained, an H1N1 subtype, was obtained from a male mallard and an H10N4 subtype was obtained from a female pintail.

All cloacal swabs collected from game birds and rural geese and ducks were negative for AIV (Table 1).

The results of the present investigation support previous studies which indicate that migratory waterfowls represent a source of infection for domestic birds. In an investigation carried out between 1992 to 1998 in which a total of 802 cloacal swabs were collected from migratory and resident waterfowl on the west coast of Italy, 22 isolates obtained (18 H1N1, 1 H3N8, 1 H5N2 and 2 H10N8).

In USA birds belonging to the order *Charadriiformes* are considered one of main reservoirs of AIVs (Stallknecht *et al.*, 1988). As part of the investigation we collected 82 cloacal swabs from dunlins *(Calidris alpina)* in order to obtain information on the role of *Charadriiformes* as reservoirs of AI viruses in Italy, since this information is currently lacking. No isolates were obtained from the samples collected.

Table 1. Results of serological and virological tests.

	Species	N. samples collected		Results of laboratory tests		
		Cloacal swabs	Sera	RRT-PCR (n. positive/n. samples tested)	Virus isolation (n. positive/ n. samples tested)	Serological tests (HI, AGID) (n. positive/ n. samples tested)
Game farms	Pheasant *(Phasianus colchicus)*	60	219	nd	0/60	0/219
	Partridge *(Perdix perdix)*	10	45	nd	0/10	0/45
	Rock Partridge *(Alectoris greca)*	0	5	nd	0/5	0/5
Ducks and Geese farms	Domestic Duck *(A. platyrhynchos var. domestica)*	62	0	nd	0/62	nd
	Muschovy Duck *(Cairina moscata)*	35	25	nd	0/35	0/25
	Mallard *(Anas platyrhynchos)*	5	5	nd	0/5	0/5
	Goose *(Anser anser var. domestica)*	10	10	nd	0/10	0/10
Wild Birds	Mallard *(Anas platyrhynchos)*	206	0	9/206	1/9	nd
	Teal *(Anas crecca)*	27	0	1/27	0/27	nd
	Pintail *(Anas acuta)*	115	0	4/115	1/115	nd
	Wigeon *(Anas penelope)*	92	0	1/92	0/92	nd
	Coot *(Fulica atra)*	2	0	0/2	0/2	nd
	Gadwall *(Anas strepera)*	6	0	0/6	0/6	nd
	Shoveler *(Anas clypeata)*	23	0	3/23	0/23	nd
	Pochard *(Aythya furina)*	5	0	0/5	0/5	nd
	Tufted Duck *(Aythya fuligula)*	2	0	0/2	0/2	nd

nd=not done

The discrepancy between the number of samples which resulted positive at the RRT-PCR and the virus isolation attempts, probably lies in the greater sensitivity of the molecular test (Foni *et al.*, 2002; Cattoli *et al.*, 2004) compared to virus isolation.

Conclusions

In conclusion, the results of this study emphasise the continuous need to monitor wild bird population in order to gain more knowledge on the ecology of AI infections.

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