

# Influenza virus circulation in wild aquatic birds in Italy during H5N2 and H7N1 poultry epidemic periods (1998 to 2000)

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Two epidemics of avian influenza due to H5 and H7 highly pathogenic viruses occurred in poultry in Italy in 1997/98 and 1999/2000, respectively. The circulation of these serotypes in wild aquatic birds was investigated examining 638 cloacal swabs and 621 sera collected from 150 gulls, 162 coots, and 326 ducks trapped in Italian wetlands from 1998 to 2000. Seroprevalences against influenza A viruses, detected by a double-antibody sandwich-blocking enzyme-linked immunosorbent assay (ELISA), were 11% in gulls, 16% in coots, and 45% in ducks. Among the *Anatidae* group, duck species wintering in Mediterranean areas showed significantly higher values than ducks wintering in South-Saharan areas of Africa. In order to detect H5 and H7 antibodies, the haemagglutination-inhibition assay and two competitive ELISA tests (H5-ELISA and H7-ELISA) using monoclonal antibodies specific for H5 and H7 subtypes were performed. None of the aquatic bird species were found seropositive for H7 subtype, whereas H5-positive sera were found by both the haemagglutination-inhibition and ELISA assays in ducks only. The highest H5 seroprevalences were detected by H5-ELISA; overall, 5% (10/201) of duck species wintering in Mediterranean areas tested positive by this assay, with annual seroprevalences ranging from 2% (2/123) to 12% (6/51). In the present study, only five viruses belonging to H1N1, H11N6, and H2N3 subtypes were isolated from ducks. However, the H5 seroconversion observed in one mallard duck at the beginning of 1998 indicates that H5 virus circulation also occurred in the study area.

## Introduction

Several epidemics of avian influenza occurred in poultry in Italy between 1997 and 2003. In two cases, the causative agents were highly pathogenic avian influenza (HPAI) viruses, belonging to the H5N2 and H7N1 subtypes, respectively (Capua & Marangon, 2000; Donatelli *et al.*, 2001). While the H5N2 outbreak arose all of a sudden and had a very limited extent, the HP H7N1 epidemic was the result of the mutation of a H7N1 avian influenza virus (AIV) from a low-pathogenicity to a high-pathogenicity phenotype, which took place after widespread and prolonged circulation in commercial poultry, thus producing a devastating outcome (more than 14 million animals killed or stamped out).

When epidemics of avian influenza of both HPAI and lowly pathogenic avian influenza viruses occur in domestic species, it is frequently hypothesized as an initial introduction of a low pathogenicity virus from free-

living birds, in particular waterfowl, that harbour large gene pools of influenza A viruses and are regarded as the natural hosts of this infection. Then, in some instances, a period of circulation among reared birds may allow the change of the H5 and H7 subtype strains to HPAI viruses (Alexander, 2000). To date, the only reported case, in the wild bird reservoir, of AIVs representing direct precursors of strains circulating in commercial poultry has been detected in Italy: during 2001, a lowly pathogenic avian influenza H7N3 strain was isolated from free-living mallards in Central Italy (Campitelli *et al.*, 2004a), and 1 year later the infection occurred in commercial poultry of Northern Italy (Capua & Alexander, 2004). The virus responsible, very similar to the wild bird strains, spread among intensively reared poultry causing heavy economic losses, and was eradicated by means of both direct and indirect prophylaxis

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measures, after 1 year of circulation as a lowly pathogenic avian influenza strain (Campitelli *et al.*, 2004a).

In order to understand the epidemiological role played by free-living bird populations in the influenza ecology, since 1992 we have conducted influenza virological and serological monitoring in various wild species, particularly aquatic birds (De Marco *et al.*, 2003a).

The present paper represents a retrospective survey, aimed at evaluating influenza A virus circulation in free-living aquatic birds (ducks, coots, gulls) sampled in Italy, between January 1998 and June 2000. In this way it was possible to clarify whether H5 and/or H7 subtype viruses, affecting commercial poultry in Italy in the same period, were also circulating in natural reservoir species.

A second objective of our study was to evaluate the epidemiological role in the influenza virus natural cycle that is played by wild duck species characterized by a different migratory behaviour. To this aim, we compared serological results obtained from duck species overwintering in Mediterranean areas (DWMA) with garga-

neys (*Anas querquedula*) that overwinter almost entirely in the South Saharan tropical regions of Africa after leaving their breeding sites in Eastern Europe and Asia (DWSSA).

