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## Serological Survey for Avian Paramyxoviruses from Wildfowl in Aquatic Habitats in Andalusia

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**ABSTRACT:** A serological survey for a range of avian paramyxoviruses (PMV) was carried out among wildfowl from southern Spain, 1990 to 1992, using the hemagglutination inhibition technique. We collected 579 sera from 24 avian families (18 aquatic and six non-aquatic). Antibodies were detected to all paramyxoviruses in waterfowl, with a notable prevalence of antibodies to PMV-8 (43%) and to a lesser extent PMV-6 (21%). By contrast, in non-aquatic species high antibody prevalences were detected only to PMV-2 (60%), particularly in sparrows (68%), while antibody prevalences to other PMV's were moderate or low.

**Key words:** Avian paramyxoviruses, wildfowl, hemagglutination inhibition test.

Paramyxoviruses (PMV) have been isolated from various avian species in a wide range of areas. Nine different serotypes, designated PMV-1 to PMV-9, currently are recognized (Alexander, 1991). However, surveillance campaigns have not been conducted in many countries, and many wildfowl species have not been monitored; thus epizootiological information available does not provide a complete view of the natural ecology of these viruses (Alexander, 1988).

Migratory fowl constitute a likely primary source of infection by which avian PMV's might be introduced into the bird population of any given country or geographical area (Kelleher et al., 1985). Introduction of PMV's also is provided by small wild birds with peridomestic habits, chiefly passeriformes (Alexander, 1985).

In view of this, and given that Andalusia is an important strategic area as the flyway and nesting-site for a large number of avi-

an species, our objective was to determine the antibody prevalence against difference avian paramyxoviruses among the available wildfowl species.

We collected 507 sera from 18 wild waterfowl species, and 72 sera from six non-aquatic species (Table 1). House sparrows and hoopoe were captured using a 3 × 5 m nylon net placed between two trees and fastened to the ground (Japanese net). Prey birds were sampled from a recuperation unit. All blood samples were obtained from the cubital vein. Sera from other species were obtained by hunting or from recuperation units. All sera were collected between February of 1990 and November of 1992 from two different ecosystems, representative of coastal wetlands (Doñana) (37°10'N, 7°W) and inland wetlands, including Lakes Zóñar, Rincón and Fuente de Piedra (37°10'N, 5°W).

The following reference strains were kindly provided by Dr. D. J. Alexander of the International Reference Laboratory for Avian Ortho- and Paramyxoviruses of the Central Veterinary Laboratory (New Haw, Weybridge, England): PMV-1/NDV, PMV-2/chicken/California/Yucaipa/56, PMV-3/turkey/Wisconsin/68, PMV-4/duck/Hong Kong/D3/75, PMV-6/duck/Hong Kong/199/77, PMV-7/dove/Tennessee/4/75, PMV-8/goose/Delaware/1053/76 and PMV-9/duck/New York/78.

Reference strains were inoculated via the allantoic route into 9-day-old specific-pathogen-free (SPF) embryonated chicken eggs and incubated for 72 hr at 37 C.

TABLE 1. Species, number of subjects analyzed, number seropositive, and antibody prevalences to avian paramyxoviruses, Andalusia, 1990 to 1992.

