# SURVEY OF COLORADO AND WYOMING BIGHORN SHEEP AND MOUNTAIN GOATS FOR PARATUBERCULOSIS

Elizabeth S. Williams, Wild Animal Disease Center, Department of Pathology, Colorado State University, Fort Collins, CO

Charles P. Hibler, Wild Animal Disease Center, Department of Pathology, Colorado State University, Fort Collins, CO

## AdSTRACT

The lymphocyte blastogenesis (LB) test was used to test 157 free-ranging bighorn sheep (Ovis canadensis) from eight herds in Colorado and Wyoming for paratuberculosis. Of 49 blood samples from bighorns in the Grant, Colorado herd where paratuberculosis has been confirmed, 33 (67%) were positive. Positive results were obtained from most bighorn herds tested, including those where paratuberculosis was presumed absent. All 155 serum samples from seven bighorn herds tested by complement fixation were negative. Fecal samples from nine herds were culture negative for Mycobacterium paratuberculosis.

Sixteen blood samples were collected from two herds of mountain goats (Oreamnos americanus); one sample from the Mount Evans, Colorado herd was positive on the LB test. Tissue from one of five hunter-killed goats was culture positive and one of 11 goats was diagnosed as having paratuberculosis by microscopic examination of tissues. Eight fecal samples from goats in the Mount Evans area were culture negative for M. paratuberculosis.

# INTRODUCTION

Paratuberculosis, or Johne's disease, is an infectious enteric disease of ruminants caused by the bacterium M. paratuberculosis. It is an important disease of domestic livestock and is responsible for economic losses in many areas of the United States, but it is seldom a problem in the intermountain west. The disease has been reported in a variety of captive wild bovids and cervids (Williams and Spraker 1979), but has been infrequently identified in free-ranging populations. An early report from France mentions paratuberculosis in free-ranging mouflon (Ovis musimon) (Lucas, cited by Thiery 1953) and several recent papers describe the disease in tule elk (Cervus elaphus nannodes) (Jessup et al. 1981), axis deer (Axis axis) and fallow deer (Dama dama) at Pt. Reye's National Seashore in California (Riemann et al. 1979). Other free-ranging

populations with paratuberculosis include the bighorn sheep and mountain goats in the Grant-Mount Evans area of Colorado (Williams et al.1979).

Paratuberulosis was diagnosed in bighorn sheep from the Grant and Mount Evans bighorn sheep herds in 1977. Movement of marked sheep between the Mount Evans and Grant herds was demonstrated by Martin and Stewart (1977). Retrospective study of tissues in files of the Wild Animal Disease Center, Colorado State University identified an additional case from Mount Evans in a bighorn ram in 1972. In 1979, paratuberculosis was identified in a ram in the Laramie Range, Wyoming (Williams 1981). An affected mountain goat from Mount Evans was examined in 1978 (Williams et al. 1979). During the period 1972 to 1981, 12 clinical cases of paratuberculosis in bighorn sheep and one mountain goat have been studied (Williams 1981).

Streeter (1969) conducted an extensive study of the demography of the Mount Evans sheep population from 1965-1968. During the 3 year study he observed seven sheep showing evidence of emaciation, scours, eye discharges and failure to shed hair coats normally. These clinical signs suggest paratuberculosis may have been present in the herd as early as 1965.

The present study was undertaken to determine the efficacy of several diagnostic tests for paratuberculosis in bighorn sheep and to apply them to free-ranging herds of sheep and goats in Colorado and Wyoming to establish the distribution of paratuberculosis in these species. Results of the tests were used to estimate the incidence of paratuberculosis in the Grant bighorn sheep.

We would like to acknowledge the assistance of Ms. Kathy Martin, Mr. Layne Adams, Mr. Gene Schoonveld, Drs. Tom Thorne, Dale Reed, William Lance and James DeMartini, members of the Wild Animal Disease Center, the Colorado Division of Wildlife and the Wyoming Game and Fish Department. This project was funded in part by a Burroughs-Wellcome Fellowship administered by the American College of Veterinary Pathologists.

