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Isolation of a Bunyamwera Group Arbovirus From a Naturally Infected Caribou [1]

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Abstract

A Bunyamwera group arbovirus was isolated from the blood and from the brain of a female caribou parasitized with meningeal worms. The virus passed through a 0.45 μ filter; was ether sensitive; possessed no hemagglutination properties; could be propagated in suckling mice, 6-day old chick embryos, and BHK-21 tissue culture; and produced plaques in chick embryo fibroblast tissue culture. Neither complement-fixation or neutralization tests were sensitive enough to determine the serotype of the virus.

Introduction

In the late summer and early fall of 1969, 7 of 14 woodland caribou *(Rangifer tarandus terraenovae)* on a commercial game reserve in northern Wisconsin died of a central nervous system disease. Six of the seven animals were adults, ranging from 3 to 6 years of age, and all had been on the reserve 1 to 5 years. The epizootiology of the die-off and the pathological findings from a three year old female caribou, which died on August 23, 1969, are reported in detail elsewhere.[16] Briefly, examination of the brain and the spinal cord of the caribou revealed nematodes, identified as *Pneumostrongylus tenuis,* in the medulla of the brain as well as in a gelatinous fluid surrounding the spinal cord. Observations made in other laboratories implicate this worm with the death of caribou.[1,16]

This paper reports on 1) the isolation of a member of the Bunyamwera group of arboviruses from the blood and from the brain of the above mentioned female caribou, and 2) serological findings in elk and deer from the game reserve as well as from Alaskan caribou.

Methods and Materials

Virus isolation was accomplished by intracerebral inoculation of suckling HaM/ICR white mice with 0.02 - 0.03 ml. of 10% suspensions of the blood and of the brain from the caribou. The blood had been collected on the day prior to the death of the animal. Following inoculation, the mice were observed daily, with the brains of morbid or dead individuals being harvested under sterile conditions. After passage through a 0.45 μ millipore filter, the harvested mouse brain

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was again inoculated into suckling mice until a reproducible pattern of sickness and death occurred in mice inoculated with material shown by culture to be free of bacteria. Reisolation of virus was attempted from an aliquot of the original caribou samples. After successful reisolation, the isolates were tested for ether sensitivity and specific hyperimmune mouse ascitic fluid (MAF) was produced against them by Dr. Ralph O. Anslow, University of Wisconsin.

Virus identification was accomplished by agar gel double diffusion, complement-fixation (CF) and neutralization tests. The agar-gel double diffusion technique employed has been previously described;[6,12] while the complement-fixation test was performed in the laboratories of Dr. Wayne Thompson, Department of Preventive Medicine, University of Wisconsin. Six-day old chick embryos, suckling mice and BHK-21 cells were used as the host systems for the neutralization tests. The following viruses and their respective MAF or antisera were used in the identification of the virus isolates from the caribou: Tensaw, Cache Valley, Wisconsin Bunyamwera group isolate W-523, LaCrosse, Snowshoe hare, Jamestown Canyon, Trivittatus, BFS-283, Silverwater and Powassan.

Ten-fold dilution series of the virus isolates from the caribou, as well as of three other Bunyamwera group viruses, were inoculated into 6-day old chick embryos via the yolk sac and into 10-day old chick embryos via the allantoic cavity. The other Bunyamwera group viruses included Tensaw,[4] Cache Valley,[7] and an isolate from a Wisconsin horse designated H-917.[11] Half of the eggs were incubated at 34 C while the other half were held at 37 C. The eggs were examined daily for 8 days with the specificity of embryonic death being determined by virus neutralization tests against reference MAF, in both mice and eggs.

Primary chick embryo fibroblast tissue cultures were tested for their ability to support plaque formation by the virus isolates from the caribou, other Bunyamwera group viruses, as well as LaCrosse virus, a member of the California encephalitis group. The procedure used was the one described for Newcastle disease virus.[3,11] The infected tissue cultures were incubated in the presence of CO_2 at 37 C for six days. Plaque formation and titer were recorded, as was preliminary data on plaque inhibition.

In order to obtain preliminary information of Bunyamwera group virus activity on the game reserve, sera from six elk *(Cervus canadensis)* and one white-tailed deer *(Odocoileus virginianus)* shot in the late fall of 1969 were tested for the presence of neutralizing antibodies against the virus from the caribou and to the California encephalitis virus group. Serum samples obtained from 52 Alaskan caribou were also tested. The metabolic inhibition test with BHK-21 cells was used as the assay system[10] and only those heat - inactivated, undiluted sera which neutralized more than 1.5 to 2.0 logs of virus were considered positive. The elk and deer sera were also titrated in a two-fold dilution series against a constant amount of the caribou virus.

Results

Virus was recovered from both the blood and the brain of the female caribou. The virus isolates, which killed mice in 5 days, passed through a 0.45 μ filter, possessed no hemagglutination (HA) properties, and were found to be ether sensitive. They titered $10^{5.5}$ in suckling mice and $10^{8.8}$ in BHK-21 cells.

Agar-gel double diffusion tests demonstrated that the isolates, designated C-69-26, were identical to each other but not related to the California encephalitis complex, Silverwater or Powassan viruses. It was then suspected that C-69-26 virus might have been a member of the Bunyamwera group of arboviruses. A neutralization test in 6-day old chick embryos inoculated via the yolk sac and incubated at 34 C reaffirmed this suspicion. The virus was neutralized with chicken antisera prepared against virus W-523, a Wisconsin mosquito isolate of the Bunyamwera group.[2] Subsequent neutralization tests in mice and eggs as well as

complement-fixation tests supported this identification (Table 1). However, since none of these procedures are sufficiently sensitive to determine the exact serotype of C-69-26, the virus has been sent to Dr. Robert Shope, Yale Arbovirus Research Center, for further identification.

