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VIRUSES ISOLATED FROM CAPTIVE AND FREE-RANGING WILD RUMINANTS IN ALBERTA[1]

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Abstract: Nasal secretions, leukocytes and preputial or vaginal swabs from a group of 15 captive wild ruminants, comprising six pronghorn antelope (*Antilocapra americana*), seven fallow deer (*Dama dama*) and two mule deer (*Odocoileus hemionus*), and from 50 free-ranging pronghorns in southern Alberta, were examined for viral agents. Captive animals were given injections of dexamethasone daily for 6 days in attempts to reactivate latent infections. Specimens were collected at 2-3 day intervals from days 0 to 18. Free-ranging pronghorns were sampled only once, at the time of capture. Fifteen viral isolates were obtained from the animals: six isolates of parainfluenza 3 (PI3) from nasal swabs from one fallow deer and one mule deer; five isolates of herpesvirus from leukocytes, vaginal, preputial and nasal swabs from three fallow deer; and four isolates of PI3 from nasal secretions of the 50 free-ranging pronghorns.

INTRODUCTION

Serologic surveys of several species of wild ruminants have provided evidence of infection with the viruses of infectious bovine rhinotracheitis (IBR) and parainfluenza type 3 (PI3)[1,3,4,6,8,10,11,13] but few isolations of these agents have been made.[6,7] To obtain more information about the host range of these agents and also to search for other viral agents, isolation attempts were made on samples collected from seven captive fallow deer, two mule deer and six pronghorns on a game farm in southern Alberta, as well as on samples from 50 free-ranging pronghorns, trapped for other purposes. The free-ranging pronghorns were from the same population in which serologic evidence of PI3 infection had been demonstrated previously.[1]

MATERIALS AND METHODS

Animals

The captive deer and pronghorns were studied over an 18-day period. During the period of study, animals were housed in a series of pens with solid plywood or canvas sides and interconnecting doors. Weighing, physical examination and specimen collection took place in a handling pen, where animals were restrained by being wrapped in a tarpaulin.

In an attempt to increase shedding of virus, dexamethasone was administered to the captive animals daily by intramuscular injection of 0.07 mg/kg body weight for 6 days. To guard against bacterial infection, animals were given intramuscular injections of procaine penicillin G (39,000 IU/kg) and dihydrostreptomycin sulfate (50 mg/kg).

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Specimens were collected from 50 free-ranging pronghorns trapped in southeastern Alberta during December, 1974. The pronghorns occupied traditional native short-grass prairie and sagebrush (*Artemisia cana*) rangeland and had no previous history of confinement.

Sampling

Specimens collected for viral isolation were blood leukocytes, nasal swabs and vaginal or preputial swabs. Immediately after collection, swabs were placed in individual screw-capped vials containing a transport medium consisting of Hanks' balanced salt solution (HBS) with 0.5% lactalbumen and 500 IU/ml penicillin, 250 ug/ml streptomycin and 100 units/ml nystatin.[4] Nasal and vaginal or preputial swabs were collected on day 0 and at 2 or 3 day intervals over the next 18 days. Blood samples for leukocyte specimens were drawn into 5 ml heparinized tubes on days 2, 7 and 16. Specimens were collected once from the 50 free-ranging pronghorns, at the time of trapping, and were flown to Lethbridge to ensure inoculation into cell cultures within 10 h of sampling.

Cell cultures

Bovine thyroid (BTh) cell cultures were prepared by trypsinization of fetal bovine thyroid glands and growth of the dispersed cells in Eagle's minimal essential medium (EMEM) in HBS, with 10% fetal bovine serum and containing 250 IU/ml penicillin and 100 ug/ml streptomycin. Cultures were maintained in the same medium with serum content reduced to 5%. For preparation of cultures for inoculation, secondary BTh cultures, obtained by trypsin dispersal of the primary monolayers, were grown in Corning 16 x 125 mm plastic tissue culture tubes with screw caps. A number of cultures from each batch prepared were incubated and observed daily for 14 days for the presence of adventitious cytopathic viruses.