## METHODS

M. paratuberculosis; blood and fecal samples were collected monthly from infected and two uninfected control sheep for 1 year postinoculation. In addition, 24 bighorns were captured by drop net near Grant, Colorado and transported to pens at the Wild Animal Disease Center. Blood and fecal samples were collected at the time of capture and at post-mortem examination. Additional samples were collected from some of these sheep during the period of captivity. Twelve cases of clinical paratuberculosis were examined by various diagnostic tests from 1977 to 1981. All sheep were examined postmortem for gross and microscopic evidence of paratuberculosis and a variety of tissues were cultured for M. paratuberculosis. Results of tests on spontaneously and experimentally infected sheep were used to determine the efficacy of diagnostic tests to be used in the field.

During winter trapping operations, serum for complement fixation (CF) tests, heparinized blood for the lymphocyte blastogenesis (LB) tests, and fecal samples were collected from sheep in eight bighorn herds in Colorado (Grant, Chalk Cliffs, Sagauche, Pikes Peak, Tarryall, Gunnison, Waterton Canyon, Poudre) and one in Wyoming (Whiskey Mountain).

A survey of hunter-killed bighorn sheep for paratuberculosis was conducted in September and October 1979. Packets containing small plastic bottles of formalin, plastic bags, blood tubes (plain and heparinized) and an instruction sheet were sent to all bighorn hunters with permits for the Grant-Mount Evans hunt areas. Packets were usually returned within 48 hours of death of the animal. Fifteen were distributed and three were returned. Serum, heparinized blood and fecal samples occasionally were obtained from mountain goats trapped in the Collegiate Range, Colorado and on Mount Evans for the LB test, CF test and fecal culture. During August and September 1979 sample collection packets were sent to goat hunters in the Mount Evans hunt area. Packets were the same as those described for bighorn sheep. Six of 12 packets were returned. In October 1980, blood tissues and fecal samples were collected from five mountain goats shot during the hunting season on Mount Evans. Samples were collected directly from the carcasses within 0.5 hours of death.

Lymphocyte blastogenesis tests were conducted essentially as described by Alhaji and coworkers (1974) and Burrells and Wells (1977). Jugular blood samples were collected into 20 milliliter glass evacuated blood tubes containing 20 USP units sodium heparin per milliliter of blood and into tubes without heparin which were allowed to clot. Serum was usually drawn off the latter within 24 hours of collection, a portion saved for use in the LB test and the remainder frozen for serologic tests. Heparinized blood was held at room temperature for 2 to 48 hours until used in the LB test. Tests were usually conducted within 24 hours after sample collection. In unusual circumstances, such as samples obtained from hunter-killed animals, blood held for as long as 72 hours was used in the assay.

Fifteen milliliters of heparinized blood was diluted 1:1 with Hank's balanced salt solution (HBSS) containing 100 units penicillin and 100 micrograms streptomycin<sup>2</sup> per milliliter. Mononuclear cells were separated from blood by the ficoll-diatrizoate gradient technique. Specific gravity of the gradient was adjusted to 1.077. Dilute blood was carefully layered onto 10 milliliters of gradient in 50 milliliter polypropylene centrifuge tubes using the technique of DeRock and Taylor (1977) which allowed 12 samples to be processed at once. All procedures were conducted in a laminar flow hood to prevent contamination of the cultures.

Sodium heparin injection, 5000 USP units/ml. Dell Laboratories, Inc., Teaneck, N.J. 07666. Benzyl alcohol preservative.
Penicillin-Streptomycin Solution, Grand Island Biological Company, 3175
St. Aley Road, Grand Island, New York 14072

Loaded tubes were centrifuged for 40 minutes at 670 relative centrifugal force units (RCF) at room temperature. The layer of mononuclear cells was drawn off using a Pasteur pipette and placed into a second tube. Cells were washed twice using HBSS and 10 minute centrifugation at 2000 RCF. Washed cells were suspended in 1 milliliter RPMI 16403 medium containing 100 units penicillin and 100 micrograms streptomycin. Cells were counted and diluted to 1 x 106 per ml in RPMI 1640 with 20% heat inactivated fetal bovine serum or autologous serum. Two hundred microliters of cell suspension (2 x 105 cells) were pipetted into round bottom wells in plastic microtiter trays containing 10 micrograms concanavalin A (con A)4 or mycobacterial antigens. Each test was run in triplicate. Purified protein derivative (PPD) of M. bovis, M. avium, and in some cases, M. paratuberculosis were used as antigens in the tests. Five micrograms M. bovis PPD or M. avium PPD or 12.6 micrograms M. paratuberculosis PPD were used per 2 x 105 mononuclear cells. Mycobacterial antigens were supplied by Dr. Dale Angus, Veterinary Services Laboratory, National Animal Disease Center, Ames, Iowa. Lymphocyte cultures were incubated at 37 C in 5 percent CO2 humid atmosphere for 5 days. Eighteen to 20 hours prior to harvest 1 microcurie thymdine methyl-3H5 was added to each well. Cultures were terminated by harvesting contents of each well onto glass fiber filter paper using a vacuum microtiter plate cell harvester. Oried filter paper discs were counted in a liquid scintillation counter. Results were recorded as counts per minute (CPM). The mean CPM of each set of triplicate cultures was used to determine a stimulation index:

