INFECTIOUS BURSAL DISEASE VIRUS ANTIBODIES IN EIDER DUCKS AND HERRING GULLS[1]

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Abstract. We measured antibodies to infectious bursal disease virus (IBDV) in blood of nesting Common Eider (*Somateria mollissima*) females and immature Herring Gulls (*Larus argentatus*) in the Baltic Sea, and in blood of Spectacled Eider (*Somateria fischeri*) females nesting in a remote area of western Alaska. Positive ($\geq$ 1:16) IBDV titers occurred in 75% of the eiders and 45% of the Herring Gull chicks. In eiders, the prevalence of positive titers differed among locations. We found no evidence that IBDV exposure impaired the immune function of Herring Gull chicks, based on their response to inoculation of sheep red blood cells. We suggest that eider ducks and Herring Gulls have been exposed to IBDV, even in locations where contact with poultry is unlikely. The presence of this virus in wild bird populations is of concern because it causes mortality of up to 30% in susceptible poultry.

Key words: Alaska, Common Eiders, Finland, Herring Gulls, infectious bursal disease, Spectacled Eiders.

Infectious bursal disease virus (IBDV) has been identified recently as a member of the family *Birnaviridae*, although the disease syndrome caused by this virus has been recognized since the 1950s in chickens (Lasher and Davis 1997). IBDV targets the developing lymphoid tissues, primarily the bursa of Fabricius, compromising the immune system of the infected bird. The tissue damage caused by the virus often leads to severe immunosuppression that renders affected birds highly susceptible to secondary pathogens. The virus also

may cause clinical illness and up to 30% mortality in older chicks (Lukert and Saif 1997). Once infected, a poultry facility often remains contaminated because the virus is very durable and can persist in the environment even after thorough disinfection procedures (Lukert and Saif 1997). Its pathogenicity, resistance to disinfectants, and ubiquitous distribution make IBDV one of the most important viruses of domestic poultry throughout the world. Chickens are the only birds currently known to develop clinical disease when exposed to IBDV (Lukert and Saif 1997).

Although little is known about the occurrence of IBDV in wild birds, the virus has been found in wild populations on at least three continents. Nawathe et al. (1978) used an agar gel precipitation test and found IBDV antibodies in 5 of 29 Village Weavers (*Ploceus cucullatus*) and 1 of 8 Cordon Bleus (*Uraeginthus bengalus*) sampled in Nigeria. Wilcox et al. (1983) found serological evidence of IBDV in seven wild avian species in western Australia using agar precipitation and plaque reduction tests. These authors considered it possible that the virus had spread to wild birds from domestic poultry. More recently, Gardner et al. (1997) reported neutralizing antibodies to IBDV in serum of two species of Antarctic penguins (*Pygoscelis adeliae* and *Aptenodytes forsteri*), speculating that this disease could threaten avian fauna in that remote part of the world. Seropositive penguins were found near areas frequented by people, but not in a rarely visited colony, and the authors suggested that the virus was introduced with poultry products and spread by human activity.

During the last decade, Common Eider (*Somateria mollissima*) populations in the Baltic Sea near coastal Finland have declined by over 50% in some breeding areas (Hario 1998). Increased juvenile mortality, resulting in low recruitment rates, is the primary cause for the decline (Hario 1998). In Alaska, the Spectacled Eider (*S. fischeri*) is listed as threatened under the U.S. Endangered Species Act because populations have declined by over 95% in the past 30 years (Stehn et al. 1993). We tested serum from adult females of Com-

mon and Spectacled Eiders to determine whether they had been exposed to IBDV. We studied the potential role of Herring Gulls (*Larus argentatus*) as carriers of the virus, because observations in Finland indicate that some Herring Gulls nesting in close proximity to eiders feed at landfills on the mainland (Kilpi and Öst 1998), where they could potentially come in contact with poultry waste. To evaluate the immunocompetence of Herring Gull chicks in relation to serologic evidence of IBDV exposure, we tested their ability to produce antibodies to heterologous erythrocytes (sheep red blood cells, SRBC). The antibody response to SRBC has been widely used in avian species to evaluate immune function *in vivo* (Schrank et al. 1990, Trust et al. 1994), and experimental infection with IBDV affects SRBC responses in poultry (Dohms and Jaeger 1988).

METHODS

In May of 1998, we collected blood from Common Eiders at three locations, Utö (59°50′N, 21°25′E, n = 22), Tvärminne (59°50′N, 23°15′E, n = 54), and Söderskär (60°06′N, 25°25′E, n = 47) in the Baltic Sea. Utö is a fairly remote archipelago approximately 50 km off the southern coast of Finland, whereas the other two study sites lie closer to the mainland. Spectacled Eiders (n = 46) were sampled in June of 1998 on Kigigak Island (60°50′N, 165°00′W) in the Yukon-Kuskokwim Delta of western Alaska. Eiders were bled by jugular venipuncture with plastic syringes and 21-gauge needles. Blood was transferred to evacuated glass (Vacutainer®, Becton Dickinson Labware, Franklin Lakes, New Jersey) or plastic (Vacuette®, Greiner Meditech, Bel Air, Maryland) tubes, allowed to clot, and serum was harvested and frozen at −80°C.

