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SEROLOGIC STUDY OF SOME INFECTIOUS DISEASES OF CANADA GEESE

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Abstract

A serologic study was conducted to investigate the exposure of captive and free-flying Canada geese *(Branta canadensis)* to five arboviruses, chlamydiae and *Mycoplasma gallisepticum*. Of more than 1200 serums screened in the metabolic-inhibition test (MIT), no serums neutralized eastern encephalitis virus (EEV), less than 1% neutralized western (WEV) or Venezuelan (VEV) encephalitis viruses, 2% neutralized St. Louis encephalitis virus (SLEV), and 29% neutralized California encephalitis virus (CEV). Supplementary serologic procedures indicated that a nonspecific inhibitor probably caused inhibition of CEV in the MIT.

Fifty-six per cent of 197 serums had hemagglutination-inhibition titers of 1:80 or higher against *M. gallisepticum* and were considered "positive". Two hundred and eight serums were negative for complement-fixing antibody against chlamydiae.

Introduction

Approximately 1,300,000 migratory Canada geese, an important wildlife resource, inhabit North America east of the Rocky Mountains. Successful goose management has markedly increased the size of many migratory Canada goose populations and has caused behavioral changes, including the congregation of larger numbers of geese on smaller areas and for longer periods of time.

Knowledge about the infectious diseases of the Canada goose, essential for continued successful management of this valuable resource, it limited.

The involvement of wild and domestic birds in the epidemiology of eastern encephalitis virus (EEV), western encephalitis virus (WEV) and St. Louis encephalitis virus (SLEV) has been well documented[2,3] although the relationship of these viruses with waterfowl has not been thoroughly studied. EEV has been isolated from white pekin ducks,[15] and EEV antibody has been reported in a captive white-fronted goose *(Anser albifrons)*, a captive black swan *(Cygnus atratus)*,[6] and a wild black duck *(Anas rubripes)*.[24] WEV antibody has been serologically detected in a captive black duck, a captive Canada goose[6] and several species of migratory ducks in South America.[37] Experimentally, domestic ducks are susceptible to SLEV.[21]

Venezuelan encephalitis virus (VEV) and California encephalitis virus (CEV) are generally considered to have mammalian reservoirs;[3] however, VEV has been isolated from several species of wild birds in Central America,[18] and chickens have been experimentally infected with CEV.

Mycoplasmas have been isolated from ducks and geese with sinusitis.[14,16,32] Chlamydiae have been isolated from numerous domestic and wild waterfowl.[27]

Materials and Methods

Between October, 1965, and January, 1967, almost 1400 serums were obtained from Canada geese in 14 collections on 10 national, state and private waterfowl refuges. Table 1 lists the refuges where blood samples were obtained, dates of collection, and references pertinent to the biology of each flock.

Serums were collected from four major populations of migratory geese (Table 1): the Mississippi Valley and Eastern Prairie populations of the Mississippi Flyway and the Western Prairie and Tall Grass Prairie populations of the Central Flyway. In addition, serums were collected from several small captive and free-flying, non-migratory flocks (Table 1).

Geese were captured in corrals during the summer molt, baited swim-in traps, or cannon-net-traps. Captured geese were sexed and aged. Geese were classified "juvenile" (less than one - year - old), "yearling" (one-year-old), and "adult" (two years and older), but sometimes all geese over one-year-old were combined as "adults".

After collection, serums were heat-treated in a water-bath 30 min. at 56°C and stored at —20°C. The metabolic-inhibition test (MIT), using HeLa cells, was used to screen more than 1200 serums for neutralizing antibodies against five arboviruses.[29,34] Antigens used were EEV (AP-128), WEV (Fleming isolate), SLEV (CDC-862), VEV (Trinidad strain), and CEV (RML-10-8-59, Snowshoe hare). Selected serums which were CEV-reactors in the MIT were retested at the Wisconsin State Hygiene Laboratory, Madison, for confirmation of antibody specificity. Fifteen MIT - positive and five MIT-negative serums were tested for hemagglutination-inhibition (HI) antibody against the LaCrosse strain of CEV. Eight MIT-positive and two MIT-negative serums were tested by mouse inoculation for neutralization antibodies against CEV (BFS 283). Six serums which were positive for CEV-neutralization in the screening procedure were titrated in the MIT against 158 $TCLD_{50}$