Species	Number of sera tested	Number infested (percent positive)								
		PMV-1	PMV-2	PMV-3	PMV-4	PMV-6	PMV-7	PMV-8	PMV-9	
Pintail ( <i>Anas acuta</i> )	6	0	0	0	0	0	0	0	0	0
Marbled teal ( <i>Anas angustirostris</i> )	3	0	2 (67)	0	0	0	0	0	0	0
Shoveler ( <i>Anas clypeata</i> )	21	0	0	0	0	0	0	0	0	0
Teal ( <i>Anas crecca</i> )	8	0	0	0	0	0	0	0	0	0
Mallard ( <i>Anas platyrhynchos</i> )	166	5 (3)	21 (13)	10 (6)	5 (3)	38 (23)	14 (8)	72 (43)	2 (25)	2 (25)
Gadwall ( <i>Anas strepera</i> )	80	2 (3)	2 (3)	10 (13)	0	10 (13)	6 (8)	22 (28)	10 (13)	10 (13)
Greylag goose ( <i>Anser anser</i> )	14	0	2 (14)	0	2 (14)	2 (14)	2 (14)	13 (93)	2 (14)	2 (14)
Pochard ( <i>Aythya ferina</i> )	29	0	2 (7)	0	0	2 (7)	3 (10)	11 (38)	0	0
Red-crested pochard ( <i>Netta rufina</i> )	16	2 (13)	0	2 (13)	0	2 (13)	0	6 (38)	0	0
White-headed duck ( <i>Oxyura leucocephala</i> )	2	0	0	0	0	2 (100)	0	2 (100)	2 (100)	2 (100)
Shelduck ( <i>Tadorna tadorna</i> )	10	0	0	0	0	0	0	6 (60)	0	0
Grey heron ( <i>Ardea cinerea</i> )	22	0	0	0	0	14 (64)	13 (59)	13 (59)	0	0
Little egret ( <i>Egretta garzetta</i> )	3	0	2 (67)	0	0	0	0	0	0	0
Crane ( <i>Grus grus</i> )	5	0	0	0	0	0	0	5 (100)	0	0
Black-headed gull ( <i>Larus ridibundus</i> )	10	0	3 (30)	0	0	5 (50)	2 (20)	0	0	0
Greater flamingo ( <i>Phoenicopterus ruber</i> )	48	0	0	0	0	0	0	18 (38)	5 (10)	5 (10)
Coot ( <i>Fulica atra</i> )	54	0	8 (15)	3 (6)	3 (6)	21 (39)	6 (11)	34 (63)	24 (44)	24 (44)
Spoonbill ( <i>Platalea leucorodia</i> )	10	0	0	0	0	5 (50)	6 (60)	6 (60)	2 (20)	2 (20)
Aquatic bird total	507	9 (2)	44 (9)	25 (5)	10 (2)	106 (21)	54 (11)	218 (43)	68 (13)	68 (13)
Booted eagle ( <i>Hieraetus pennatus</i> )	2	0	0	0	0	0	0	0	0	0
Woodpigeon ( <i>Columba palumbus</i> )	5	0	3 (60)	3 (60)	0	0	0	0	0	0
Woodchat shrike ( <i>Lanius senator</i> )	2	0	2 (100)	0	0	0	0	0	0	0
House sparrow ( <i>Passer domesticus</i> )	56	0	38 (68)	5 (9)	0	0	2 (4)	11 (20)	0	0
Song thrush ( <i>Turdus philomelo</i> )	5	0	0	0	0	0	0	0	0	0
Hoopoe ( <i>Upupa epops</i> )	2	0	0	0	0	0	0	0	0	2 (100)
Non-aquatic bird total	72	0	43 (60)	8 (11)	0	0	2 (3)	11 (15)	2 (3)	2 (3)
Grand total	579	9 (2)	87 (15)	33 (6)	10 (2)	106 (18)	56 (10)	229 (40)	70 (12)	70 (12)

Amnio-allantoic fluid (AAF) then was harvested, and tested for bacterial sterility by inoculation of a sample into blood agar base (Oxoid, Basingstoke, Hampshire, England) to which was added 7% sterile defibrinated sheep blood. The sample was incubated for 18 hr at 37 C. The AAF was centrifuged at 2,500 rpm for 15 min at 4 C and stored at -80 C until used in the hemagglutination inhibition test (Alexander, 1991).

Positive control sera were obtained from 3-mo-old SPF Leghorn chicks hyperimmunized by successive inoculations, following the method of Arenas et al. (1991). Negative control sera were obtained from untreated chicks under the same conditions.

Hemagglutination micro- $\beta$ -inhibition tests were carried out as described by the World Health Organization Expert Committee on Respiratory Virus Diseases (1959). All sera inhibiting hemagglutination at a dilution of 1/20 were considered positive.

