ANTIGENIC COMPARISONS BETWEEN HERPESVIRUSES ISOLATED FROM FALLOW DEER IN ALBERTA AND THE VIRUSES OF INFECTIOUS BOVINE RHINOTRACHEITIS, EQUINE RHINOPNEUMONITIS AND DN-599, A NON-IBR BOVINE HERPESVIRUS

Authors: KINYILI, J.H., and THORSEN, J.

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J.H. KINYILI [1] and J. THORSEN, Department of Veterinary Microbiology and Immunology, University of Guelph, Guelph, Ontario, Canada N1G 2W1.

Abstract: Antigenic comparison studies of three herpesviruses isolated from fallow deer *(Dama dama)* in Alberta and herpesviruses from some domestic species were carried out by the alpha serum-virus neutralization test. Complete cross neutralization was demonstrated among the deer herpesviruses and equine herpesvirus type 1.

INTRODUCTION

Serologic surveys conducted on several species of wild ruminants in Canada and USA have provided evidence of infection of these animals with infectious bovine rhinotracheitis virus (IBRV).[1,2,3,4,5] Virus isolation attempts have yielded herpesviruses from some species of wild ruminants in North America.[4,6] The herpesviruses included in this study were isolated from fallow deer *(Dama dama)* on a game farm in Alberta. Isolations were made after dexamethasone injections.[6] Preliminary antigenic comparisons by plaque-reduction tests showed these herpesviruses to be unrelated to IBRV but closely related to each other.[6]

In an attempt to obtain further information on the antigenic relationships of the herpesviruses from deer to known herpesviruses of domestic animals, further studies were undertaken. The three herpesviruses found in Alberta were compared with the viruses of infectious bovine rhinotracheitis, equine herpesvirus type 1 (EH1) and DN-599, a bovine herpesvirus unrelated to IBRV.

MATERIALS AND METHODS

Viruses

The three herpesviruses from fallow deer in Alberta included in this study were given the laboratory numbers, 17, 47 and 67. The IBRV was a Colorado strain, obtained from Dr. J.B. Derbyshire, University of Guelph. The EH1, obtained from Dr. J.T. Bryans, University of Kentucky, was the Army 183 strain. The non-IBR bovine herpesvirus, strain DN-599, was obtained from Dr. D.E. Reed, Iowa State University.

All the viruses were grown in Madin-Darby bovine kidney (MDBK) cell cultures, except strain DN-599, which did not propagate in MDBK cell cultures and was grown in embryonic bovine lung (EBL) primary cell culture.

Cell cultures

Madin-Darby bovine kidney cells were grown in Eagle's minimum essential medium (EMEM) and Earle's salts supplemented with 10% fetal calf serum (FCS) and containing antibiotics at a final concentration of 200 IU/ml penicillin and 80 μg/ml streptomycin.

[1] Present address: Veterinary Research Laboratories, P.O. Kabete, Kenya.

For maintenance, the serum concentration was reduced to 5%. Secondary cell cultures were established by dispersing confluent monolayers of MDBK cells with ATV (antibiotic-trypsin-versene) solution containing 0.05% trypsin, 0.02% versene, 100 IU/ml penicillin and 100 μg/ml streptomycin.

Antiserum preparation

Antisera were prepared by inoculating each virus strain into three rabbits. Pre-inoculation sera were obtained by removing blood from the rabbits via the ear vein. Virus with a titre of $10^{6.5}$ $TCID_{50}$/ml was mixed 1:1 with Freund's complete adjuvant and each rabbit was given 2 ml intramuscularly. Each rabbit also was given 1 ml of virus suspension intraperitoneally (IP); and then four booster doses of 2 ml of virus suspension IP at weekly intervals. Rabbits were exsanguinated by cardiac puncture 7 days after the last inoculation. The blood was kept at room temperature for 2 h, after which it was centrifuged at 12000 × g for 30 min to separate the serum.

Serum was heat inactivated at 56 C for 30 min, dispensed into vials in 1 ml volumes and stored at -20 C.

Neutralization tests

The cross neutralization tests were performed in plastic tissue culture plates using standard micro-titre techniques. All sera were used at a dilution of 1:50 and the alpha method was utilized in which an equal volume of each serum, diluted 1:50, was mixed with serial ten-fold dilutions of each of the different viruses. Serum-virus mixtures were incubated at room temperature for 1 h prior to inoculation into the cell cultures. Each virus also was titrated without hyperimmune serum, to establish the titre. Tests were read after 4-5 days incubation.

For each serum-virus mixture the neutralization index was calculated. The neutralization index is the difference in titre, in log $TCID_{50}$, between viruses titrated in the presence and absence of hyperimmune serum.

RESULTS

The neutralization indices calculated from the results of neutralization tests are recorded in Table 1. The three herpesviruses from fallow deer are antigenically closely related to each other and to EH1. There was slight neutraliza-

TABLE 1. Neutralization indices calculated from cross neutralization tests of herpesviruses from Alberta fallow deer and IBR, EH1 and DN-599 viruses.

	Antisera					
Virus	17	47	67	IBRV	EH1	DN-599
17	3*	2.5	2	0.5	2.5	0.25
47	3	3	3	0	3	1.00
67	2	2	3	0	2	1.00
IBRV	0	0	0	2	0	0
EH1	3	3	3	0	3	0

17, 47, 67 } Fallow deer herpesviruses
IBRV - Infectious bovine rhinotracheitis virus.
EH1 - Equine herpesvirus type 1.
DN-599 - A non-IBR bovine herpesvirus.
*Difference in titre, in log $TCID_{50}$, between viruses titrated in the presence and absence of antiserum.

tion of the deer herpesvirus by antiserum to DN-599 virus, but little cross reaction occurred between IBRV and the deer herpesviruses.

DISCUSSION

The close antigenic relationship of the deer herpesviruses to EH1, as revealed by these studies, was unexpected. Prior to this, EH1 infection had been recognized only in horses. Horses were kept on the same game farm as the deer from which the herpesviruses were isolated and the deer may have acquired the infection from horses. Serologic surveys of free-living and captive deer populations should be undertaken to determine the prevalence of infection with EH1 in the various species of deer. Also, horses in Southern Alberta should be tested for antibodies to the deer EH1 isolates.

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