## Materials and Methods

**Sample collection and area description.** From January 1998 throughout December 1999 a total of 488 serum samples and 488 cloacal swabs were collected from 326 wild ducks (sampled species are presented in Table 1) and 162 coots (*Fulica atra*) caught for ringing purposes, in two neighbouring wildlife protected areas ("Laguna di Orbetello" and "Lago di Burano" WWF Oases) located on the West coast of Italy (De Marco *et al.*, 2003b). Bird age was identified whenever possible; in this report the term juveniles refers to birds hatched during the last breeding season, whereas the term adults refers to individuals hatched any year before the last breeding season. Due to the bands used during ringing operations, which allow identification, ducks captured twice during a single sampling period were also analysed; the second capture occurred at least 3 weeks from the first one. A total of 11 recaptured ducks were sampled.

In June 1999 and June 2000 both serum samples and cloacal swabs were collected from 133 nestling yellow-legged gulls (*Larus michahellis*

**Table 1.** Virological and serological results for influenza A viruses in wild ducks, coots, and gulls caught between January 1998 and June 2000 in the wetlands of Italy

Sampling periods	Bird groups	Number of isolates/number examined (isolation prevalence%) <sup>a</sup>	Virus subtype (number of isolates)	Number of NP-ELISA-positive sera/number examined (type A Seroprevalence%) <sup>a</sup>	Number of sera with HI antibodies <sup>b</sup>			Number of H5-ELISA-positive sera/number examined <sup>b</sup> (H5-ELISA seroprevalence%) <sup>a</sup>
					H5N2 <sup>c</sup>	H5N2 <sup>d</sup>	H5N9 <sup>e</sup>	
Jan-Mar 1998	DWSSA <sup>f</sup>	0/125	n.i. <sup>k</sup>	12/125 (10%)	0	0	0	0/125
	Coots <sup>g</sup>	0/54	n.i.	1/54 (2%)	0	0	0	0/54
	DWMA <sup>h</sup>	0/51	n.i.	20/51 (39%)	3	3	0	6/51 (12%)
Dec 1998-Jan 1999	Coots	0/8	n.i.	2/8 (25%)	0	0	0	0/54
	DWMA	2/123 (2%)	H1N1 (1) H11N6 (1)	99/123 (80%)	1	1	1	2/123 (2%)
Jun 1999	YL-Gulls <sup>i</sup>	0/64	n.i.	15/64 (23%)	0	0	0	0/64
Dec 1999	Coots	0/100	n.i.	23/100 (23%)	0	0	0	0/100
	DWMA	3/27 (11%)	H2N3 (2) H11N6 (1)	14/27 (52%)	0	0	1	2/27 (7%)
Feb 2000	YL-Gulls	0/11	n.i.	n.d. <sup>l</sup>	n.d.	n.d.	n.d.	n.d.
	BH-Gulls <sup>j</sup>	0/6	n.i.	n.d.	n.d.	n.d.	n.d.	n.d.
Jun 2000	YL-Gulls	0/69	n.i.	0/69	n.d.	n.d.	n.d.	0/69
Total	DWSSA	0/125	n.i.	12/125 (10%)	0	0	0	0/125
Jan 1998-Jun 2000	Coots	0/162	n.i.	26/162 (16%)	0	0	0	0/162
	DWMA	5/201 (2%)	H1N1 (1) H11N6 (2) H2N3 (2)	133/201 (66%)	4	4	2	10/201 (5%)
	YL-Gulls	0/144	n.i.	15/133 (11%)	0	0	0	0/133
	BH-Gulls	0/6	n.i.	n.d.	n.d.	n.d.	n.d.	n.d.

<sup>a</sup>Prevalence data were rounded off to the nearest whole number.

<sup>b</sup>Only NP-ELISA-positive sera were tested.

<sup>c</sup>A/Ruddy Turnstone/Delaware/253/91 (H5N2).

<sup>d</sup>A/Mallard/Italy/80/93 (H5N2).

<sup>e</sup>A/Chicken/Italy/9097/97 (H5N9).

<sup>f</sup>Duck species wintering in South Saharan areas of Africa (*Anas querquedula*,  $n = 125$ ).

<sup>g</sup>Coots (*Fulica atra*,  $n = 162$ ).