Stimulation index = Mean CPM of wells containing mitogen or antigen
Mean CPM of wells without mitogen or antigen

Fecal and/or tissue samples were obtained for mycobacterial culture from bighorn sheep and mountain goats when trapped or during postmortem examinations. Most samples were cultured within 2 days of collection; some samples were frozen at -70C for up to 2 years prior to culture. Cultures were conducted as described in Laboratory Methods in Veterinary Mycobacteriology<sup>6</sup> using Herrold's egg yolk medium. Three tubes containing mycobactin and one without were used for each sample. Tubes were incubated at 37C for 16 weeks. Identification of M. paratuberculosis. was based on slow growth, acid-fast staining properties and mycobactin dependence.

<sup>&</sup>lt;sup>3</sup>RPMI-1640 with L-Glutamine, Grand Island Biological Company, Grant Island, New York, 14072

<sup>4</sup>Highly purified lyophilized powder, Sigma Chemical Co., P.O. Box 14508, St. Louis, MO. 63178.

<sup>5</sup>Thymidine Methyl-3H, 6.0 Ci/mmol spec. act., Schwartz-Mann, Orangeburg, New York 10962, or Thymidine Methyl-3H, 6.7 Ci/mmol, New England Nuclear, 549 Albany Street, Boston, Mass. 02118.

<sup>6</sup>Veterinary Services Diagnostic Laboratory, Animal Plant Health Inspection Service, U.S. Department of Agriculture, Ames, Iowa, 77 pp.

One milliliter of frozen serum from selected animals, including known infected and known uninfected animals was submitted to Dr. B. D. Blackburn, Veterinary Services Diagnostic Laboratory, Ames, Iowa for CF testing for paratuberculosis. A microtiter method was used and a CF titer of less than or equal to 1:8 was considered negative, 1:16 suspect and 1:32 or greater positive.

Sensitivity and specificity of the tests were determined on experimentally infected bighorn hybrid sheep (Rogan and Gladen 1978, Brown and Newman 1979). Sensitivity is defined as the chance a test will be positive when applied to animals which are infected and specificity is the chance a test will be negative when applied to uninfected animals. Estimates of prevalence of paratuberculosis in the Grant bighorn sheep were determined by the method of Rogan and Gladen (1978).

#### RESULTS

EFFICACY OF TESTS IN EXPERIMENTAL AND SPONTANEOUSLY INFECTED BIGHORN SHEEP.

In experimentally infected hybrid sheep, highest mean stimulation indexes on the LB test to antigen were obtained using M. avium PPD; thus, M. avium antigen was used in all subsequent tests. Tests using fetal calf serum and a stimulation index greater than or equal to 3.5 as positive had 82 percent sensitivity and 94 percent specificity (Table 1). By two months postinoculation, most infected hybrid sheep responded to the test.

The CF test was not useful in diagnosis of paratuberculosis in hybrid sheep (Table 1); sensitivity was 0 and specificity was 100 percent. No false positive responses occurred; however, there were no true positive responses either. Fecal culture of subclinically affected hybrid sheep was 3 percent sensitive and 100 percent specific.

The LB test was run on 16 bighorn sheep from Grant which were found to be infected with M. paratuberculosis either by culture or visualization of acid-fast bacteria within typical lesions of paratuberculosis. Twelve responded positively (stimulation index 23.5) when fetal calf serum and M. avium PPD was used (Table 1). When the CF test was conducted on sera of 13 known infected bighorns a positive response was obtained on only one occasion (8% sensitivity). Eight fecal cultures from known infected bighorn sheep were negative for M. paratuberculosis (0 sensitivity).

