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Authors: ZARNKE, RANDALL L., and YUILL, THOMAS M.

Source: Journal of Wildlife Diseases, 17(3) : 453-461

Published By: Wildlife Disease Association

URL: https://doi.org/10.7589/0090-3558-17.3.453

SEROLOGIC SURVEY FOR SELECTED MICROBIAL AGENTS IN MAMMALS FROM ALBERTA, 1976

RANDALL L. ZARNKE [1] and THOMAS M. YUILL, Department of Veterinary Science, University of Wisconsin, Madison, Wisconsin 53706, USA.

Abstract: Blood samples were taken from humans and several species of free-ranging wild mammals from five different geographic areas of Alberta, Canada. Sera were tested for antibody to eastern equine encephalitis (EEE) virus, western equine encephalitis (WEE) virus, St. Louis encephalitis (SLE) virus, Powassan (POW) virus, the snowshoe hare (SSH) strain of the California group (CAL) of viruses, Northway (NOR) virus, Klamath (KLA) virus, infectious bovine rhinotracheitis (IBR) virus, and two bacteria, *Brucella abortus* and *Francisella tularensis*. CAL antibody was found in 63% of 11 snowshoe hares *(Lepus americanus)*, 33% of 167 black bears *(Ursus americanus)*, and 19% of 55 humans *(Homo sapiens)*. NOR antibody was found in 0.4% of 258 hares, 11% of 9 bighorn sheep *(Ovis canadensis)*, 20% of 44 moose *(Alces alces)*, 4% of 56 bears, 14% of 22 woodland caribou *(Rangifer tarandus)*, and 2% of 50 humans. IBR antibody was detected in 14% of 14 moose. *B. abortus* antibody was found in 1% of 283 bears. *F. tularensis* antibody was detected in 2% of 52 humans. These findings represent extension of: (1) the natural host range for IBR, CAL, and NOR; (2) the geographical distribution of NOR infection in North America; and (3) the geographical distribution of CAL infection within Alberta.

INTRODUCTION

Evidence for infection of wild mammals in northern North America by the following microbial agents has been reported: eastern equine encephalitis (EEE) virus,[3,10,22] western equine encephalitis (WEE) virus,[3,10,22] St. Louis encephalitis (SLE) virus,[10,17,18,22,33] Powassan (POW) virus,[10,17,35] the California group (CAL) of viruses,[10,18,22,33] Northway (NOR) virus,[26] Klamath (KLA) virus,[4] infectious bovine rhinotracheitis (IBR) virus,[6] *Brucella abortus* bacteria,[24] and *Francisella tularensis* bacteria.[10,35] All of these agents, except NOR and KLA, were previously reported from Alberta, Canada.

Humans *(Homo sapiens)* are known to be susceptible to infection with all of these agents except IBR, NOR, and KLA.[10,13,14,15,22] All except SLE, NOR, and KLA are of economic importance because they infect domesticated animals.[3,10,15,22,24,34] The natural host range of NOR and KLA has not been thoroughly investigated.

The objective of this study was to determine the prevalence of these microbial agents in populations of humans and wild, free-living mammals from different geographic areas of Alberta. Prevalence was assessed by means of serologic tests. This communication reports extension of the (1) natural host range for IBR, CAL, and NOR; (2) geographic distribution of NOR in North America; and (3) geographical distribution of CAL within Alberta.

MATERIALS AND METHODS

All samples were collected in Alberta, Canada. The primary study area 45 km

[1] Present address: Alaska Department of Fish and Game, 1300 College Road, Fairbanks, Alaska 99701, USA.

north of Fort McMurray (111° West longitude; 57° North latitude) was described previously.[9,29]

Animal trapping and sampling. All small mammals were captured in live traps.[2] Snowshoe hares *(Lepus americanus)* were bled by cardiac puncture using the Vacutainer system.[3] All other small animals were bled from the retro-orbital sinus of the eye with sterile 15 cm Pasteur pipettes. Blood was immediately transferred to sterile sealed centrifuge tubes and transported to the field laboratory in a styrofoam cooler. A sealed plastic container filled with a chilled, saturated brine solution was used to keep samples cold during transport. The blood samples were kept at 20-23 C for 4 to 6 h, and then transferred to 4 C for 18 to 20 h. After centrifugation, serum was collected by aspiration, inactivated at 56 C for 30 min, and stored at -20 C until the time of testing.

