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Authors: Lundström, Jan O., Turell, Michael J., and Niklasson, Bo

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Antibodies to Ockelbo Virus in Three Orders of Birds (Anseriformes, Galliformes and Passeriformes) in Sweden

Jan O. Lundström,[1,2] **Michael J. Turell,**[3] **and Bo Niklasson,**[1,4] [1] Department of Virology, National Bacteriological Laboratory, S-10521 Stockholm, Sweden; [2] Department of Virology, Karolinska Institute % National Bacteriological Laboratory, S-10521 Stockholm, Sweden; [3] Department of Arboviral Entomology, Virology Division, United States Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, Maryland 21702, USA; [4] Swedish Defence Research Establishment, FOA-5, S-17290 Sundbyberg, Sweden

ABSTRACT: Sera from 324 birds collected in an Ockelbo virus disease endemic area in central Sweden were examined for the presence of specific antibodies to Ockelbo virus by a plaque reduction neutralization test. Birds examined belonged to the orders Anseriformes (n = 207), Galliformes (n = 66) and Passeriformes (n = 51). Ockelbo virus neutralizing antibodies were detected in 26 (8%) of the specimens, including species from each of the three orders tested. Specific antibodies found in caged birds and in 6- to 10-week-old birds suggested local transmission. The highest antibody prevalence (27%, 14/51) was observed in the Passeriformes in which 5 of 9 species tested contained antibodies. The high antibody prevalence in passeriforms and the very large population of this group in relation to other avian groups in Sweden gives them a high potential as amplification hosts for Ockelbo virus.

Key words: Alphavirus, Ockelbo virus, antibodies, birds, serosurvey, amplification hosts.

A clinical syndrome in humans with rash and arthralgia as major symptoms was first described in Sweden three decades ago. Serological studies showed that the disease was caused by Sindbis or a Sindbis-like virus (Skogh and Espmark, 1982). In 1982, a virus was isolated from mosquitoes collected in central Sweden. This virus, designated Ockelbo virus, was implicated as the causative agent of Ockelbo disease and shown to be closely related to, but distinguishable from, Sindbis virus (Niklasson et al., 1984).

Sindbis virus is transmitted primarily by *Culex* mosquitoes in a mosquito-bird transmission cycle (Taylor et al., 1955; Doherty et al., 1963; McIntosh et al., 1967, 1968; Jupp and McIntosh, 1970; Marshall et al., 1982). Isolation of Ockelbo virus primarily from ornithophagic *Culex* spp. and *Culiseta* spp. mosquitoes, detection of Ockelbo virus neutralizing antibodies in several species of Passeriformes, and the ability of *Culex* sp. mosquitoes to transmit Ockelbo virus to domestic chickens (*Gallus gallus*) under experimental conditions indicate that Ockelbo virus is maintained in a zoonotic cycle similar to that of Sindbis virus (Francy et al., 1989; Lundström et al., 1990). The role of bird involvement in Ockelbo virus transmission was evaluated by examining native anseriform, galliform and passeriform birds for virus specific neutralizing antibodies.

In the present study, both wild birds and birds held in captivity were sampled at the Boda Wildlife Research Station (61°32′N, 17°52′E), in an Ockelbo disease endemic area in central Sweden. This study site was located in a mixed deciduous (*Alnus glutinosa, Betula pubescens, Prunus padus, Salix caprea, Sorbus aucuparia, Populus tremula*) and coniferous (*Picea abies, Pinus sylvestris*) forest. The Enångersån River meandered through the area and produced a biotope suitable for both mosquitoes and birds. Native anseriforms and galliforms were held either in captivity within a 150 × 100 m fenced area which included several dams for water-birds or in out-door wire cages within this area. These birds had been in captivity for generations, they were individually banded, and records were kept on the bird's ages. Passeriforms were collected with Japanese mist nets within or adjacent to the fenced area during 2 to 3 August 1988, and galliforms and anseriforms were sampled from 26 July to 3 August 1988. Captured passeriforms were identified to species and the age was estimated on the basis of a bird's

plumage and body-size. Passeriforms were bled from the jugular vein (0.1 ml), marked by feather clip, and released as previously described (Francy et al., 1989). The blood was diluted 1:10 in Hanks' balanced salt solution containing 10% heat-inactivated (30 min at 56 C) fetal bovine serum, Hepes buffer and antibiotics. A 1- to 8-ml sample of blood was obtained from the jugular vein (adult galliforms) or from the tarsal vein (anseriforms). In contrast, 0.1 ml was obtained from young galliforms and these blood samples were diluted 1:10 as described above.

In addition, wild Canada geese (*Branta canadensis*) were collected in lakes in the Stigsjö (62°38'N, 17°40'E) and Ullånger (63°00'N, 18°11'E) areas during 19 to 25 July 1988. These shallow lakes were surrounded by either farmland or mixed deciduous (*Salix caprea, Alnus glutinosa, Betula pubescens, Populus tremula*) and coniferous (*Picea abies*) forests. The age of wild Canada geese was estimated on the basis of plumage, size and body weight. They were bled (2 to 8 ml) from the tarsal vein and then released.

All blood specimens were transported on wet ice to the National Bacteriological Laboratory where sera were separated and the samples stored at −20 C until tested. Sera were heat-inactivated and tested by a plaque-reduction neutralization (PRN) test (Earley et al., 1967; Francy et al., 1989) for neutralizing antibodies to Ockelbo virus, Edsbyn 82/5 strain (Niklasson et al., 1984). The plaque dose was 40 to 80 plaques per each 2 cm^2 well and an 80% reduction in the number of plaques was considered positive. All sera were screened at a 1:10 dilution, and those found positive were retested at four-fold dilutions up to 1:640 (sera from blood diluted 1:10 during the initial processing were tested undiluted).