Viral isolation

After collection, specimens were cooled but not frozen prior to inoculation into BTh cultures, usually within 2-4 h of collection and within 10 h in the case of samples from the free-ranging pronghorns. After inoculation, cell cultures were incubated for 24 h at 37 C, then held at approximately 4 C for a week or less and air-shipped under refrigeration to Guelph. Subcultures were then made in BTh cultures, using cells and medium from first passage cultures as inoculum. The cells were removed from the growing surface by scraping. Freezing between passages was avoided and cultures were observed as long as uninoculated control cultures remained in healthy condition, usually about 2 weeks. Further passages were made only if cytopathic effects were noted in second passage cultures.

RESULTS

Clinical observations

Except for injuries, no clinical signs of disease were observed in captive or free-ranging animals. Most of the captive animals tolerated the handling and dexamethasone injections with little evidence of stress. The largest male fallow deer (1406) had suffered an antler puncture of the thorax in fighting with a rival male the day before the experiment began. The animal was treated with chloramphenicol, 500 mg intramuscularly, on days 7, 8, 9 and 11. He remained strong throughout the experiment, although hematology revealed elevated leukocyte counts and reduced erythrocyte numbers and packed cell volumes (Barrett, unpublished data).

One mule deer (1408) developed partial posterior paralysis and became recumbent on day 3, apparently as a result of an injury during handling. The animal continued to eat and drink and gradually regained almost full mobility by day 16.

[4] Mycostatin: E. R. Squibb and Sons, Inc., New York, New York 10022, USA.

Viral isolation and identification

Fifteen isolations of viruses were made from the specimens inoculated into cell cultures. Five of the isolates were identified as herpesviruses on the basis of characteristic cytopathic effect in cell cultures, sensitivity to lipid solvents and morphology as observed by electron microscopic examination of negatively stained preparations (Fig. 1).[9] All herpesviruses were isolated from captive fallow deer: from a vaginal swab and a nasal swab on days 0 and 5, respectively, from one fallow deer; from buffy coat cells of another on day 2; and from a preputial swab and a nasal swab on days 0 and 5, respectively, from a third fallow deer. The herpesviruses could be subcultured from supernatant fluid from cell cultures that had been frozen and thawed, indicating that the viruses were not cell associated.

The remaining 10 isolates were identified as P13 virus on the basis of hemadsorption of guinea pig erythrocytes by infected cultures, and inhibition of hemadsorption by specific antiserum against P13 virus.[2] One P13 isolate was obtained from a nasal swab from a fallow deer on day 5 and five were recovered from nasal swabs from a mule deer on successive sampling intervals from days 7 through 16. The remaining four isolations were obtained from nasal swabs from the free-ranging pronghorns.

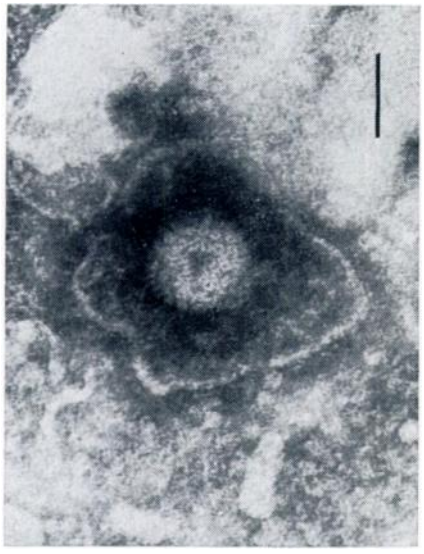

FIGURE 1. Electron micrograph of Alberta herpesvirus, 2% phosphotungstic acid negative stain. Bar is equivalent to 100 nm.

The results of viral isolation attempts from the captive animals are tabulated in Table 1.

Studies on the antigenic relationships of the herpesviruses have not yet been completed. One isolate was compared with the virus of IBR by reciprocal plaque reduction tests with antisera produced in rabbits. Results indicated that the two viruses were unrelated (Table 2). By means of a varying virus—constant serum technique, the remaining herpesviruses isolated were shown to be antigenically identical to the first Alberta isolate and unrelated to IBR.

