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SEROLOGICAL SURVEY OF VIRAL PATHOGENS IN BEAN AND WHITE-FRONTED GEESE FROM GERMANY

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ABSTRACT: Sera from wild geese were tested for antibodies to selected viral pathogens at a resting site for wild waterfowl in Germany. Serum samples from both bean geese (*Anser fabalis*) and white-fronted geese (*Anser albifrons*) collected in October 1991 were examined using serological methods licensed for routine diagnosis in domestic poultry. Of 130 sera tested, antibodies to several infectious agents were found including Newcastle disease virus (45%), goose parvovirus (48%), avian reovirus (29%), and avian adenovirus or egg drop syndrome 76 virus (6%). Antibodies against duck hepatitis virus were not detected. Differences in seroprevalences were not detected between the two geese species. While role and significance of wild geese in the epidemiology of avian diseases remains to be determined, it is possible that they could be of some importance as reservoirs and carriers of certain viral diseases of domestic poultry.

Key words: *Anser albifrons*, *Anser fabalis*, avian reovirus, bean goose, duck hepatitis virus, egg drop syndrome 76 virus, goose parvovirus, Newcastle disease virus, seroepidemioloy, seroprevalence, white-fronted goose, wild geese.

INTRODUCTION

Wild geese are one of the best known bird species according to their population dynamics and their interesting migration behavior (Curry-Lindahl, 1982). Due to the more intensive agriculture in central Europe the feeding conditions for wintering wild geese have improved and populations of wild geese have increased continuously during the last decades (Rutschke, 1987). In the eastern parts of Germany more than one million bean geese (*Anser fabalis*) and white-fronted geese (*Anser albifrons*) were reported in 1994 and 1995, respectively (Rutschke, 1996).

Due to the general decrease of wetlands in central Europe, there also are changes observed in the migratory behavior of wild geese. Depending on the climatic conditions geese may remain for longer periods at their traditional resting sites (Rutschke and Liebherr, 1996). These environmental changes have created new opportunities for wild geese to introduce and transmit pathogens. In general, the high mobility of the migrating wild bird is important in the spread of various avian viruses (Ottis and Bachmann, 1983; Hopkins et al., 1990; Stallknecht et al., 1991; Astorga et al., 1994; Ziedler et al., 1995), and wild waterfowl are considered both as reservoirs and/or carriers of several infectious agents (Sinnecker et al., 1985; Slemons et al., 1991; Maldonado et al., 1994; Swayne and Slemons, 1995). However, the role of wild birds, especially wild waterfowl, in the epidemiology of avian pathogens and the transmission of economically important avian diseases is not defined in detail.

The aim of this study was to examine wild geese sera for specific antibodies against a panel of avian viruses including Newcastle disease virus, avian reovirus, avian adenovirus/EDS 76 virus, duck hepatitis virus, and goose parvovirus. These infectious agents also are relevant to the poultry industry. In previous studies we demonstrated the presence of specific antibodies against these viruses in domestic waterfowl, chickens, and other birds in east Germany (Hlinak et al., 1992c; Ziedler and Hlinak, 1993; Ziedler et al., 1995). Herein our objectives were to determine (1) if viral diseases of domestic fowl also

are present in wildlife and (2) whether or not wild geese represent a virus reservoir for domestic birds. To this end, we evaluated the seroprevalence of these selected viral pathogens in one of the most important resting sites for wild geese in Germany.

MATERIALS AND METHODS

The serological survey was initiated as a completion to a zoological study on food ecology, influence on agriculture and migration of bean geese (Liebherr, 1993). Sera were collected from resting wild geese caught at the Lake Gülpe in the lowland of the Havel River in October 1991. The Lower Havel River valley occupies an area of about 5,800 ha along the Havel and Rhine Rivers situated in the eastern part of Germany (52°45′N; 12°18′E), between Rathenow and Havelberg, about 80 km northwest of Berlin.

A slight modification of a rocket-netting method described by Stubbe et al. (1995) and Rutschke (1987) was applied to catch wild geese. The north and the west shores of the lake are covered with wide reed belts but the south shore is immediately bordering a grassland. A 800 m^2 synthetic fibre net piloted by two rockets was installed on the sandy shore, an ideal place for capturing wild waterfowl spending the night in shallow waters. Immediately after being captured, the birds were fixed and inspected externally. Species were identified using bill colour and shape as well as wing and leg length. Age and sex was determined as described in Rutschke (1987). Blood was collected by puncture of the brachial vein and allowed to clot. Serum was separated by centrifugation (300 rpm), heat inactivated at 56 C for 30 min, and frozen at −20 C until it was tested. One hundred thirty blood samples were collected from wild geese, representing 65 bean geese and 65 white-fronted geese.