Twenty-six of 324 birds, including species of all three orders investigated, contained neutralizing antibodies (Table 1). Of the 26 positive sera titrated, one contained neutralizing antibodies at a 1:10 dilution, two at a 1:20 dilution, three at a 1:40 dilution, four at a 1:80 dilution, nine at a 1:160 dilution, five at a 1:320 dilution, and two at a ≥1:640 dilution.

Two-hundred-seventy-three birds were sampled at Boda, including 156 Anseriformes, 66 Galliformes and 51 Passeriformes (Table 1). The highest antibody prevalence rate (27%) was observed in the Passeriformes where 5 of 9 species tested contained antibodies. This is in agreement with a previous report from an Ockelbo virus endemic area in central Sweden (Francy et al., 1989).

Six (4%) of 156 Anseriformes from Boda and two of 51 (4%) (both Canada geese) sampled at Stigsjö and Ullånger contained Ockelbo virus antibodies. Similarly, four (6%) of the 66 specimens obtained from four species of Galliformes at Boda were positive. However, the capercaillie (*Tetrao urogallus*) was the only galliform species with detectable antibodies to Ockelbo virus. The antibody prevalence rate in this species was four of 24 (17%).

Specific Ockelbo virus neutralizing antibodies were detected in 6 anseriforms and 4 galliforms at Boda. These birds were hatched at Boda, had spent their entire life there and were the offspring of birds held in captivity at Boda for several generations. The occurrence of specific antibodies in these birds shows that virus was transmitted locally. Buescher et al. (1959) found that an immune bird may transfer antibodies to her offspring, but these passively acquired antibodies are only detectable during the first three to 5 weeks after the young bird is hatched. Thus, the presence of specific antibodies in two 10-wk-old Canada geese from Boda and two 6-wk-old Canada geese from Stigsjö suggests that these birds were infected with Ockelbo virus in these areas during the 1988 summer.

The overall antibody prevalences of Ockelbo virus by PRN testing were 27% and 4% for Passeriformes and Anseriformes, respectively. In contrast, McIntosh et al. (1968) found Sindbis virus hemagglutination-inhibition (HI) antibodies more

TABLE 1. Ockelbo virus neutralizing antibodies in birds sampled in central Sweden during July and August 1988.

Order Species (common name)	Number of positive/ number tested (%)	Location
Anseriformes		
Branta canadensis (Canada goose)	2/7 (29)*	Boda
Branta canadensis (Canada goose)	2/21 (10)*	Stigsjö
Branta canadensis (Canada goose)	0/30	Ullånger
Branta leucopsis (barnacle goose)	0/15	Boda
Anser anser (greylag goose)	0/6	Boda
Anser fabalis (bean goose)	3/26 (12)	Boda
Anas platyrhynchos (mallard)	0/82	Boda
Anas penelope (wigeon)	1/3 (33)	Boda
Aythya fuligula (tufted duck)	0/15	Boda
Aythya ferina (pochard)	0/1	Boda
Brucephala clangula (goldeneye)	0/1	Boda
Subtotal	8/207 (4)	
Galliformes		
Lagopus lagopus (willow grouse)	0/4	Boda
Tetrao urogallus (capercaillie)	4/24 (17)	Boda
Tetrao tetrix (black grouse)	0/11	Boda
Phasianus colchicus (pheasant)	0/27	Boda
Subtotal	4/66 (6)	
Passeriformes		
Phoenicurus phoenicurus (redstart)	0/1	Boda
Erithacus rubecula (European robin)	1/3 (33)*	Boda
Turdus philomelos (song thrush)	0/1	Boda
Motacilla alba (white wagtail)	1/5 (20)	Boda
Parus major (great tit)	0/2	Boda
Sitta europea (nuthatch)	0/1	Boda
Passer domesticus (house sparrow)	2/4 (50)	Boda
Fringilla coelebs (chaffinch)	7/16 (44)	Boda
Emberiza citrinella (yellowhammer)	3/18 (17)	Boda
Subtotal	14/51 (27)	
Grand total	26/324 (8)	

* All positive in this species were young birds, born in the area of collection.

frequently among Anseriformes (9%, n = 160) than among Passeriformes (0.7%, n = 1,652) in South Africa. Similarly, Ernek et al. (1975, 1977) found the prevalence of neutralizing antibodies to Sindbis virus to be 15% (n = 144) among Anseriformes and 4% (n = 82) among Passeriformes in Czechoslovakia. In Australia, antibody rates of 22 to 33% to Sindbis virus in Anseriformes have been detected by HI (Hore et al., 1973; Marshall et al., 1982). The relative difference between antibody prevalence in Passeriformes and Anseriformes in Sweden compared with Czechoslovakia and South Africa may reflect a difference in the mode of natural transmission.

In Sweden, the passeriforms appear to be the principal vertebrate hosts for Ockelbo virus. More than 90% of the breeding bird population in Sweden are passeriforms (Ulfstrand and Högstedt, 1976), and Swedish passeriforms have frequent contact with infective mosquito vectors as shown by the high prevalence of virus specific antibodies. However, we must still determine if Ockelbo virus can produce a viraemia of sufficient titre and duration in these birds for them to serve as efficient amplification hosts.

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