RESULTS OF DIAGNOSTIC TESTS ON FREE-RANGING BIGHORN SHEEP AND MOUNTAIN GOATS

The LB test was used to test 157 free-ranging bighorn sheep for paratuberculosis from eight herds in Colorado and Wyoming (Table 2). Positive results were obtained from most herds, including herds where paratuberculosis was presumed absent (Chalk Cliffs, Pikes Peak, Tarryall, and Whiskey Mountain). Thirteen percent (10/77) of the samples from these herds reacted positively (stimulation index 23.5) using M. avium PPO as antigen.

Over a 2 year period, 45 percent (14/31) of bighorns samples from the Saguache herd responded positively to the LB test. Of 49 blood samples collected from bighorns at Grant over 3 years, 33 (67%) were positive. Twenty-seven percent (3/11) of blood samples from bighorn lambs at Grant were positive. The reactor rate increased in the yearling and older age classes.

All 155 serum samples from seven bighorn sheep herds tested with the CF test for paratuberculosis were negative (Table 2). In 1978, fecal samples collected at the time of trapping from nine bighorn sheep herds were culture negative for M. paratuberculosis.

Examination of tissues collected from hunter-killed bighorn sheep in 1979 did not result in the diagnosis of paratuberculosis. One sheep, however, had granulomas suggestive of paratuberculosis in the intestinal tract and mesenteric lymph nodes but acid-fast organisms could not be demonstrated.

Results of tests for paratuberculosis in samples from mountain goats are shown in Table 3. Four heparinized blood samples collected from mountain goats trapped in the Collegiate Range were negative on the LB test for paratuberculosis. One positive LB test response was obtained from mountain goats trapped on Mount Evans; culture of eight fecal samples from this herd was negative for paratuberculosis.

Examination of tissues collected from hunter-killed mountain goats in 1979 and 1980 resulted in the diagnosis of paratuberculosis in two of 11 mountain goats on the basis of histopathology in one case and culture of  $\underline{\mathsf{M}}$ .  $\underline{\mathsf{paratuberculosis}}$  from the mesenteric lymph node in the other case.

# DISCUSSION

Specificity and sensitivity of the LB test were calculated to be 75 percent and 87 percent, respectively, based on test responses of known infected bighorns from Grant and responses of free-ranging bighorns from herds thought not to be infected with M. paratuberculosis. These responses are lower but comparable to the 82 percent sensitivity and 94 percent specificity of the LB test in experimentally infected bighorn x mouflon sheep. Thus false positive and false negative results may cause problems in use of the LB test in bighorn sheep. The test, however, appears to be more accurate than other available tests.

False positive responses to the LB test could be explained by prior exposure to another antigenically related organism. Corynebacterum renale (Gilmour and Goudswaard 1972), C. equi (McKenzie and Ward 1981), acid-fast saprophytic organisms (Hole and Maclay 1959, Jensen 1956), and other mycobacteria, especially M. avium (Wilks et al 1981), have been shown to cause false positive reactions on tests for paratuberculosis. Free-ranging bighorn sheep could easily come in contact with saprophytic mycobacteria, M. avium from free-flying birds or with Corynebacterum spp.

Corynebacterium spp. frequently have been recovered from bighorn sheep in

Colorado and Wyoming. False negative responses on the test would be expected before an immune response to M. paratuberculosis is counted. This period may be several months long for most mycobacterial diseases and would result in a lowered sensitivity of the test. By any mechanism, suppression of the LB test response in individuals infected with paratuberculosis, as has been described in other species (Williams 1981), could result in false negative responses.

The CF test was unreliable as a diagnostic test for paratuberculosis in spontaneously and experimentally infected bighorn sheep and could not be recommended for this species. Few fecal samples from experimentally infected hybrid sheep and none from known infected bighorn sheep were culture positive for M. paratuberculosis. The low rate of positive fecal cultures in infected animals could be explained by low sensitivity of the cultural procedure; 50 to 100 organisms per gram of feces must be present before culture will be positive (Merkal 1970). Many fecal samples from bighorn and hybrid sheep were frozen prior to culture which may have decreased titer of viable organisms (Richards and Thoen 1977).

Most bighorn herds surveyed contained at least one sheep that responded positively to the LB test. These were assumed to be false positive responses because there was no clinical or cultural evidence of M. paratuberculosis within these herds. As described previously, false positive reponses were probably due to sensitization of an animal by an antigenically related organism. If these herds are actually free of paratuberculosis, a false positive result occurred in 13 percent of the free-ranging bighorn sheep tested.

