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# Serosurvey for Newcastle Disease and Avian Influenza A Virus Antibodies in Great Cormorants from France

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ABSTRACT: Inland great cormorants (*Phalacrocorax carbo*) culled in France were examined in the winter of 1997–98 and 1998–99 for antibodies to Newcastle disease (ND) and influenza A strains H5 and H7 by the hemagglutination inhibition test. Antibodies to influenza A group antigen were tested by agar gel precipitin test. Ten of 53 adult individuals were seropositive for ND virus. All sera were negative for influenza A antibodies. It is speculated that ND occurred in the sampled population.

*Key words:* Great cormorant, influenza A, Newcastle disease, *Phalacrocorax carbo,* serological survey.

Among the various factors explaining emerging or reemerging diseases of veterinary importance are the expansion of host populations and new migratory pathways (Brown, 1997). Both factors are occurring in the case of the great cormorant (*Phalacrocorax carbo*) which has increased in population (at a rate close to 15% a year) and range (visible expansion on the mainland) in Europe (Baccetti and Cherubini, 1997; Trolliet, 1999). Several viruses are associated with transmission of infection from wild to domestic birds (Nuttall, 1997), particularly Newcastle disease virus (NDV; Fenner et al., 1987) and avian influenza virus.

Shags (*P. aristolelis*) and great cormorants (*P. carbo*) were implicated in outbreaks of Newcastle disease (ND) in poultry in Scotland (Blaxland, 1951). An epidemiologic study mentioned NDV antibodies (by ELISA titration) in 18.9% of 291 great cormorants sampled in Switzerland, but attempted virus isolation proved negative (Schelling et al., 1999). Since 1992 outbreaks of ND in the USA and Canada have been associated with widespread mortality of double-crested cormorants (*P. auritus*) (Kuiken et al., 1998) and evidence of transmission to poultry recorded. It is reasonable to expect therefore that NDV can circulate in natural population of cormorants in Europe.

The question of reservoirs of avian influenza of human health or veterinary importance is debated (Webster et al., 1993). For instance, Iftimovici et al. (1980) reported 11 H1N1 strains isolation from six bird species, including a great cormorant in Romania. Similar subtypes of influenza virus were reported by the authors as having circulated among the human population in the preceeding season.

Nevertheless, in this paper we have limited our investigation to avian influenza A strains of which certain viruses may possibly be pathogenic for poultry (Murphy and Webster [1993] report only one account of mortality in wild species by this virus type in the common tern *Sterna hirundo* in The Republic of South Africa [Nuttal, 1997]).

In the present study 53 samples from inland great cormorants were tested by hemagglutination inhibition test (HI) for antibodies to NDV and influenza A virus subtypes H5 and H7, and by agar gel precipitin (AGP) test for influenza A group specific antibodies. The samples became available following culling during routine population control in the Lorraine region of Eastern France. All birds were sampled by shooting at roost in eight localities, along the Mosel river within a 30 km radius north and south of the town of Nancy (48°40 N, 06°10 E). After death, blood or serous fluid was collected from heart or



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TABLE 1. Newcastle disease virus antibody titers (HI) in great cormorants in France classified by season and age.

| Season | Age | Positive | Negative | Suspect |
|---|---|---|---|---|
| 1997/98 | unknown | 3 | 14 | 5 |
| | juveniles | 0 | 2 | 0 |
| | adults | 1 | 2 | 0 |
| 1998/99 | unknown | 0 | 1 | 0 |
| | juveniles | 0 | 12 | 0 |
| | adults | 6 | 5 | 2 |
| Total | — | 10 | 36 | 7 |

abdominal cavity, centrifuged, and sera were kept frozen before titration.

Hemagglutination inhibition test for NDV and Influenza A H5 and H7 antibodies and influenza A group specific antibody were carried out according to European Union directives (Commission of European Communities, 1992); HI titers were expressed as the $\log_2$ of the reciprocal of the highest dilution of serum causing complete inhibition of 4HA units of antigen. Titers $\geq 4$ were regarded as positive (titres $\leq 2$ as negative).

Data were analyzed using chi-square tests or exact tests for low sample sizes (Sokal and Rohlf, 1995). Logistic regression was performed when several risk factors were to be considered (Crawley, 1993).

A summary of the NDV results is presented in Table I. Ten samples were seropositive with titers of $\geq 2$. Despite some differences in the sampling, greater number of sampling areas in 1989–99 together with a more accurate determination of sex and age (results not shown), the difference between seasons was not significant (Fisher statistic = 0.33; 1 df, $P = 0.5631$; only positive and negative, not suspect were taken into account). Sex had no obvious influence (Fisher statistic = 2.752, 2 df, $P = 0.2526$). Only age (juveniles, unknown, or adults) seemed to have a significant impact on the probability of being infected (Fisher statistic = 10.09, 2 df, $P = 0.0064$); none of the juveniles were found to have significant antibody levels. Notice only one bird was quoted as juvenile during the first season, several individuals remaining of unknown age. Because age determination was more accurate in 1998–99 than in 1997–98, a potential confusion between age and season was studied using logistic regression. A comparison of all possible models using likelihood ratio test (LRT) and Akaike Criterion (AIC) showed no significant interaction and no effect of season. These results remained unchanged when discarding individuals of unknown age, considering doubtful results, or both.

Avian influenza antibodies were not detected. It is assumed that influenza A virus subtypes H5 and H7 should not have circulated at a significant level to be detectable within the sampled cormorant population, whereas infection with NDV was evident although only the adult birds were found positive. A seroprevalence close to 20% (10/53) or even less, suggests a significant infection which should have occurred within the months preceding the capture of these birds. During the years 1996 and 1997 several outbreaks of ND were recorded in Europe: namely an outbreak in a flock of 550 breeding pheasants (species not mentioned) in Great Britain in May 1996 (Alexander et al., 1997), another in a group of approximately 12,000 released pheasants (*Phasianus colchicus*) on one island in Denmark during the summer of 1996 (Jorgensen et al., 1999), and a major epizootic in poultry in the UK between January and April 1997, with no isolation of PMV 1 in wild birds (Graham et al., 1999). Even in absence of any recent die-off or morbidity in freely born and free-ranging wild birds in Europe, the vulnerability of birds reared in open air to NDV infection from feral and wild birds and the potential role of wild birds as reservoir has been evoked. Our results confirm that it would be worthwhile improving NDV surveillance in great cormorants, by improving serologic and clinical monitoring and attempting virus isolation to determine the source of infection and if fur-

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ther outbreaks can be linked with recorded infection in poultry.

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