Specific antibodies against a panel of avian viruses were detected using established serological test systems and defined test antigens. Positive and negative reference sera originating from domestic geese were obtained for the serological tests (a generous gift from the former Institute of Poultry Disease of the Veterinary Department of the Humboldt–University, Berlin, Germany, and the State Veterinary and Food Investigation Centre, Frankfurt/Oder, Germany).

In all enzyme immunoassays a Peroxidase (POD)-labeled anti-goose IgG-antibody was used as the anti-species-conjugate. Briefly, we immunized two goats threefold with purified geese IgG. After the extraction of the goat IgG from sera, the POD-coupling followed the procedure described by Wilson and Nakane (1978).

Screening of the goose sera for antibodies against the Newcastle disease virus (NDV) was performed using an established enzyme immunoassay based on the NDV strain "La Sota" (Minning et al., 1993). Sera also were investigated in the standard hemagglutination inhibition test (HI-test) using chicken erythrocytes (0.5% in PBS). The HI-test was carried out with a twofold dilution of sera in the standard way using four hemagglutination units of the NDV (Starke, 1968). The end-titer was defined as the last dilution with complete inhibition of the hemagglutination activity. The correlation of the results from the enzyme immunoassay and the HI-test for detection of antibodies against the NDV was as reported by Hlinak et al. (1992b).

Serum antibodies against avian reoviruses (REO; strain U conn 1133) were detected with an established enzyme-linked immunosorbent assay (ELISA) based on the avian reovirus strain U conn 1133. The test procedure and the interpretation of the results are described by Hlinak et al. (1992a).

Antibodies against goose parvovirus (GPV; strain "Dessau") were detected using a standard micro-neutralization test (Mayr et al., 1977). Primary goose embryo fibroblasts cultured in 96 well cell culture plates (Greiner, Frickenhausen, Germany) were the biological basis for the determination of the antibody titre in this neutralization test.

The method of serological determination of antibodies against an avian adenovirus (EDS virus 76) was the standard hemagglutination inhibition test (Starke, 1968).

We established an ELISA for the examination of goose sera for specific antibodies against the duck hepatitis virus. In this assay we used a duck hepatitis virus (DHV) field isolate (isolate Storkow) as the solid phase coated antigen. The antigen preparation procedure was based on a method described by Zhao et al. (1991). In the first step of the assay microtiterplates (Nunc, immunoplate F 96, Polysorp; Wiesbaden-Biebrich, Germany) were coated with 50 µl per well of duck hepatitis virus antigen at a concentration of 10 µg/ml in carbonate/bicarbonate buffer (pH 9.6), over night at 4 C. After washings, 50 µl of goose serum dilution were added in each well. The sera were diluted 1: 500 in PBS (pH = 7.6) containing 0.05% Tween 20 and 10% horse sera. Following an incubation of 60 min at 37 C, the plates were washed three times and 50 µl/well POD-labeled anti-goose IgG-antibody were added (1:

2,500 in PBS/0.05% Tween 20/10% horse serum). The plates were incubated for 60 min at 37 C. Following this, the plates were washed and 50 µl/well substrate chromogen solution (TMB substrate solution/ready for use; Seramun, Dolgenbrodt, Germany) was added. The reaction was stopped with 1M H_2SO_4 (50 µl/well) after 15 min. The absorbency was measured at 450 nm using a SLT-reader system (SLT Labinstruments, Crailsheim, Germany). The determination of the results was based on positive/negative ratio (P/N) analysis as described before (Hlinak et al., 1992a).

Chi square and Fishers exact test was used to evaluate differences in antibody prevalence between the two waterfowl species and the age groups (Software EPI-INFO version 6.03/January 1996, Centre for Disease Control and Prevention, Epidemiology Program Office, Atlanta, Georgia, USA). The confidence intervals (CI) for the determination of the true seroprevalence within the wild geese populations were determined as described elsewhere (Willer, 1982). The significance level was set at $P \leq 0.05$.

RESULTS

In October 1991, 130 sera were collected from resting wild bean and white-fronted geese caught at the Lake Gülpe in the lowland of the Havel River. All sera were tested for the presence of specific antibodies against a panel of avian viruses using established serological tests. Serum antibodies against Newcastle disease virus (NDV), avian reovirus (REO), avian adenovirus/EDS 76 virus, and goose parvovirus (GPV) were demonstrated in both wild geese populations. Antibodies against the duck hepatitis virus (DHV) could not be detected in any of the sera (Table 1).