TABLE 1. *Waterfowl Areas Where Canada Goose Blood Samples Were Obtained*

Waterfowl Area*	Flock Status**	Vicinity	Collection Date
Mississippi Flyway			
Horicon Marsh[31]	M-MV	Horicon, Wis.	10-65, 4-66
Horseshoe Lake[31]	M-MV	Miller City, Ill.	1-67
Seney[33]	M	Seney, Mich.	7-66
Kellogg[23]	F	Battle Creek, Mich.	7-66
Swan Lake[30]	M-EP	Sumner, Mo.	1-66, 10-66
Crex Meadows[22]	C	Grantsburg, Wis.	7-66
Green Bay[22]	F	Green Bay, Wis.	7-65, 7-66
Central Flyway			
Squaw Creek[19]	M-TG	Mound City, Mo.	1-66
Squaw Creek[19]	M-WP	Mound City, Mo.	11-66
Sand Lake[19]	M-TG	Columbia, S. Dak.	10-66
Trimble[5]	F	Trimble, Mo.	6-66

*Including pertinent references on the biology of each flock.
**M—migratory; C—captive; F—free-flying, essentially nonmigratory.
MV—Mississippi Valley population; EP—Eastern Prairie population;
TG—Tall Grass Prairie population; WP—Western Prairie population,
predominantly.

CEV. Doubling dilutions of serum began at 1:2 and were tested in replicates of three.

Almost 200 serums collected from geese on three refuges were tested for HI antibodies against *Mycoplasma gallisepticum*.[35] Canada goose erythrocytes were satisfactorily agglutinated by the Salsbury HI antigen and were used to avoid agglutination of test erythrocytes by serums. A HI end-point of 1:80 is considered positive evidence of *M. gallisepticum* infection in poultry, and this standard was arbitrarily accepted as the criterion for Canada goose serums.

Serums from 208 Canada geese collected at Swan Lake in January, 1966, were tested for chlamydial antibodies with a modified complement - fixation test[8] conducted at the Regional Animal Diagnostic Laboratory, Barron, Wisconsin.

Results

More than 1200 Canada goose serums were screened in the MIT for neutralizing antibodies against five arboviruses. No serums neutralized EEV in the MIT; less than 1% neutralized WEV or VEV; 29% neutralized CEV; and 2% neutralized SLEV. No serum neutralized more than one group A arbovirus. The distribution of virus-neutralizing serums by refuge is summarized in Table 2. There were no apparent sex-associated differences in reactor-prevalence to any of the arboviruses. Serums from twice as many juveniles (42%) as adults (21%) neutralized CEV; however, not enough reactors against the other arboviruses were detected to make meaningful comparisons of reactor-prevalence by age.

Fifteen goose serums which neutralized CEV in the MIT were tested against CEV HI antigen. Although serums from one adult and one juvenile reacted at 1:10 and 1:20, respectively, the remainder were negative ($<$1:10). The two serums which had low titers and six other serums which were "positive" in the MIT were negative (titer, $\log_{10}<1$) in the mouse neutralization test. Three of six serums titrated against 158 $TCLD_{50}$ of CEV in the MIT inhibited virus growth in at least one of three replicates at an end-point dilution of 1:8; two had end-points at 1:4 and one at 1:2.

Fifty-six per cent of 197 serums had HI titers against *M. gallisepticum* of 1:80 or higher and were considered to be "positive" (Table 3). The prevalence of "positive" males (56%) and females (55%) were similar within the juvenile and adult age-groups, but there was an empirically higher reactor - prevalence among juveniles (69%) than among adults (47%). Twenty-three per cent of all serums had *M. gallisepticum* HI titers of 1:160 or higher.

The 208 serums collected at Swan Lake were negative for complement-fixing antibodies against chlamydiae.

Discussion

The lack or low prevalence of EE, WE and VE vrius-neutralizing substances in this study were similar to results reported in a study of Crex Meadows and Horicon Marsh geese.[4] Although WEV and EEV activity were not serologically detected in this study at the W. K. Kellogg Bird Sanctuary, Brown[6] reported serologic evidence of exposure to these viruses in several waterfowl species, including a Canada goose, during an epizootic of WE and EE in horses in southern Michigan.

A portion of the Eastern Prairie population, in which WEV-reactors were twice detected, nests within the geographical range of *Culex tarsalis*[10] and WEV activity. Although many of the other migratory and sedentary flocks spend the summer within the general geographical range of WEV, only the Trimble collection contained a WEV-reactor. The overall low prevalence of WEV-exposure reflects either a minimal involvement of Canada geese in the epizootiology of WEV or possibly a low level of WEV activity in these specific habitats during the study period.

SLEV-neutralization was detected in serums from four collections in southern

Illinois and Missouri (Table 2). Since the Trimble flock spends the summer "arbovirus season" in Missouri, and since there has been SLEV-activity in the area of Kansas, Nebraska and Missouri during the period of 1964-1966,[11,12,13] these SLEV-reactors may indicate exposur:e to SLEV. The flocks tested at Horicon Marsh, Swan Lake, and Squaw Creek are not within the generally accepted range of SLEV during the mosquito season. Although SLEV has twice been isolated 50 miles north of Calgary, Alberta,[7] the virus is generally not considered to be widespread in the far north. Since arbovirus studies have been most extensive in the populous areas of southern Canada, a bird-mosquito cycle of SLEV might occur without detection in the far north.