In Finland, blood also was collected from 4-week-old Herring Gull chicks in 1998 and 1999 at Tvärminne (n = 20) and Söderskär (n = 22), where gull colonies are in close proximity to eider nesting areas. At Söderskär, the ability of B-lymphocytes of the gull chicks to react to exogenous challenge with SRBC also was tested (Grasman et al. 1996). There, the gull chicks were first captured at approximately four weeks of age, bled from the ulnar vein, and inoculated intravenously (i.v., n = 11) with 0.1 ml of 1% suspension or intraperitoneally (i.p., n = 11) with 0.3 ml of 20% suspension of SRBC (#R3378, Sigma, St. Louis, Missouri). The chicks were recaptured and rebled after six days, when SRBC antibody titers peak in gulls (Grasman et al. 1996). Antibody titers to IBDV serotype 1 (Spafas® Inc., Preston, Connecticut) were measured in all serum samples using a standard virus neutralization test (Thayer and Beard 1998). Briefly, 100 infectious virus units were incubated for 30 min at 37°C with serial dilutions of heat treated (30 min at 56°C) serum samples. All samples were run in duplicate, and the antibody titer was determined as the highest dilution of serum that inhibited viral activity. Titers of ≥ 1:16 were considered as evidence of exposure (Giambrone 1980). The serum samples from the Herring Gulls at Söderskär also were tested for the presence of agglutinating antibodies to SRBC by a microtiter method (Gross and Siegel 1980). Serial 2-fold dilutions of serum were incubated with equal volume of 0.25%
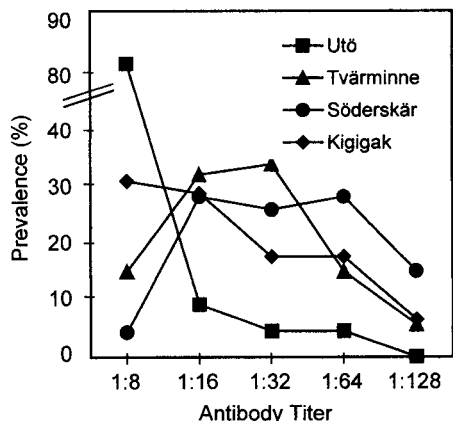

FIGURE 1. Prevalence of antibody titers to infectious bursal disease virus (IBDV) in Common and Spectacled Eiders at study sites in the Baltic Sea (Utö, Tvärminne, Söderskär) and the Yukon-Kuskokwim Delta of Alaska (Kigigak). Titers of ≥ 1:16 are considered evidence of exposure.

SRBC suspension for 3 hr at 37°C, and the antibody titer was determined as the highest dilution of serum agglutinating the SRBC. We compared prevalence of positive (≥ 1:16) IBDV titers in eiders among locations with Chi-square and Fisher's exact tests (Sokal and Rohlf 1995). In gulls, a 4-fold increase in IBDV titer between the first and the second bleeding was considered evidence of exposure to the virus (Hermann and Erdman 1995). Separate Wilcoxon two-sample tests (Sokal and Rohlf 1995) were used to compare the response of Herring Gulls to i.v. and i.p. SRBC treatments, and to compare SRBC results with IBDV titer. We used an alpha level of 0.05 as an indication of statistical significance.

RESULTS

We found positive (≥ 1:16) IBDV titers in 75% of the eiders tested, and the prevalence of positive titers differed among locations. At Utö, 18% of the eiders were seropositive, which was significantly lower than the prevalences at Söderskär (96%), Tvärminne (85%), and Kigigak (70%) (combined prevalence of 75% in eiders). The prevalence of seropositive eiders at Söderskär and Kigigak also were significantly different. In the Baltic Sea, high antibody titers (≥ 1:128) were detected at Tvärminne (6%) and Söderskär (15%) (Fig. 1).

Forty percent of Herring Gull chicks at Tvärminne and 50% of those at Söderskär had IBDV titers of ≥ 1:16 (combined prevalence of 45%). Of the gull chicks bled twice, 36% showed a 4-fold increase in IBDV titer between the first and the second bleeding. The method of SRBC inoculation (i.v. or i.p.) had no effect on the response of gull chicks to SRBC, so the i.v. and i.p. results were combined. We found no significant difference in the SRBC responses between the chicks that showed a 4-fold rise in IBDV titer and those with negative (< 1:16) IBDV titers.