Whereas 58 (45%) of the 130 sera were positive for anti-NDV specific antibodies by ELISA, only 11 (8%) samples also showed positive reactions in the standard HI-test. The end-titer of the positive sera in the HI-test ranged between 1:2 and 1:128.

We found in 38 (29%) sera specific anti-reovirus antibodies and in 8 (6%) samples specific antibodies against the EDS 76 virus. The highest titer of antibody against EDS 76 virus was 1:16 using the standard HI-test.

Testing for specific antibodies against goose parvovirus in the serum neutralization test (SNT) yielded 62 (48%) positive sera having titers ranging up to 1:64 (Table 1).

There were no statistical differences in seroprevalences to the selected avian pathogens between the two geese species when the confidence intervals were compared (Table 2).

DISCUSSION

Although more than 40 resting sites for wild waterfowl are known in eastern Germany, the lowland of the Havel River including Lake Gülpe is one of the most important inland resting sites for wild geese (Rutschke, 1987; Warthold, 1993).

Wild waterfowl can be a natural reservoir or a carrier of infectious agents that may have relevance for domestic birds; their migration may facilitate transmission of viral pathogens over long distances (Sinnecker, 1985; Ziedler et al., 1995). However, the possible role of wild birds in the epidemiology of avian pathogens and in the dissemination and transmission of economically important avian diseases is not defined in detail.

Using indirect evidence we have demonstrated the presence of the Newcastle disease virus in wild geese. There was a rather high percentage (45%) of goose sera containing NDV-specific antibodies. Newcastle disease has been the economically most important disease of domestic poultry. A wide range of captive and free-living, semidomestic birds including migratory waterfowl is susceptible to NDV and can be a primary source of infection. Newcastle disease virus also has been recovered from shags (*Phalacrocorax aristotelis*), cormorants (*Phalacrocorax carbo*), and gannets (*Sula bassana*) suggesting that sea birds also can be one source of infection for domestic poultry (Banerjee et al., 1994). However, the problem of involvement of wild waterfowl and other wild birds in the epidemiology of Newcastle disease and its consequences to disease

TABLE 1. Results of the serological survey on selected viral pathogens in bean and white-fronted geese from Germany.

Species	Age structure	Number of geese examined	Viral pathogens[a] (test method)									
			NDV (ELISA)[c]		EDS 76 (HIT)[c]		GPV (SNT)[c]		REO (ELISA)		DHV (ELISA)	
			Positive	(%)	Positive	(%)	Positive	(%)	Positive	(%)	Positive	(%)
Bean goose	1	36	19	53	3	8	18	50	8	22	0	—
	2	3	2	67	0	—	0	—	1	33	0	—
	3	24	11	46	2	8	11	46	7	29	0	—
	n.d.	2	0	—	0	—	0	—	0	—	0	—
	total	65	32	50	5	8	29	45	16	25	0	—
White-fronted goose	1	35	12	34	2	6	23	66	13	37	0	—
	2	5	3	60	0	—	1	20	3	60	0	—
	3	24	11	46	1	4	9	38	6	25	0	—
	n.d.[b]	1	0	—	0	—	0	—	0	—	0	—
	total	65	26	40	3	5	33	51	22	34	0	—

[a] See text for abbreviation of viral agents.
[b] n.d. = not determined.
[c] ELISA = enzyme-linked immunosorbent assay; HIT = hemagglutination inhibition test; SNT = serum neutralization test.

TABLE 2. Confidence intervals (95%–CI) of true seroprevalence of selected viral pathogens within two geese populations from Germany ($P \leq 0.05$).

Viral pathogen[a]	Species	Positive/examined	Confidence interval
NDV	Bean goose	32/65	36.6–61.9
	White-fronted goose	26/65	27.9–53.0
EDS 76	Bean goose	5/65	2.5–16.7
	White-fronted goose	3/65	1.1–12.9
GPV	Bean goose	29/65	32.2–57.6
	White-fronted goose	33/65	37.5–63.9
REO	Bean goose	16/65	14.7–36.9
	White-fronted goose	22/65	22.6–46.3
DHV	Bean goose	0/65	—
	White-fronted goose	0/65	—

[a] See text for abbreviation of viral agents.

control are often discussed, but not yet solved (Palmer and Trainer, 1970; Friend and Trainer, 1972; Spalatin and Hanson, 1975; Ziedler et al., 1993, 1995).

Previous studies have shown that the ELISA is more sensitive than the HI-test for detecting anti-NDV-antibodies in poultry sera (Hlinak et al., 1992b, c; Ziedler et al., 1993). The present results are consistent with those previously reported. The ELISA is a sensitive and effective tool for seroepidemiological studies in domestic and wild bird populations.