The CEV-neutralizing-substance detected in the screening procedure was probably nonspecific inhibitor since it was low titer or undetectable in quantitative supplementary tests.

Natural arbovirus infections of indigenous North American birds are usually inapparent,[2] so natural infections of Canada geese would probably not cause significant mortality. Although the sedentary flocks which were studied could potentially be involved in the epidemiology of human arbovirus infections, the large migratory goose populations nest in remote areas during the summer.

TABLE 2. *Summary of MIT Results of Canada Goose Serums for Antibody Against Five Arboviruses*

Flock	Number Tested	Per Cent Reactors*				
		WE	VE	SLE	CE	EEV
Horicon Marsh	34	0	0	21	12	0
Horseshoe Lake	183	0	1	0	26	0
Swan Lake	86	2	0	3	5	0
Swan Lake	292	1	0	0	31	0
Squaw Creek	4	0	0	25	0	0
Squaw Creek	61	0	0	0	5	0
Sand Lake	34	0	0	0	76	0
Seney	142	0	0	0	12	0
Kellogg	136	0	1	0	36	0
Green Bay	131	0	0	0	64	0
Crex Meadows	68	0	0	0	25	0
Trimble	93	1	0	14	27	0
Total	1264	1	1	2	29	0

*Reactors neutralized $10^{1.5}$ to $10^{3.5}$ $TCLD_{50}$ of specific antigen in HeLa cells.

TABLE 3. *Age Distribution of Canada Geese Which Were HI-reactors Against* Mycoplasma Gallisepticum

Refuge	Collection Date	Results*		
		Juvenile	Adult	Total
Horicon Marsh	10-65	8/11 (73)	18/40 (45)	26/51 (51)
Green Bay	7-65	16/27 (59)	21/39 (54)	37/66 (56)
Swan Lake	1-66	30/41 (73)	17/39 (44)	47/80 (59)
Total		54/79 (68)	56/118(47)	110/197(56)

*Number of reactors/number tested (per cent reactors).

The high prevalence of *M. gallisepticum* HI-substance in Canada goose serums is of potential importance. *M. gallisepticum* may cause chronic debilitating disease in Canada geese, as it does in domestic poultry.[3b] The high prevalence of reactors must be considered with caution because frozen serums sometimes cause nonspecific hemagglutination-inhibition of *M. gallisepticum*.[1] The large number of high-titer reactors, however, suggests the presence of antibody. If the HI-substance detected in this study is antibody, it would probably be specific for *M. gallisepticum* since the HI test has been reported to be serologically specific for *Mycoplasma sp.*[25,30] The recent isolations of, as yet uncharacterized, *Mycoplasma sp.* from a Canada goose at Crex Meadows and from migratory Canada geese at the Pine Island Wildlife Area in Wisconsin[17] give additional credence to the validity of these serologic results. The high prevalence of high-titer reactors in geese of both the migratory Mississippi Valley and Eastern Prairie populations and the essentially non-migratory Green Bay flock appear to indicate widespread exposure; however, further serologic study, characterization of isolates, and experimental exposure of Canada geese to these isolates would be required to clarify the status of the pathogen.

Two previous serologic searches for *M. gallisepticum* antibody in Canada geese have been reported. During the period 1964 to 1967, none of 193 serums from the wintering goose flock at Rochester, Minnesota, were positive for *M. gallisepticum* in the plate agglutination test.[29] There were no *M. gallisepticum* HI-reactors among 12 geese collected in 1964 from the Crex Meadows flock.[4] Because of the diversity of the flocks tested, the results of the three studies are not necessarily contradictory; however, differences in the three serologic tests allow speculation about the comparative validity of the results.

The lack of detectable ornithosis antibody in waterfowl serums in this study and earlier studies[4,25] suggests that chlamydial infection is not widespread in wild waterfowl in the midwestern United States; however, the possibility of occasional infection should not be dismissed since only a limited number of serums was tested in comparison with the size of the study populations.

Epizootiological information which can be obtained from an exploratory serological study such as this has limitations. The number of geese collected from an individual flock is often insufficient, and the collection technique may bias the composition of the collection so that the geese tested are not representative of the flock present or the population as a whole.[29,30] Even though limited, a study of this type gives direction to future investigation of the diseases of Canada geese. The results of this study indicate a need for increased study of the prevalence of *M. gallisepticum* antibody in Canada geese, for isolation of *Mycoplasmas* from these geese and experimental study of the effect of *Mycoplasmas* on Canada geese.

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