# FURTHER STUDIES ON TRYPANOSOMES IN GAME ANIMALS IN WYOMING 1 2

Authors: MORTON, JAMIE K., and KINGSTON, NEWTON

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# FURTHER STUDIES ON TRYPANOSOMES IN GAME ANIMALS IN WYOMING[1,2]

JAMIE K. MORTON[3] and NEWTON KINGSTON[4]

*Abstract:* Blood samples were collected from captive and free-ranging elk (*Cervus canadensis*), mule deer (*Odocoileus hemionus*), white-tailed deer, (*Odocoileus virginianus*), black-tailed deer (*Odocoileus hemionus columbianus*), pronghorn (*Antilocapra americana*), moose (*Alces alces*), and bighorn sheep (*Ovis canadensis*) for cultural evidence of *Trypanosoma* sp. infection. Eleven of 88 (12%) hunter-killed elk, 22 of 37 (59%) free-ranging elk, and 79 of 119 (66%) captive elk were culture positive in 1973-74. Parasitemia in adult captive elk showed seasonal variation. Other captive or live-trapped animals found positive included 16 mule deer, two white-tailed deer, and one black-tailed deer. No pronghorn, moose, or bighorn sheep were positive. A 0.25 ml inoculum of elk blood was sufficient to give positive culture results. Small sample size may have contributed to negative results from elk trapped in March, 1973.

## INTRODUCTION

Epimastigote stages of trypanosomes were detected by blood culture in an elk (*Cervus canadensis*) at the Wyoming Game and Fish Department's Sybille Big Game Research Unit in May, 1972. Subsequent sampling of elk in March and April, 1973 at the Unit indicated 89% of the adults, but none of the calves were infected with trypanosomes. None of 21 live-trapped elk sampled at the National Elk Refuge, Jackson, Wyoming, in March 1973 were positive by NNN culture.[3] Because the elk tested at Sybille originated from this same Jackson Hole herd, sampling technique was considered to be a factor in the negative results.

Using culture techniques, trypanosomes have been reported in elk in Michigan[10,11] and New Mexico,[2] in white-tailed deer (*Odocoileus virginianus*) in Michigan,[10,11] New York,[8] and the southeastern U.S.,[7] and in mule deer (*Odocoileus hemionus*) in Colorado and New Mexico.[1] Blood stream trypomastigotes have been recovered from elk in Wyoming and described as a new species, *Trypanosoma cervi*.[4] Multiplication stages have been found in the spleen of elk.[5] Blood stream trypomastigotes have been recovered from 16 mule deer in Wyoming.[6]

Blood samples were collected from captive ungulates at the Sybille Big Game Research Unit and from free-ranging animals throughout the state to compile information pertaining to the prevalence and distribution of the organism in Wyoming. Also, trypanosome detection techniques were evaluated.

## MATERIALS AND METHODS

Venous blood was periodically collected throughout the year from elk at the Sybille Big Game Research Unit. Mule deer, white-tailed deer, pronghorn (*Antilocapra americana*), and one black-tailed deer (*Odocoileus hemionus columbianus*) at the Unit were sampled when

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[3] Wyoming Game and Fish Department Research Laboratory, Laramie, Wyoming 82071, USA.

[4] Division of Microbiology and Veterinary Medicine, Box 3354, University Station, University of Wyoming, Laramie, Wyoming 82071, USA.

possible. Blood was inoculated onto NNN agar slants (1.4% agar, 0.6% sodium chloride, defibrinated rabbit blood plus 100 units penicillin and 0.1 mg streptomycin/ml) or veal infusion medium (VIM).[4] Cultures were maintained at 20-25 C and examined weekly for 3 weeks by bright field or phase microscopy.

Hunter cooperation was solicited in the fall of 1973 to collect blood samples from game animals. NNN culture tubes and directions for collection were distributed to volunteers. Samples from elk, deer, pronghorn and one moose (*Alces alces*) were returned and examined as above.

Venous blood (approximately 0.5 ml) collected from free-ranging elk trapped at the National Elk Refuge and Greys River feedgrounds in December, 1973 and January, 1974 was cultured and examined. Blood from four young, male bighorn sheep (*Ovis canadensis*) captured by chemical immobilization in Fremont County for transplant in May, 1974 was inoculated into NNN media.

Graduated quantities (1.0 ml, 0.5 ml, 0.25 ml, 0.1 ml, and 0.05 ml) of blood from several elk at Sybille were inoculated into NNN culture media as described above. Heparinized hematocrit tubes were also used to collect blood for culture inoculation. Duplicate blood samples were inoculated into NNN media with defibrinated bovine blood substituted for rabbit blood.