DISCUSSION

Mortality events caused by infectious agents have been reported frequently in wild birds, although relatively little is known about long-term effects of diseases on avian populations (Gulland 1995). A virus that persists in the nesting environment and weakens the immune defense of the young could potentially have an impact on the host population size through reduced survival of offspring, which is one of the most important factors influencing population densities of wild animals (Krebs and Myers 1974, Loudon 1985, White 1993). Recently, it has been suggested that alterations in host immunocompetence caused by environmental stressors, especially among the neonatal age classes, may act as an intrinsic self-regulatory process leading to population fluctuations (Lochmiller 1996). An immunosuppressive virus with high transmission rates could lead to such effects.

Seroprevalence of IBDV in adult eiders in Finland and Alaska was higher than that found in adult Antarctic penguins (Gardner et al. 1997). We found antibody titers of $\geq$ 1:128 in 7% and 9% of eiders in Alaska and Finland, respectively, compared with the findings of Gardner et al. (1997), who reported titers of $\geq$ 1:80 in up to 2.6% of the adult penguins they sampled. In the Baltic, the antibody prevalence was highest in eiders nesting at Tvärminne and Söderskär, where low duckling survival (1–5%) is the primary factor in recent population declines (Hario 1998). In poultry, IBDV causes disease and mortality particularly in juvenile birds and, if the virus has similar pathological effects in eiders, it may be associated with poor fledging success.

The presence of gulls and proximity to poultry wastes may contribute to the differences in the prevalence of IBDV antibodies that were found among the eiders nesting along the Finnish coast. Of 42 Herring Gull chicks sampled at Tvärminne and Söderskär, 45% had IBDV antibody titers of $\geq$ 1:16. In these areas, 90% of eiders had a serum IBDV titer of 1:16 or greater. In contrast, we found little evidence of IBDV exposure at Utö, the most remote of our study sites in Finland and the one with the fewest nesting Herring Gulls (Miettinen 1996). The landfills nearest Utö are 80 km distant and any Herring Gulls nesting at Utö are unlikely to feed at them. Herring Gulls that nest nearer to the Finnish coast feed at landfills on the mainland where they may come in contact with poultry wastes (Kilpi and Öst 1998). They carry food back to their nesting island to feed their young, and chicks could become infected through exposure to contaminated landfill waste. Gulls nest on the same or nearby islands as the eiders at Tvärminne and Söderskär and, potentially, may be carriers of IBDV and could transmit the virus to eiders. The pathogenic infectious bursal disease viruses affect primarily the function of host B-lymphocytes, but gull chicks that had serological evidence of active IBDV infection were able to respond to SRBC immunization as well as the ones that did not show evidence of recent IBDV exposure. Thus, in our study, we found no evidence that Herring Gull chicks suffered immunosuppression induced by the virus they had been exposed to.

Although our results from Finland lead us to suggest that gulls may be carriers of IBDV, potentially through their exposure to poultry waste, it is difficult to use similar reasoning to explain the findings in Alaska. The Spectacled Eiders in Alaska had a prevalence of IBDV exposure similar to the Finnish eiders, but they nest in remote areas of the Yukon-Kuskokwim Delta where human habitation occurs in sparsely populated villages. Although landfills are maintained at these villages and visited by sympatric nesting Glaucous Gulls (*Larus hyperboreus*), we found no evidence to suggest that those disposal sites are important sources of food for gulls. Glaucous Gulls nesting in coastal areas of western Alaska, including Kigigak Island, feed heavily on marine invertebrates and fish (Schmutz and Hobson 1998), and an examination of approximately 400 stomachs collected from Glaucous Gulls in the Yukon-Kuskokwim Delta revealed no evidence of landfill waste materials (T. Bowman, unpubl. data). The Spectacled Eiders winter in the Bering Sea, far from any potential sources of poultry waste. Therefore, the finding of antibodies to IBDV in Spectacled Eiders raises speculation that eiders, in Alaska and possibly Finland, may be carriers of IBDV. Although poultry may be a source of new IBDV infections, this or a closely related virus may have established itself at some earlier time in populations of wild birds through some other means.

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RAPD-PCR AMPLIFICATION OF DNA EXTRACTED FROM AVIAN BLOOD INFECTED WITH *HAEMOPROTEUS* FAILS TO PRODUCE FALSE POSITIVE MARKERS[1]

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Abstract. We tested whether contamination of avian blood by the parasite *Haemoproteus* could cause the amplification of false positive genetic markers generated by random amplified polymorphic DNA (RAPD). DNA was extracted from blood taken from five Rock Doves (*Columba livia*) infected with *Haemoproteus,* and one uninfected control individual. A drug treatment was administered to clear the infection, and a second DNA sample extracted. The parasitized and unparasitized DNA samples were used in RAPD reactions with 20 different 10-nucleotide primers, which produced 178 well-resolved markers. In no case were any of the reaction products specific to the parasitized samples, and in almost all cases the products