Reoviruses are ubiquitous in poultry worldwide. They have been associated with a variety of avian disorders, including viral arthritis/tenosynovitis; enteritis, myocarditis, hepatitis, and respiratory-associated syndromes (van der Heide et al., 1981; Al Afaleq and Jones, 1989; Jones et al., 1989). The so-called malabsorption syndrome and other reovirus associated infectious complexes have been demonstrated in turkeys, pheasants, muscovy ducks, guinea fowl and certain ornamental bird species, but reoviruses isolated from affected birds have been inconsistent (Hieronymus et al., 1983; Adair et al., 1987; Ziedler et al., 1988). The multifaceted nature of these diseases, the inconsistent replication, and the variety of terms used to describe reovirus-associated conditions have resulted in confusion over the importance of reovirus-induced disease (Shapouri et al., 1994; Ni and Kemp, 1995). Nevertheless, reovirus infections are routinely blamed for major economic losses to the poultry industry. Based on 38 (29%) serologically positive reactants, we have evidence that reoviruses also occur in wild geese, but we do not know anything about their impact on the health status of free-living geese.

We also found specific antibodies against the egg drop syndrome 1976 (EDS 76) virus in the geese sera. This virus is an avian adenovirus and the cause of inconsistency in egg yield and problems in quality of the egg shell in laying chickens. EDS 76 virus does not have common antigens with fowl adenoviruses and adenoviruses of turkeys. EDS 76 virus has been reported to be distributed widely in wild waterfowl (Bouquet et al., 1982; Bartha, 1984; Brugh et al., 1984). Our serological results also indicate waterfowl as a possible source of this adenovirus, but waterfowl are not the direct source of this infection for laying hens.

The parvovirus infection of goslings is an acute and protracted disease with high economic losses. Derzsy's disease is a special infection only of geese and the muscovy duck, especially in juveniles (Kisary, 1993; Takehara et al., 1994; Zadori et al., 1994). We demonstrated seroprevalences of 45% and 51% for bean geese and white-fronted geese, respectively. White-fronted geese <1-yr-old had the highest percentage of parvovirus specific antibodies indi-

cating a slight shift toward decreased susceptibility with age. However, this difference between age classes was not significant when tested. The serological findings demonstrated a high prevalence of antibodies in wild geese and implicated wild geese in the epidemiology of this special goose infection. However, nothing is known about the morbidity or mortality in wild goose populations due to this virus infection.

The duck virus hepatitis is only an infection of ducklings including young muscovy ducks, caused by a picornavirus. The disease is highly contagious and shows high losses in juveniles (Asplin, 1965; Ulbrich, 1971). Adult ducks are resistant to this viral agent. In the present study, we were not able to detect specific antibodies against this enterovirus using described diagnostic method. Results were negative, although the wild geese have direct contact to wild and domestic ducks in the lowlands of the Havel River.

The results of our serological study clearly show that pathogens of domestic poultry also can occur in wild waterfowl. The defined seroprevalences demonstrate possible epidemiological relationships between domestic and wild birds in the circulation of certain viruses. Long distance migration of wild waterfowl may be another important reason for the spread of infectious agents and the transmission of important avian diseases to domestic poultry (Sinnecker, 1985; Ziedler et al., 1995). Alternatively, it also is possible, that large poultry farms can be a source of avian viruses to wild birds. The vaccination programs in domestic poultry farmed in open systems are a real source of circulation of specific avian viruses. The association of systematically immunized poultry, especially chickens and turkeys vaccinated against the Newcastle disease, to the number of serological positive wild birds has been discussed (Alexander et al., 1979; Maldonado et al., 1994). The potential crossover of avian diseases is most likely where open range flocks of domestic poultry are located near wild waterfowl habitats (Hopkins et al., 1990).

In a previous study we showed the presence of specific antibodies against the same panel of viral agents in large flocks of domestic ducks and geese in the neighboring regions to the present study area (Hlinak et al., 1992c). We also demonstrated the occurrence of antibodies against infectious agents in 21 species of zoo and wild birds, particularly in wild ducks from eastern Germany (Ziedler et al., 1995).

Now we show the indirect, serological evidence of viral agents in another important group of waterfowl, geese. Although direct proof of these infections by virus isolation was not presented, serological results allow for the conclusion that the birds had been in contact with the relevant pathogens. The origin of these infections in the wild geese populations is unknown. The prevalence of serum antibodies against the panel of selected avian viruses suggests an involvement of these wild birds in the epidemiological frame work of maintenance and spread of the pathogens. Also wild birds could be a relevant factor in the transmission of these infectious agents. Most importantly, the present serological investigation demonstrates the need for additional work in order to better understand the potential role of wild waterfowl in the epidemiology of these important avian diseases.

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