There was a widespread presence of antibodies against PMV-6 and PMV-8 in waterfowl, almost all the eighteen species surveyed had some seropositive birds (Table 1). The low prevalence of PMV-1 in wildfowl (2%) may reflect the fact that in the years prior to this study, and during the study itself, there were no reports of Newcastle disease in this area.

Paramyxovirus type 2 appears to be enzootic in Passeriformes (Alexander, 1988). The prevalence recorded here for PMV-2 in wildfowl as a whole was 15%, while the results obtained for non-aquatic species, chiefly Passeriformes, was 60%. Antibody prevalences of PMV-2 among waterfowl were substantially lower (8%) than among other birds and close to the 4% reported by Fleury et al. (1985).

Paramyxovirus type 3 frequently has been isolated from turkeys, although not from wildfowl (Alexander, 1991); but the overall antibody prevalence of 6% detected here, was slightly higher in non-aquatic

species (12%) than in waterfowl. This difference may stem from the fact that non-aquatic species, many of them having peridomestic habits, may have been in contact with turkeys on intensive farms, where Arenas et al. (1991) recorded infection rates of 34%.

Serotypes PMV-4, PMV-6 and PMV-8 have been isolated primarily from waterfowl (Alexander, 1991); PMV-9 has not been reported previously from wildfowl (Alexander, 1991), although the virus has been reported in domestic ducks (Hinshaw et al., 1978). In the present study, all subjects with antibodies against serotypes PMV-4 and PMV-6 were waterfowl, with antibody prevalences of 2% and 21%, respectively. This latter figure agrees with the 29% reported by Fleury et al. (1985). Antibodies to PMV-8 and PMV-9 were detected in both aquatic and non-aquatic birds though in both PMV-8 and PMV-9 antibody prevalences were much higher in waterfowl (43% and 13%, respectively).

Finally, PMV-7 previously has been reported only in Columbiformes (Alexander, 1988). In our study, antibodies to serotype PMV-7 were considerably higher in waterfowl (11%) than in non-aquatic species (2%). No doves had antibodies against PMV-7.

The hypothesis that some avian PMV serotypes may be widespread in duck populations in the form of non-apparent infections has not been ruled out (Alexander et al., 1979). Most serotypes in the present study (PMV-1, 4, 6, 7, 8, and 9) had higher antibody prevalences in wild waterfowl (mostly Anatidae) than in non-aquatic species (Table 1).

Kelleher et al. (1985) suggested that some avian species appear to play a significant role in PMV epizootiology. We found that prevalence of PMV-2 in sparrows (*Passer domesticus*) was strikingly high (68%) (Table 1); this might be evidence for the importance of this species in the epizootiology of PMV-2, as suggested by Alexander (1991). Our results support this idea. There

was a significant ( $P < 0.05$ ) correlation and an Odds Ratio (OR) of 20.4 (10.4 to 40.5) (Dean et al. 1990).

We also found that some species such as the mallard (*Anas platyrhynchos*) had antibodies against all eight serotypes surveyed, with a notably high level of antibodies to PMV-6 (23%) and PMV-8 (43%) (Table 1). These data support the assertion by Hinshaw et al. (1980) that the mallard is one of the most important species in avian PMV epizootiology. Coots (*Fulica atra*) also had antibodies against most serotypes, with a notable prevalence of PMV-6 and PMV-8, as did the gadwall (*Anas strepera*).

In coastal marshlands, the nesting-site of migratory fowl, prevalence was detected for all serotypes, whereas in inland wetlands no birds registered positive to PMV-1 and PMV-4, probably because waterfowl density in this area was much lower. Conversely, the presence of poultry farms in this area may have accounted for the greater prevalence of serotypes 2 and 3.

Seasonal variations in antibody prevalences are related to the life-cycle of the avian species concerned. Stallknecht et al. (1991) reported that the prevalence of PMV decrease from autumn to winter, while Kelleher et al. (1985) found that infection rates peaked during July and August. A similar trend was reported by Fleury and Alexander (1979), who found the greatest prevalence between May and September. In the present study, the highest antibody prevalences were reached between July and September, coinciding with the greatest density of young birds; after September, the time of the greatest density of young, they disappeared during the winter months.

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