Other wildlife sera included: 283 black bear *(Ursus americanus)* from Cold Lake, 247 snowshoe hare from Rochester, 35 moose *(Alces alces)* from Fort MacKay, 22 woodland caribou *(Rangifer tarandus)* from Fort MacKay, 11 moose from Swan Hills, 9 bighorn sheep *(Ovis canadensis)* from the Rocky Mountain Forest Preserve, and 3 wolves *(Canis lupus)* from Fort MacKay. These sera were collected by other biologists as part of established research projects. These sera were stored and processed as above.

Human serum samples. Blood samples were taken from the brachial vein of 55 residents of Fort MacKay, Alberta, an American Indian community of approximately 250 people located 15 km north of the study area. The sampled residents ranged in age from 5 to 72 years; 18 were males and 37 were females. Serum was collected, processed and stored as above.

Cell culture. An established, continuous line of M-199-adapted baby hamster kidney cells (BHK-21) was used for all tests, except for infectious bovine rhinotracheitis (IBR) virus. For the IBR tests, primary bovine kidney cells were used.

Cell culture medium. Cell culture medium consisted of: medium M-199,[4] 10% heat-inactivated fetal calf serum,[4] 0.1% gentamicin sulfate,[5] 1.0% sterile tricine buffer, and 2.0% sterile sodium bicarbonate. Cell culture medium was used as a diluent throughout this study.

Viruses. The viruses used in neutralization tests were: strain AP-218 of EEE, strain TD6-53/W of WEE, strain CDC-904 of SLE, L.B. strain of POW, prototype snowshoe hare (SSH) virus, strain 61-V-2235 of Jamestown Canyon (JC) virus, strain 993 of trivittatus (TVT) virus, protype LaCrosse (LAC) virus, strain 0234 of NOR, strain 1056 of KLA, and the Omro strain of IBR.

Bacterial antigens. A stained *F. tularensis* antigen was obtained commercially.[6] A stained, buffered *B. abortus* antigen was obtained from the Diagnostic Reagents Section, Veterinary Services Laboratory, APHIS, Ames, Iowa 50010.

Antisera and hyperimmune mouse ascitic fluids. Hyperimmune mouse ascitic fluid (HI-MAF) was prepared for both POW and SLE by the method of Feild and Kalter for epizootic hemorrhagic disease of deer and Semliki Forest viruses.[8] HI-MAF for JC, LAC, SSH, and TVT were prepared by the

[2] National Live Trap Co., Tomahawk, Wisconsin 54487, USA.

[3] Becton, Dickinson and Company, Rutherford, New Jersey 07070, USA.

[4] International Scientific Industries, Inc., Cary, Illinois 60013, USA.

[5] Schering Corp., Kenilworth, New Jersey 07033, USA.

[6] Difco Laboratories, Detroit, Michigan 49232, USA.

same method except that the first two injections of antigen were administered subcutaneously (SC), and the final injection of antigen and Freund's complete adjuvant was omitted.

Antisera for EEE,[7] WEE,[7] and IBR[8] were prepared in domestic rabbits. HI-MAF for NOR and KLA were obtained from C.H. Calisher of the Center for Disease Control, Ft. Collins, Colorado 80522, USA. *F. tularensis* antiserum was obtained from Difco. *B. abortus* antiserum was prepared in a cow.[9]

Serological tests. Sera were tested by the constant virus/serum dilution neutralization test (NT) method.[21] In preliminary tests, bighorn sheep, moose, black bear, wolf, woodland caribou, and human sera were diluted 1:4 for all viruses except IBR. Deer mouse *(Peromyscus maniculatus),* snowshoe hare, red squirrel *(Tamiasciurus hudsonicus),* least chipmunk *(Eutamius minimus),* redback vole *(Clethrionomys gapperi),* and flying squirrel *(Glaucomys sabrinus)* sera were initially tested at a dilution of 1:10. IBR testing of bighorn sheep and moose sera was done with two-fold serum dilutions beginning at 1:3.[10] Wild mammal sera were tested against only SSH of the CAL group. Human sera were tested against SSH, JC, TVT, and LAC of the CAL group. A serum was considered positive if cytopathic effect (CPE) was inhibited in either or both of the test wells at the minimum serum dilution at which the serum was tested. Sera positive in the preliminary NT were then titrated by NT to their end-point, in serial two-fold dilutions.