It was possible to propagate C-69-26 virus and other members of the Bunyamwera virus group in 6-day old chick embryos inoculated via the yolk sac and incubated at 34 C (Table 2). No significant differences in titers were observed with the use of either suckling mouse or chick embryo passed viruses. None of the viruses were capable of producing mortality in 10-day old embryos inoculated in the allantoic cavity and incubated at 34 C or 37 C.

Preliminary studies demonstrated the ability of C-69-26 and other Bunyamwera group viruses as well as LaCrosse virus to produce plaques in chick embryo fibroblast tissue culture within 3 to 4 days (Table 3). However, only members of the Bunyamwera complex were neutralized by hyperimmune mouse ascitic fluids prepared against C-69-26 virus.

Neutralizing antibodies to C-69-26 virus were found in all seven animals sampled from the game reserve and each serum titered greater than 1:320. Only one of the elk and the one white-tailed deer reacted positively to the California encephalitis virus complex. Six of the 52 Alaskan caribou possessed neutralizing antibodies to the Bunyamwera group of viruses, while 10 reacted positively to the California encephalitis group. Only two of the caribou were considered positive to both virus groups.

TABLE 1. *Complement-fixation relationship of C-69-26 virus to members of the Bunyamwera group of viruses.*

M.A.F. or Sera	Antigens					Normal Mouse Brain
	C-69-26	H-917	523	Tensaw	Cache Valley	
C-69-26	7/6*	8**	8	8	7	< 2
H-917	6	7/5	6	5	5	< 2
523	8	7	7/7	7	6	< 2
Tensaw	6	7	6	6/7	5	< 2
Cache Valley	10	10	10	9	10/5	< 2
M.A.F. Control	< 2	< 2	< 2	< 2	< 2	< 2
Serum Control	< 2	< 2	< 2	< 2	< 2	< 2

* Serum titer/antigen titer. All titers indicated by dilution number: $< 2 = 8$, $2 = 8$, ..., $12 = 8112$.

** Serum titer versus optimal dilution of antigen.

TABLE 2. *Propagation of C-69-26 virus and other Bunyamwera group viruses in 6-day old chicken embryos inoculated via yolk sac and incubated at two temperatures.*

Virus	Titer Indices*		Incubation Period
	@ 34 C	@ 37 C	
C-69-26	7.0	0.0	3 to 8 days
H-917	4.0	0.0	3 to 8 days
Cache Valley	5.0	0.0	3 to 8 days
Tensaw	7.0	0.0	3 to 8 days

* Chicken embryo LD_{50} calculated for three trials for each virus.

TABLE 3. *Plaque formation and neutralization by C-69-26 virus and other Bunyamwera super-group viruses in chicken embryo fibroblast tissue culture.*

Virus	Plaque Formation	Plaque Neutralization*	Virus Titer/PFU	Time of Appearance
C-69-26	Yes	Yes	4.5	3 to 4 days
H-917	Yes	Yes	3.5	3 to 4 days
Cache Valley	Yes	Yes	3.5	3 to 4 days
Tensaw	Yes	Yes	5.0	3 to 4 days
LaCrosse**	Yes	No	2.0	3 to 4 days

* Plaque neutralization attempted only with anti-C-69-26 M.A.F.
** California group virus.

Discussion

Bunyamwera group arboviruses in North America have been isolated almost exclusively from mosquitoes,[2,4,9] although isolations have also been made from the caribou in the present study, a cotton mouse,[5] a fox,[13] a dog,[4] and a horse.[11] Of the mammalian isolates, three have been associated with animals exhibiting clinical illness, however none of the viruses isolated were established as the etiologic agent.

Serological surveys have shown that cervids are commonly infected by arboviruses,[8,17] although isolation of virus from cervids have been rare events, such as the isolation in the present study and that of tick-borne encephalitis virus (TBE) from a Swedish moose.[15] Based on 1) the fact that the isolation procedures in this investigation were conducted in facilities separated from those where known stocks of Bunyamwera group arboviruses were being used or stored, 2) that the virus was isolated from both the blood and from the brain of the caribou, 3) that these samples were collected in the field on separate days, 4) that reisolation attempts from both the blood and from the brain were successful, and 5) that the histopathology of the caribou's central nervous system was consistent with what might be expected in a viral encephalitis,[16] it is apparent that the caribou had an active arboviral infection prior to and at the time of death. Experimental infection studies are necessary to determine the degree to which Bunyamwera group viruses are pathogenic for caribou. The presence of neutralizing antibodies to Bunyamwera group viruses in Alaskan caribou suggests that infection is not necessarily lethal.

Further complicating the situation in the present study is the presence of the nematode, *Pneumostrongylus tenuis,* in the central nervous system of the caribou. *P. tenuis* is introduced into the animal by ingestion of infected gastropods and subsequently develops in the central nervous system, primarily the spinal cord. In white-tailed deer, the development of the parasite generally does not result in clinical illness, while in caribou the development is aberrant and it is reported that the resulting damage to the nervous system leads to the death of the caribou.[1] To the best of our knowledge, complicating factors, such as microbial agents or toxic substances, have in the past not been associated with *P. tenuis* parasitism. Whether the Bunyamwera group virus isolated in this investigation represents such a situation or whether it was a fortuitous isolation or whether it was the cause of illness can only be established following additional experimental infection studies in caribou.

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