Positive responses on the LB test in the Grant bighorn sheep were 56 percent (5/9), 62 percent (16/26) and 86 percent (14/16) for the years 1978, 1979 and 1980, respectively. These were considerably higher than the 13 percent expected false positive rate and correctly reflect the fact that this herd is infected with M. paratuberculosis. It is unclear if the increase in positive responses over 3 years reflected increased prevalence of disease. In this herd, prevalence of M. paratuberculosis infection was estimated to be 87 percent using the method of Rogan and Gladen (1978) which takes sensitivity and specificity of the test into account in determining results of screening tests.

It is interesting to speculate on the high rate of positive LB test responses in the Saguache bighorn sheep herd. Fifty percent (9/18) of sheep responded positively in 1978 and 38 percent (5/13) in 1980. This 45 percent reactor rate is considerably greater than the expected 13 percent false positive response rate. The sheep might be sensitized to cross-reacting organisms, or they could be infected with M. paratuberculosis or M. avium. This herd comes into close contact with domestic cattle. A similar situation exists in the Laramie Range in Wyoming where a clinical case of paratuberculosis has been diagnosed. An emaciated diarrheic ewe was observed in the summer of 1980 at Saguache, but was not collected for postmortem examination. Although there is no

definitive evidence, the Saguache bighorn sheep herd may be infected with M. paratuberculosis. and deserves further study.

Evidence from hunter-killed and trapped mountain goat tissues and blood samples indicate M. paratuberculosis is present within the goat population on Mount Evans. This is of concern because goats appear to be emigrating from the area of original transplant on Mount Evans and could be carrying the bacteria into new areas. The significance of mountain goats in the epizootiology of paratuberculosis on Mount Evans deserves additional study.

with a few exceptions, it seems unlikely that paratuberculosis is of much importance in free-ranging wildlife. Most wild ruminants are susceptible to M. paratuberculosis infection and once introduced into a free-ranging population the probability of the disease remaining in that population would depend upon habitat and behavioral characteristics of the species. The free-ranging bighorn sheep in the Grant-Mount Evans herd are able to maintain M. paratuberculosis within the population because the sheep maintain a relatively high ecological density within certain areas of their range. This serves to enhance transmission of M. paratuberculosis between animals. The importance of congential infection with M. paratuberculosis is unknown but it has been shown to occur in bighorn sheep (Williams 1981). Congenital infection with M. paratuberculosis could serve to maintain infection in the population without the bacteria being exposed to harsh environmental conditions.

The effect of paratuberculosis on the Grant-Mount Evans bighorn sheep population is unknown because data on the dynamics of the herd are insufficient to determine what impact the disease has on the population. The population grew since the 1920's to approximately 200-225 animals but has been relatively stable for the last 10 years (Streeter 1969, Loessberg 1972, Martin and Stewart 1977, Goforth 1979, 1980). If bighorn sheep are like domestic sheep, mortality in adult bighorns due to paratuberculosis is unlikely to exceed 10 percent per year (McEwen 1939, Doyle 1956). Approximately three cases of paratuberculosis per year have been examined for 4 years from a population of about 200 animals. This gives a known mortality rate of 1.3 percent per year. This undoubtedly represents only a portion of the sheep which have died of paratuberculosis. In domestic animals, prevalence of infection may be 20 times greater than actual clinical disease (Buergerlt et al 1977). If an 87 percent infection rate is accurate, based on LB test survey data, nine sheep a year would be expected to die of paratuberculosis in the Grant-Mount Evans herd for an annual mortality rate of 5 percent. This is comparable to mortality rates in domestic species.

Table 1. Sensitivity and specificity of diagnostic tests for paratuberculosis in sheep spontaneously or experimentally infected with <a href="Mycobacterium">Mycobacterium</a> paratuberculosis.

Test animals	Diagnostic Test	Sensitivity	Specificity
Subclinically affected experimentally infected	Lymphocyte blastogenesis <sup>1</sup> (n = 119)	82%	94%
bighorn x mouflon sheep	Complement fixation (n = 119)	0	100%
	Fecal culture (n = 119)	3%	100%
	Lymphocyte blastogenesis (n = 16)	75%	T and
Spontaneously infected sighorn sheep	Complement fixation (n = 13)	8%	-
	Fecal culture (n = 8)	0	12
ree-ranging bighorns	Lymphocyte blastogenesis (n = 77)	2	87%
from herds assumed free of paratuberculosis	Complement fixation (n = 100)	~	100%
entresse verst i <b>#</b> erent Kuste i italien trissellist ett ett	Fecal culture <sup>2</sup> (n = 30)	12	100%

 $<sup>^1</sup>$ All lymphocyte blastogenesis test results are for tests run using  $\underline{\text{M.}}$  avium PPD as antigen, fetal calf serum and a value of 3.5 positive test.