Sera were tested at a dilution of 1:40 (large mammals) or 1:400 (small mammals) for *B. abortus* and *F. tularensis* antibodies by the rapid slide agglutination technique[28] (small mammal sera were initially diluted 1:10 in the field). Sera that agglutinated the antigens were considered positive and were titrated to determine the agglutination end-point. All bear, moose, caribou, and sheep sera were also tested for *B. abortus* complement fixing (CF) antibody.[11] The cold fixation procedure was employed in a micro-adaptation of the standard CF test.[1] A serum sample was considered positive if it fixed complement at a minimum serum dilution of 1:8.

RESULTS

NOR antibody was found in sera from six species, and sera from five species had SSH antibody. *Brucella abortus* antibody occurred only in bear sera, *F. tularensis* antibody was found in one human serum, and two moose had IBR antibody (Table 1). Ten of 50 human sera had antibody to CAL group viruses (Table 2), including SSH, LAC, TVT, and JC. None of the sera had antibody to EEE, WEE, SLE, POW, or KLA. Large mammals, except wolves, had antibodies to several agents. No antibody to any of the 10 agents was found in sera from: *Peromyscus maniculatus, Tamiasciurus hudsonicus, Eutamius minimus,*

[7] Provided by B.D. Nassif, Department of Veterinary Science, University of Wisconsin, Madison, Wisconsin 53706, USA.

[8] Provided by S.J. Hyland, Department of Veterinary Science, University of Wisconsin, Madison, Wisconsin 53706, USA.

[9] Provided by L.M. Jones, Department of Veterinary Science, University of Wisconsin, Madison, Wisconsin 53706, USA.

[10] Tests performed by S.J. Hyland, H. Peterson and J. Homan, Department of Veterinary Science, University of Wisconsin, Madison, Wisconsin 53706, USA.

[11] Tests performed by L.M. Jones and S. Le, Department of Veterinary Science, University of Wisconsin, Madison, Wisconsin 53706, USA.

TABLE 1. Serum antibody prevalence and titers in mammals for five microbial agents.

		Northway		Snowshoe Hare		Infectious Bovine Rhinotracheitis		*Brucella abortus*		*Francisella tularensis*	
Common Name	Scientific Name	Prev.[1] (N[2])	Titer[3] (Range)	Prev. (N)	Titer (Range)	Prev. (N)	Titer (Range)	Prev. (N)	Titer (Range)	Prev. (N)	Titer (Range)
Human	*Homo sapiens*	2(50)	12	20(50)	4-32	ND[4]		0(55)		2(52)	80
Black bear	*Ursus americanus*	4(56)	8-16	17(167)	4-16	ND		1(283)	4-16[5]	0(251)	
Moose	*Alces alces*	20(44)	4-128	11(44)	8-128	14(14)	3-16	0(44)		0(44)	
Bighorn sheep	*Ovis canadensis*	11(9)	12	33(9)	4-16	0(9)		0(9)		0(9)	
Woodland caribou	*Rangifer tarandus*	14(22)	16-128	0(22)		ND		0(22)		0(22)	
Wolf	*Canis lupus*	0(3)		0(3)		ND		0(3)		0(3)	
Snowshoe hare	*Lepus americanus*	1(258)	12	64(11)	16-256	ND		0(11)		0(11)	

[1]Prev. = (Number positive/number tested) × 100.
[2]N = number of serum samples tested.
[3]Range of inverse of serum dilutions which (a) neutralized 100 TCD_{50} of virus, or (b) agglutinated bacterial antigens. For all tests except *B. abortus* in bears.
[4]ND = not done.
[5]Range of inverse of serum dilutions which fixed complement.

TABLE 2. Serum antibody titers in 10 humans for four members of the California encephalitis group of viruses.

Serum Designation	Snowshoe Hare	La Crosse	Jamestown Canyon	Trivittatus
36	32[1]	4	8	8
53	16	4-8	16-32	4-8
23	8-16	16-32	16	8
50	8	16	8-16	8
6	8	8	16	4
47	8-16	8-16	4	4-8
45	4-8	4	4	8
29	8	4	4-8	2
52	4-8	2-4	ND[2]	ND
19	8	0	0	ND

[1]Inverse of serum dilution which neutralized 100 TCD_{50} of virus.
[1]ND = not done.