<sup>h</sup>Duck species wintering in Mediterranean areas (*Tadorna tadorna*,  $n = 3$ ; *Anas platyrhynchos*,  $n = 174$ ; *Anas crecca*,  $n = 8$ ; *Anas Penelope*,  $n = 1$ ; *Anas strepera*,  $n = 1$ ; *Aythya nyroca*,  $n = 3$ ; *Aythya ferina*,  $n = 9$ ; *Aythya fuligula*,  $n = 2$ ).

<sup>i</sup>Yellow-Legged Gulls (*Larus michahellis michahellis*,  $n = 144$ ).

<sup>j</sup>Black-Headed Gulls (*Larus ridibundus*,  $n = 6$ ).

<sup>k</sup>No isolation.

<sup>l</sup>Not done.

*michahellis*) sampled during ringing operations in a breeding area located on the eastern coast of Italy (Emilia Romagna region). Moreover, cloacal swabs were obtained from 17 adult gulls (11 yellow-legged gulls and six black-headed gulls, *Larus ridibundus*) that died during a botulism outbreak occurring during February 2000 in neighbouring sites.

Serum samples were stored at  $-20^{\circ}\text{C}$  until tested. Cloacal swabs were stored at  $-80^{\circ}\text{C}$  in phosphate-buffered saline/glycerol (1:1) with antibiotics.

**Serological tests.** Sera were assayed for antibodies against influenza A virus nucleoprotein using a double-antibody sandwich-blocking enzyme-linked immunosorbent assay (NP-ELISA), as previously described (De Marco *et al.*, 2004). Two-fold dilutions of the serum samples were tested and NP-ELISA titres  $\geq 8$  were considered positive.

Serum samples that had tested positive in the NP-ELISA were assayed by the haemagglutination-inhibition (HI) test for anti-H5 and H7 antibodies. Five different avian influenza strains were employed as H5 and H7 antigens, including both reference North-American strains (A/Ruddy Turnstone/Delaware/253/91, H5N2; A/Ruddy Turnstone/New Jersey/65/85, H7N3) and Eurasian low pathogenic isolates (A/Mallard/Italy/80/93, H5N2; A/Chicken/Italy/9097/97, H5N9; A/Turkey/Italy/6423-1/99, H7N1). In order to reduce false-positive or false-negative reactions (Easterday *et al.*, 1997) serum samples were treated both with receptor-destroying enzyme, as previously described (World Health Organization, 2002), and at  $37^{\circ}\text{C}$  for 1 h with 10% (v/v) chicken red blood cells, respectively. The HI assay was performed as described elsewhere (Alexander, 1989). HI titres  $\geq 16$ , detected against an antigen dose of 4 haemagglutinating units, were considered positive.

Moreover, to detect both anti-H5 and anti-H7 specific antibodies, two competitive ELISA tests were used to assay the NP-ELISA positive sera. The H7-ELISA test was carried out as described by Sala *et al.* (2003), using the low-pathogenicity H7N1 influenza strain A/Turkey/Italy/BS-2676/99 for the production of monoclonal antibodies (mAbs). Briefly, the H7N1 antigen was adsorbed onto microplates, and then two dilutions (1:10 and 1:20) of each serum were incubated with conjugated mAbs onto the antigen-coated plates; serum samples were classified as positive when they gave a reduction of absorbance value of at least 50% ( $\pm 0.1$ ) compared with the control wells. The H5-ELISA test was developed in a similar way, using A/Turkey/England/28/73 (H5N2) virus as the antigen to produce anti-H5 mAbs and to coat ELISA microplates; serum samples were classified as positive when they gave a reduction of absorbance value of at least 75% ( $\pm 0.1$ ) compared with the control wells (Cordioli, personal communication).

**Virus isolation.** Cloacal swabs were tested for influenza viruses by inoculation into embryonated specific pathogen free eggs, according to standard procedures

Influenza isolates were identified by both haemagglutination assay (World Health Organization, 2002) and a double-antibody sandwich ELISA for detection of influenza A viral nucleoprotein (Siebinga & de Boer, 1988). Influenza viruses were characterized by HI assay and neuraminidase-inhibition test (World Health Organization, 2002) using reference virus strains and antisera.

**Statistical analysis.** Wild ducks trapped on the West coast of Italy were divided into two groups: garganeys (*Anas querquedula*), a highly migratory duck species wintering almost entirely in South Saharan areas (DWSSA) of Africa; and other duck species wintering in Mediterranean areas (DWMA) (Table 1). The chi-square test was used in order to detect significant differences in influenza A seroprevalences between DWMA and DWSSA, during the first sampling period.