DISCUSSION

Herpesviruses were isolated from vaginal and preputial swabs from two fallow deer prior to dexamethasone treatment and subsequently from nasal swabs from the same fallow deer after 5 days of the same treatment. Dexamethasone may have induced increased shedding of virus, a suggestion supported by the results of other investigations.[5,7,12]

The dosage level of dexamethasone was chosen to correspond to a high therapeutic dose for a domestic ruminant. However, the effect of this treatment on viral shedding could not be assessed accurately, as no untreated controls were used in the study.

One of the fallow deer (1406) from which a herpesvirus was isolated was the animal injured by fighting. The most consistent viral shedding was observed in the partially paralyzed mule deer 1408, from which PI3 was isolated from nasal swabs at each sampling between days 7 and 16. The stresses of injuries superimposed on dexamethasone injections and handling may have increased viral shedding by these two deer.

TABLE 1. Captive animals sampled, sampling intervals and viruses isolated.

	Animal No.	Age	Sex	Days 0	2	5	7	9	11	14	16	18
	1401	Adult	M	—	—	—	—	—	—	—	—	—
Fallow deer	1402	Juvenile	F	V(H)	—	N(H)	—	—	—	—	—	—
	1403	Adult	F	—	BC(H)	—	—	—	—	—	—	—
	1404	Adult	F	—	—	N(P)	—	—	—	—	—	—
	1405	Juvenile	M	—	—	—	—	—	—	—	—	—
	1406	Adult	M	P(H)	—	N(H)	—	—	—	—	—	—
	1407	Juvenile	M	—	—	—	—	—	—	—	—	—
Mule deer	1408	Adult	F	—	—	—	N(P)	N(P)	N(P)	N(P)	N(P)	—
	1409	Adult	F	—	—	—	—	—	—	—	—	—
	1410	Adult	F	—	—	—	—	—	—	—	—	—
Pronghorns	1382	Adult	M	—	—	—	—	—	—	—	—	—
	1383	Adult	M	—	—	—	—	—	—	—	—	—
	1384	Adult	M	—	—	—	—	—	—	—	—	—
	1331	Adult	M	—	—	—	—	—	—	—	—	—
	1238	Adult	F	—	—	—	—	—	—	—	—	—

N = nasal
V = vaginal
P = preputial
BC = buffy coat

(H) = herpesvirus
(P) = parainfluenza 3

TABLE 2. Results of reciprocal plaque reduction tests between Alberta herpesvirus and IBR.

	Antiserum and dilution							
	Alberta herpesvirus				IBR			
Virus	1:1024	1:256	1:64	1:16	1:1024	1:256	1:64	1:16
Alberta herpesvirus	183*	170	108	82	—	170	124	154
IBR	120	119	123	115	168	61	6	0

* Number of plaques/0.2 ml of virus-serum mixture

— = not done

To the authors' knowledge, this is the first reported isolation of PI3 virus from deer and pronghorns and the first isolation from free-living wild ruminants. Serological evidence of PI3 infection in pronghorns[1] and in white-tailed deer (*O. virginianus*)[11] and PI3 isolations from captive bighorn sheep (*Ovis canadensis*)[8] have been reported previously. The high serum titers to PI3 reported previously[1] and the isolations of PI3 virus from pronghorns in this study, were obtained from animals in the same free-ranging population in 1970 and 1974, respectively. Taken together, these findings suggest that PI3 virus is infective for pronghorns. Potential sources of PI3 virus for free-ranging pronghorns include other big game and domestic species with which these pronghorns are sympatric.[1]

Further antigenic comparisons of the herpesvirus isolated have yet to be carried out but it may represent a distinct species indigenous to fallow deer. It is also possible that this virus may have been contracted from other sources, as the game farm also houses bison (*Bison bison*), wapiti (*Cervus canadensis*), white-tailed deer, bighorn sheep, mountain goats (*Oreamnos americanus*) and llamas (*Lama peruana*).

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