Clethrionomys gapperi, Glaucomys sabrinus, and *Canis lupus.*

DISCUSSION

The absence of NT antibody for EEE, WEE, and SLE is not surprising. In Canada these diseases are characterized by dramatic epizootics and/or epidemics during a given year, and virtual absence of cases in subsequent years. During 1976 there were no such epidemics reported in Alberta (O. Morgante, Provincial Laboratory of Public Health, Edmonton, Alberta, pers. comm.). Approximately 200 cases of WEE in horses were confirmed by the Veterinary Services Division of the Alberta Department of Agriculture during 1975. These cases occurred primarily in the southern portion of the province. Seven cases were confirmed in 1976. No cases of WEE were found in any wildlife species during these years. There were no documented cases of EEE or SLE in Alberta in 1975 or 1976 (R. Christian, Veterinary Services Division, Alberta Agriculture, Edmonton, Alberta, pers. comm.).

POW antibody was not found in any of the species tested. In contrast, two previous serologic surveys reported low antibody prevalence in snowshoe hares from Rochester.[10,35] This discrepancy in prevalence may have been due to the small sample size in the present study, or to the absence of POW transmission in the areas we studied. Other studies have reported antibody prevalence ranging from 0-28% for deer mice,[18,19,21] red squirrels,[17,21] voles,[1,19] and chipmunks,[17,19,21] and 0-4% for humans.[13,14] In our study, sample sizes for all but the voles were adequate to detect POW infection if it were common in these populations. The previous studies were conducted in areas where these species were living in close proximity to yellowbelly marmots *(Marmota flaviventris),* the species which appears to be the primary mammalian host of POW in western Canada.[17] Perhaps deer mice, red squirrels, voles, chipmunks, and humans only acquire POW infection tangentially from the primary transmission cycle. There have been no reports of previous POW serologic surveys involving bighorn sheep, moose, bear, woles, or caribou.

Occurrence of antibodies to CAL group viruses in wildlife is not surprising. Previous studies have reported antibody prevalence of 11% for CAL in bighorn sheep from the U.S.,[32] 70% for SSH in Alberta moose,[33] 12-60% for CAL, LAC, and SSH in humans from British Columbia,[13,14] and Alberta,[10] and 0-9%

(most commonly 50-60%) for SSH and CAL in hares from throughout Canada.[10,16,18,21] The 63% antibody prevalence for SSH in snowshoe hares in our study agrees with the results in these prior reports. Our results represent the first report of SSH antibody in bighorn sheep from Canada, and extend the range of SSH infection in moose and CAL group infection in humans within Alberta. There have been no previous reports of SSH antibody in bears, but the Cold Lake area is within the range of SSH,[22] and bears are believed to be bitten by *Aedes* spp. mosquitoes, the primary vectors of SSH.[16] The possibility that the SSH antibody detected in wildlife may actually have been cross-reacting antibody to some other member of the CAL group cannot be ignored. Other studies have reported SSH or CAL group antibodies in red squirrels or chipmunks.[10,18] We can offer no explanation for the absence of antibody in squirrels and chipmunks in our study.

Twenty percent of the human sera had antibody to members of the CAL group of viruses. Both SSH and JC have been isolated in Alberta.[22] Although neither LAC nor TVT have been isolated in Canada, they do occur in the north-central United States and may extend into southern Canada.[30] Members of the CAL group are serologically cross-reactive, especially SSH and LAC.[27] This serologic cross-reactivity made it difficult to determine which member of the CAL group was causing human infection (Table 2). Sera which were considered positive for the CAL group often neutralized more than one member of the group. Titers ranged from 1:4 to 1:32. Only serum number 36 had a titer significantly higher (four-fold or more) to one of the four CAL viruses (namely, SSH) (Table 2). Apparently, SSH was the infecting agent in this case.