Statistical tests were performed in order to detect significant age-related differences in overall NP-ELISA seroprevalences within each bird group. The chi-square test was used for DWMA and coots, and the Fisher's exact test was used for DWSSA. Significance was set at  $\alpha = 0.05$ .

## Results

**Serological tests.** With regard to ducks and coots, the overall NP-ELISA antibody frequencies to type A influenza viruses as well as the seasonal seroprevalences are presented in Table 1. In total, 66% of the DWMA and 16% of coots (rail species wintering in Mediterranean areas) were seropositive. Considering the first sampling period, when garganeys (DWSSA) were trapped, a significant difference was observed between this duck group and the DWMA. In particular, DWMA results were more seropositive (39%) than DWSSA results (10%). No significant age-related differences in NP-ELISA seroprevalences were observed within each group (DWMA, DWSSA, coots) during the study period. Both NP-ELISA-positive sera reported in Table 1, and the 11 serum samples obtained from recaptured ducks (data not included in Table 1) were tested by the HI assay and by both ELISA tests.

From January 1998 through December 1999, no positive sera were found by either the H7-ELISA test or the HI assay, performed using two low pathogenic H7 antigens (data not shown). On the other hand, the sampled ducks showed HI antibodies against the H5 subtype; data obtained from the first individual captures are presented in Table 1, where the different results observed yearly are also indicated; six ducks showed HI antibodies against H5 strains, including one recaptured mallard that seroconverted against the H5 subtype (Table 2).

A total of 10 DWMA birds were H5-positive by ELISA assay (Table 1). As shown in Table 2, all six HI-positive sera (including the recaptured duck) were also positive when tested by the H5-ELISA test. The higher number of ELISA-positive sera compared with the HI-positive ones is probably due to a higher sensitivity of the former test that could detect the early stages of serum-antibody response as the recapture data indicate.

In Table 2, HI titres to the three H5 antigens for each of the six H5-positive birds are also shown. All HI antibody titres were in the low range of 16 to 32; these results agree with previous studies, showing scarcely detectable HI antibody responses in ducks primarily infected with AIVs (Kida *et al.*, 1980; Alexander *et al.*, 1986). Five of six ducks reacted with both H5N2 strains, A/Ruddy Turnstone/Delaware/253/91 and A/Mallard/Italy/80/93. Two of these birds were also seropositive to A/Chicken/Italy/9097/97 (H5N9), whereas one mallard caught during December 1999 showed antibody against this H5N9 strain only.

In nestling gulls, the overall antibody frequency to type A influenza viruses, detected by NP-ELISA assay, was 11% (15/133). All the seropositive birds were sampled during June 1999, when the observed seroprevalence was 23% (15/64). None of the gull sera was found positive to the H5 (Table 1) and H7 (data not included in Table 1) strains, using both the HI and ELISA assays.

**Virus isolation.** As presented in Table 1, five influenza A viruses were isolated from the group of DWMA birds. No viruses were obtained from garganeys and coots. From the 11 recaptured ducks, no virus was isolated (data not shown). Among the DWMA group, the level of virus isolation was 2% (5/201), ranging from 0% (0/51) during the period January to March 1998 and 11% (3/27) in December 1999. All the isolates were obtained from

**Table 2.** Serological data obtained from wild ducks (*Anas platyrhynchos*) seropositive to type A influenza viruses and showing H5 antibodies by HI test and/or H5-ELISA assay

Sampling periods	Bird identification numbers	HI titres detected using as antigen different H5 strains			H5-ELISA results
		H5N2 <sup>a</sup>	H5N2 <sup>b</sup>	H5N9 <sup>c</sup>	
Jan–Mar 1998	Number 1	16	16	–	+
	Number 2	16	16	–	+
	Number 3	– <sup>d</sup>	–	–	+
	Number 4	16	16	–	+
	Number 5 (Feb) <sup>e</sup>	–	–	–	+
	Number 5 (Mar) <sup>e</sup>	32	32	16	+
Dec 1998–Jan 1999	Number 6	–	–	–	+
	Number 7	–	–	–	+
Dec 1999	Number 8	32	32	16	+
	Number 9	–	–	16	+
	Number 10	–	–	–	+

<sup>a</sup>A/Ruddy Turnstone/Delaware/253/91 (H5N2).