## RESULTS AND DISCUSSION

Results of elk tested at Sybille indicated the prevalence in adults was high in the spring and summer months but dropped in the fall and winter (Table 1). Of 36 elk sampled over a period of several months, 23 had parasitemias from March through July, 1973 but were negative when tested in September through January. Three of these 23 were rechecked in March, 1974 and were positive, suggesting a cyclic pattern of infection. Four elk remained positive throughout the winter. Although elk tested were being used in studies on brucellosis or nutrition, there was no relationship of parasitemias to the disease or nutritional status of the animals. Calves were usually negative until they were 1 year old or until they had gone through a horsefly season (July-August in Wyoming). Davies and Clark[2] reported trypanosomes in horseflies in New Mexico and putatively assigned them a role as a vector. A horsefly harboring trypanosmal stages was collected at the Sybille Unit (unpublished data) in the summer of 1973, and trypanosome infected horseflies have been reported in New York state.[9]

Of 135 samples collected from various game animals killed by hunters (Tables 1 and 2) only a few elk were positive (Table 1). The seven positive samples from elk in Teton County were collected by parasitologists at the University of Wyoming. The other hunter-killed positive samples were collected by University of Wyoming scientists in the Sierra Madre Mountains and the Snowy Range. Negative results may have been influenced by bacterial and fungal contamination of samples collected in the field. Many samples were not returned until several days after the kill and may have been subjected to adverse temperature conditions during that time. In addition, results from captive elk indicate there may be a naturally low parasitemia at that time of the year.

Quantitation trials indicated 0.25 ml of blood inoculated into NNN was sufficient to give 100% positive culture results. Eighty percent of the 0.1 ml and 50% of the 0.05 ml inoculum samples collected in heparinized hematocrit tubes with a capacity of approximately 0.07 ml were positive. Heparinized hematocrit tubes were used to collect blood for culture inoculation from elk on the National Elk Refuge in 1973. Negative results could have been due to the small sample size or other unknown factors. A larger inoculum (0.5 ml whole blood) was used from elk live-trapped in 1974 when 22 of 37 were positive. All duplicate samples inoculated into NNN with defibrinated bovine or rabbit blood were positive. Bovine blood was used in the medium for all subsequent sampling.

TABLE 1. Elk blood cultures positive for trypanosomes in 1973 and 1974.

| | 1973 | | | | | | | | | | 1974 | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | M | A | M | J | Jy | A | S | O | N | D | J | F | M | A | M | J | Jy |
| Captive Elk | | | | | | | | | | | | | | | | | |
| Adults | 22/22 | 38/44 | 5/6 | 4/4 | 35/40 | | 0/5 | 2/15 | 10/29 | | 2/23 | | 13/13 | | 3/3 | | |
| Yearlings | | 1/1 | | 0/7 | 1/6 | 0/4 | 0/3 | | 0/1 | | 0/1 | | | | | | |
| Calves | 0/3 | 1/3 | 0/8 | 0/2 | 5/10 | 1/6 | 0/3 | 0/2 | 0/12 | | 0/4 | | | | | 0/3 | 3/8 |
| TOTAL CAPTIVE ELK POSITIVE 79/119 | | | | | | | | | | | | | | | | | |
| Hunter-Killed | | | | | | | | | | | | | | | | | |
| Teton County | | | | | | | | | 7/37 | | | | | | | | |
| Statewide | | | | | | | | 4[a]/51 | | | | | | | | | |
| Live-trapped | 0/21[b] | | | | | | | | | | | | 22/37[b] | | | | |
| TOTAL FREE-RANGING ELK POSITIVE 33/125 | | | | | | | | | | | | | | | | | |

[a] Snowy Range (1)
Sierra Madre (3)

[b] Teton and Lincoln counties

TABLE 2. Species other than elk cultured for trypanosomes in Wyoming in 1973-1974.

| | Positive/cultured | |
|---|---|---|
| | Hunter-killed | Live |
| Mule Deer | 0/8 | 16/20 |
| White-tailed Deer | 0/13 | 2/3 |
| Black-tailed Deer | | 1/1 |
| Pronghorn | 0/22 | 0/8 |
| Moose | 0/4 | 0/1 |
| Bighorn Sheep | | 0/4 |

Trypanosomes were detected by culture (VIM) and/or examination of blood concentrated in hematocrit tubes in eight mule deer, two white-tailed deer, and one black-tailed deer held in captivity at Sybille and in eight other free-ranging mule deer from southeast Wyoming. None of four 2-year-old bighorn rams captured for transplant in May, 1974 were positive. No trypanosomes were found in samples from moose or pronghorn (Table 2).

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