This is the first serologic evidence of NOR infection of boreal forest mammals, and the first indication that this virus is present in Alberta. NOR has been previously isolated from *Culiseta* spp.[6] and *Aedes*[6,20] spp. mosquitoes, sentinel rabbits *(Oryctolagus cuniculus),*[26] and northern redback voles *(Clethrionomys rutilus)*[26] in arctic North America. Apparently, in Alberta NOR infection is not limited to small mammals. Our results (Table 1) indicate that large ungulates (and perhaps humans, bears, and hares) may also be infected by NOR. The highest titers were found in moose from the Swan Hills area and from caribou from the Fort MacKay area. However, the role of these large ungulates in NOR transmission is not known.

NOR neutralization appeared to be specific. Some of the sera neutralized NOR at high serum dilutions (1:128), which is evidence for the specificity of the neutralization. In addition, those sera which neutralized NOR did not neutralize any unrelated viruses, as would be expected if non-specific neutralizing substances were present. A recent report documented the close antigenic relationship between NOR and Cache Valley (CV) viruses.[12] Therefore, the possibility that this was actually cross-reactive CV antibody cannot be ignored.

KLA apparently did not infect any of the species we tested. Isolations of KLA have been made from three small rodent species in Oregon and Alaska,[4] but no additional natural host range information is available.

This is the first report of IBR antibody in moose. Previous serologic studies revealed IBR antibody in pronghorn antelope *(Antilocapra americana)* sera from southeast Alberta[3] but not from 22 moose from the same general area.[31] This discrepancy could be explained by (1) the introduction of IBR into Alberta since the time of the previous study in 1970; (2) discontinuity of the moose population in Alberta over the 670 km between the two study areas, and hence a lack of IBR transmission between the areas; or (3)

the small sample sizes. There is no significant difference (X^2) between the prevalences of the two studies ($P>0.05$). Wyoming-Montana[11] and Wyoming-Colorado[25] bighorn sheep herds had no IBR antibody.

Although the two positive moose sera had low antibody titers for IBR, they are positive. Any measurable titer indicates past infection.[2] These two moose were from the Swan Hills area. Although this is a remote area, the moose are not isolated. Studies of moose movements in central Alberta indicate that Swan Hills moose could move distances sufficient to allow for contact with large cattle herds to the south (Thomas Hauge, Dept. of Wildlife Ecology, University of Wisconsin-Madison, pers. comm.). Members of the Cervidae have been suggested as potential carriers of IBR.[7] If, in fact, IBR infection can be maintained in the moose population, and transmission between moose and cattle can occur, then vaccination of cattle in Alberta should be continued to prevent re-introduction of IBR into the cattle population.

Brucella spp. infection has been reported previously in bears.[5,23] Arctic grizzly bears *(Ursus arctos)* are believed to acquire *B. suis* IV infection by eating infected caribou.[23] Black bears in the Cold Lake area have ample opportunity for contact with domestic animals from which they might become infected, but we do not know the actual source of infection in these bears. Cold Lake bears also frequent garbage dumps where they might encounter *Brucella*-contaminated tissues from a dead and discarded animal. The possibility of transmission from bears to domestic animals seems unlikely. Perhaps of greater potential risk is the threat to hunters and bear researchers when handling infected bears. As with the arctic grizzly bears,[23] the effect of *Brucella* infection on Cold Lake bears, particularly on their reproduction, is unknown. The complement fixation test used in this study is considered to be the most specific test presently available for measuring *Brucella* spp. antibody.

The significance of the single human with tularemia antibody is not clear, as it was not possible to determine when or where this 56-year-old American Indian woman acquired her infection. None of the mammals we tested had tularemia antibody, nor was there widespread small mammal mortality suggestive of tularemia infection[15] in the Fort MacKay area during 1976. However, our serologic test was not very sensitive for small mammal sera, as it was necessary to test these sera at a dilution of 1:400. Sera with significant titers below 1:400 would have been incorrectly considered negative.

Acknowledgements

The authors are grateful to Todd Fuller, Tom Hauge, Mike Vaughn, Brian Pelchat, Barry Young, and Gerry Lynch for supplying sera from their collections for use in this study.

Financial support for these studies was provided by NIAID Grants A100771, A209801, NSF Grant GB-332OX, and the College of Agricultural and Life Sciences, University of Wisconsin. Food and lodging in the Fort MacMurray area during summer 1976 were provided by the Alberta Oil Sands Environmental Research Program.

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Received for publication 18 April 1979