<sup>b</sup>A/Mallard/Italy/80/93 (H5N2).

<sup>c</sup>A/Chicken/Italy/9097/97 (H5N9).

<sup>d</sup>HI titres < 16.

<sup>e</sup>Results obtained from mallard number 5 indicate an HI seroconversion in a bird captured twice, on 5 February 1998 and on 14 March 1998. Both HI-positive and ELISA-positive results of this latter capture are not included in Table 1.

mallards and belonged to the H1N1, H11N6, and H2N3 subtypes.

No virus isolations were obtained from cloacal swabs collected from both the 133 nestling gulls and the 17 adult ones (Table 1).

## Discussion

The overall influenza A seroprevalence observed in DWMA birds was 66%; this result confirmed the key epidemiological role of ducks in perpetuating influenza viruses in nature (Hinshaw *et al.*, 1985; Süß *et al.*, 1994).

The significant differences in type A seroprevalences observed during the first sampling period between DWMA birds (39%) and DWSSA, represented by garganeys (10%), could be due to the fact that during fall migration garganeys congregate in small flocks that, making few stops, quickly fly south to African wintering quarters (Brichetti & Fracasso, 2003). Thus, the low seroprevalence value observed in the DWSSA birds could be due to low virus circulation levels among these small and isolated garganey groups, that during the flight towards wintering quarters move in advance of mallards (the most frequently tested DWMA in the present study). This early seasonal movement of garganeys could allow them to escape the annual influenza epidemic occurring in mallards (Stallknecht & Shane, 1988). This situation parallels a similar one hypothesized in North America with Blue-winged teal (*Anas discors*), which is the Nearctic ecological counterpart of the Palearctic *Anas querquedula* (Cramp & Simmons, 1977; Stallknecht *et al.*, 1990b). Moreover, wintering sites of garganeys (Scott & Rose, 1996) could provide another explanation for their low seroprevalences: climatic conditions of tropical African habitats, such as high water temperatures and high intensity of sun ultraviolet rays, could negatively influence virus environmental survival (Stallknecht *et al.*, 1990a) compared with climatic conditions of the Mediterranean wintering sites. However, a different species-related susceptibility for influenza infection cannot be ruled out. It is worth noticing

that this monotypic duck species, *A. querquedula*, comprises four different wintering populations (ranging from West Africa to South Eastern Asia) that utilize a wide and continuous breeding area, ranging from Europe to North Eastern China (Wetlands International, 2002). Further investigations could elucidate the epidemiological involvement of this highly migratory duck in maintaining and spreading avian influenza strains belonging to Eurasian lineages.

With regard to the role of wild ducks and coots as a source of viruses potentially pathogenic for domestic poultry, we observed some differences: in coots no evidence of H5 and H7 virus circulation was found either at serological or virological level, whereas ducks serologically positive to H5 virus were detected every year. The latter result confirms the data obtained in the same study area from 1992 through 1998, showing that the antibodies against the H5 virus were annually detected (De Marco *et al.*, 2004). Even if no H5 viruses were isolated during the present study period, seroconversion observed in a mallard captured in February 1998 and recaptured 6 weeks later indicated that circulation of H5 subtype viruses occurred in the sampling area during the wintering period (Table 2). Finally, HI data seem to suggest the existence of antigenic differences among the H5 viruses circulating in the sampled duck population, since different antibody responses were shown against the H5 strains used (Table 2).

Unlike H5 AIVs, no evidence of H7 virus circulation was found either at the serological or virological level in ducks trapped in the area under study (Table 1). Thus it appears that no H7 viruses circulated among ducks sampled in the study area since 1992 (De Marco *et al.*, 2004). In particular, duck sera resulted negative for the H7 subtype both before and after the beginning of the catastrophic Italian poultry epidemic due to the H7N1 serovar (Capua & Marangon, 2000).

With regard to virus circulation, the H1N1 subtype appears to be endemic in Italian wetlands since 1992. The H2N3 and H11N6 strains were isolated for the first time, even though ducks serologically positive to both

subtypes had been found in previous years in the same study area (De Marco *et al.*, 2004).

It is probable that the influenza A-positive sera detected in nestling gulls during June 1999 were due to passive maternal immunity and not to infection, since birds sampled in 1999 were younger (2 to 3 weeks old) than those sampled in 2000 (3 to 5 weeks old), since positive samples derived from small groups of consecutively sampled birds suggest that they belong to few different broods, and since no AIVs were isolated from nestling gulls sampled in a colony of about 200 young birds living in high-density conditions. Neither H5 nor H7 HI-positive birds were found, and therefore other haemagglutination subtype viruses have presumably been circulating in these aquatic bird populations.

During an important surveillance study performed from 1975 to 1999 in the northern part of the Caspian sea basin, among 264 AIVs isolated from different gull species (*Larus ichthyæus* and *Larus argentatus*) no H7 strains were found and only one H5N2 subtype was detected in 1981 (Lvov *et al.*, 2001). In Italy, H7N2 viruses were obtained from gulls (*Larus* species) in 1986/87 and from yellow-legged gulls (*L. michahellis michahellis*) in 1992 (Fioretti *et al.*, 1988, 1994). Further investigations will be important to elucidate the epidemiological role of these birds in AIV perpetuation in Mediterranean areas.

In conclusion, the present results, together with data obtained before and after this study period in the same study area (De Marco *et al.*, 2004; Campitelli *et al.*, 2004b), suggest a continuous circulation of H5 AIVs in the wild duck reservoir in Italy. This evidence is all the more relevant when we consider that the duck populations we analysed are representative of waterfowl populations that very probably make use of wetlands of Northern Italian regions (where H5N2, H7N1 and H7N3 poultry epidemics occurred in recent years) during their migrations northward in spring and southward in autumn (De Marco *et al.*, 2000). Thus, even if neither virological nor serological data obtained in the period 1992 to 1999 could link wild migrating ducks trapped in Central Italy to the H7N1 poultry epidemic (suggesting a possible implication of other bird reservoirs), the identification in 2001, in these wetlands, of H7N3 duck viruses almost identical to H7N3 strains affecting domestic poultry in Northern Italy in 2002/03 (Campitelli *et al.*, 2004a) supports the existence of an epidemiological connection between these two areas and confirms the importance of maintaining an active influenza surveillance programme in wild waterfowl reservoirs.

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## Non-English Abstracts

### Influenza virus circulation in wild aquatic birds in Italy during H5N2 and H7N1 poultry epidemic periods (1998 to 2000)

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Circulation de virus influenza chez les oiseaux aquatiques sauvages, en Italie au cours des épisodes épidémiologiques de H5N2 et H7N1 observés chez les volailles entre 1998 et 2000

Deux épidémies d'influenza aviaire dues à des virus H5 et H7 hautement pathogènes sont respectivement apparues chez les volailles en Italie en 1997/98 et en 1999/2000. La circulation de ces sous types chez les oiseaux aquatiques sauvages a été étudiée en examinant 638 écouvillons cloacaux et 621 sérums récoltés à partir de 150 mouettes, 162 foulques, et 326 canards piégés dans les marécages entre 1998 et 2000. Les séroprévalences contre les virus influenza, détectées par un test ELISA bloquant, système sandwich avec deux anticorps, ont été de 11% chez les mouettes, 16% chez les foulques, et 45% chez les canards. Parmi le groupe des *Anatidae*, les espèces de canards hivernant dans les zones méditerranéennes (DWMA) ont montré des valeurs significativement supérieures à celles des canards hivernant dans les zones Sud Sahariennes de l'Afrique (DWSSA). Dans le but de détecter des anticorps H5 et H7, le test d'inhibition de l'hémagglutination (HI) et les deux tests ELISA de compétition (ELISA-H5 et ELISA-H7) utilisant des anticorps monoclonaux (Mabs) spécifiques des sous types H5 et H7 ont été appliqués. Aucune de ces espèces d'oiseaux aquatiques, n'a été trouvée séropositive vis-à-vis du sous type H7, alors que seuls des sérums de canards ont été trouvés positifs vis-à-vis de H5 par les tests HI et ELISA. Les séroprévalences les plus élevées vis-à-vis de H5 ont été détectées par l'ELISA H5. Au total, 5% (10/201) des DWMA se sont révélés positifs par ce test, avec des séroprévalences annuelles allant de 2% (2/123) à 12% (6/51). Dans cette étude, seuls 5 virus appartenant aux sous types H1N1, H1N6, et H2N3 ont été isolés à partir de canards. Toutefois, la séroconversion H5 observée chez un canard col vert au début de l'année 1998 indique que la circulation de virus H5 était également présente dans la zone où a été réalisée l'étude.

Influenzaviruszirkulation in Wild-Wasservögeln während der H5N2- und H7N1-Epidemien (1998-2000) beim Geflügel in Italien

In Italien traten beim Geflügel in den Jahren 1997/98 und 1999/2000 zwei durch die hochpathogenen H5- bzw. H7-Viren verursachten Influenzaepidemien auf. Die Zirkulation dieser Subtypen im wildlebendem Wassergeflügel wurde durch die Untersuchung von 638 Kloakenabstrichen und 621 Serumproben von 150 Möwen, 162 Wasserhühnern und 326 Enten, die in italienischen Feuchtgebieten zwischen 1998 und 2000 eingefangen wurden, überprüft. Das Vorkommen spezifischer Antikörper gegen Influenza A-Viren wurde mittels eines Doppel-Antikörper-Sandwich-Blocking-ELISA bei 11 % der Möwen, 16 % der Wasserhühner und 45 % der Enten nachgewiesen. Innerhalb der Familie der Anatidae wiesen die Entenspezies, die im Mittelmeerraum (DWMA) überwintern, signifikant höhere Werte auf als die Enten, die den Winter im Bereich der südlichen Sahara (DWSSA) in Afrika verbringen. Zum Nachweis von H5- und H7-Antikörpern wurden der Hämagglutinationshemmungs (HAH)- Test und zwei kompetitive ELISA-Tests (H5-ELISA und H7-ELISA) mit H5- und H7-Subtyp spezifischen monoklonalen Antikörpern (Mabs) durchgeführt. Keine der Wasservogelspezies war seropositiv gegen den H7-Subtyp, während H5-positive Seren sowohl mit dem

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HAH als auch den ELISAs nur bei Enten festgestellt wurden. Die höchsten H5-Seroprävalenzen wurden mittels des H5-ELISA ermittelt., insgesamt waren 5 % (10/201) der DWMA positiv in diesem Test mit jährlichen Seroprävalenzen zwischen 2 % (2/123) und 12 % (6/51). In der vorliegenden Studie wurden nur 5 Influenza A-Viren isoliert, die als H1N1, H1N6 und H2N3-Viren typisiert worden sind. Die H5-Serokonversion bei einer Stockente Anfang 1998 zeigt jedoch, dass das H5-Virus auch in dem untersuchten Areal zirkuliert.

Circulación de virus de influenza en aves salvajes acuáticas en Italia durante los periodos de epidemia con H5N2 y H7N2 en aves de producción (1998-2000)

Dos epidemias de influenza aviar debidas a virus altamente patogénicos H5 y H7 ocurrieron en aves de producción en Italia en 1997/98 y 1999/2000, respectivamente. La circulación de estos serotipos en aves salvajes acuáticas fue investigado mediante el examen de 638 hisopos cloacales y 621 sueros recogidos de 150 gaviotas, 162 fochas, y 326 patos atrapados en humedales italianos desde 1998 al 2000. Las seroprevalencias frente a virus de influenza A, detectados mediante un ELISA de *sandwich* de bloqueo con doble anticuerpo fueron del 11.3% en gaviotas, 16% en fochas y 45% en patos. Entre el grupo de *Anatidae*, las especies de patos invernando en las áreas del Mediterráneo (DWMA) mostraron valores significativamente mayores que los patos invernando en las áreas Subsaharianas (DWSSA) de África. Con el objetivo de detectar anticuerpos frente a H5 y H7, se utilizaron la técnica de inhibición de la hemaglutinación (HI) y dos técnicas de ELISA competitivas (H5-ELISA y H7-ELISA) mediante el uso de anticuerpos monoclonales (MAbs) específicos para los subtipos H5 y H7. Ninguna de las especies de aves acuáticas fueron seropositivas al subtipo H7, mientras que sí que se encontraron sueros positivos H5 tanto mediante HI como de ELISA en patos únicamente. Las seroprevalencias más altas frente a H5 fueron detectadas mediante H5-ELISA; en general, 5% (10/201) de los DWMA fueron positivas mediante esta técnica, con seroprevalencias anuales del 1.6% (2/123) hasta 11.8% (6/51), en 1998 y 1999 respectivamente. En el presente estudio, únicamente 5 virus pertenecientes a los subtipos H1N1, H1N6, y H2N3 fueron aislados de patos. Aún así, la seroconversión a H5 observada en un ánade real a inicios del 1998 indica que la circulación de virus H5 también ocurrió en el área de estudio.