<sup>2</sup>R. Keiss, personal communication. Fecal samples collected from 9 bighorn sheep herds in 1978.

Table 2. Lymphocyte blastogenesis and complement fixation test results from bighorn sheep herds in Colorado and Wyoming, 1978 to 1980.

Herd	Year	No. positive/No. tested (% positive)	Complement fixation tests No. positive/No. tested (% positive)
Grant	1978	5/9 (56%)	0/21 (0)
Grant	1979	16/26 (62%)	0/26 (0)
Grant	1980	12/14 (86%)	0/5 (0)
Chalk Cliffs	1978	4/17 (24%)	0/16 (0)
Chalk Cliffs	1979	1/9 (11%)	NT
Saguache	1978	9/18 (50%)	0/2 (0)
Saguache	1980	5/13 (38%)	0/23 (0)
Pikes Peak	1978	3/10 (30%)	0/9 (0)
Tarryall	1978	0/4 (0)	0/33 <sup>3</sup> (0)
Tarryall	1980	1/11 (9%)	NT
Gunnison	1978	0/2 (0)	0/7 (0)
Waterton			
Canyon	1979	0/12 (0)	NT
Whiskey			
Mountain	1980	1/12 (8%)	NT
Poudre	1978	NT	0/13 (0)

<sup>&</sup>lt;sup>1</sup>Tests performed by Veterinary Services Diagnostic Laboratory, Ames, Iowa.

<sup>2</sup>Not tested.

<sup>&</sup>lt;sup>3</sup>One test was anticomplementary.

Table 3. Paratuberculosis test results on samples collected from mountain goats, 1979 to 1980.

Herd	Date	No.	Histopathology No. positive/No.tested (% positive)	Lymphocyte biastogenesis No. positive/No. tested (% positive)	No. positive/No. tested (% positive)
Mount Evans	Sept 1978	8	ND.1	(0) 1/0	ON
Collegiate			6		9
Range	outy 197		ON.	0/4 (0)	2
Mount Evans	Sept 1979	0.	1/6 (17%)	9	9
Mount Evans	March 19	62	9	0/1 (0)	Q
Mount Evans	Sept 1980	0	0/2 (0)	0/3 (0)	1/5 (20%) <sup>2</sup>
Mount Evans	Sept & Oct 1980	ct 1980	Q	1/7 (14%)	0.8 (0)3

Not determined.

Culture of tissues.

Sculture of feces.

## LITERATURE CITED

- Alhaji, I., D.W. Johson, C.O. Muscoplat and C.O. Thoen. 1974. Diagnosis of Mycobacterium bovis and Mycobacterium paratuberculosis infections by in vitro lymphocyte immunostimulation. Am. J. Vet. Res. 35:725-727.
- Brown, J. and M.A. Newman. 1979. Lesions of swine lymph nodes as a diagnostic test to determine mycobacterial infection. Appl Environ. Microbiol. 37:740-743.
- Buergelt, C.D., C.E. Hall, R.S. Merkal, R.H. Whitlock and J.D. Duncan. 1977. Lymphocyte transformation: an aid in the diagnosis of paratuberculosis. Am. J. Vet. Res. 38:1709-1715.
- Burrells, C. and P.W. Wells. 1977. In vitro stimulation of ovine lymphocytes by various mitogens. Res. Vet. Sci. 23:84-86.
- DeRock, E. and N. Taylor. 1977. An easy method of layering blood over ficollpaque gradients. J. Immunol. Methods 17:373-374.
- Doyle, T. M. 1956. Johne's disease. Vet. Res. 68:8690878.
- Gilmour, N.J.L. and J. Goudswaard. 1972. <u>Corynebacterium renale</u> as a cause of reactions to the complement fixation test for Johne's disease. J. Comp. Pathol. 82:333-336.
- Goforth, B. 1979. Mt. Evans goat-sheep trend count 1979, Unpublished manuscript. Colorado Division of Wildlife, Fort Collins. 11 pp.
- Goforth, B. 1980. Mt. Evans goat-sheep trend count 1980. Unpublished manuscript. Colorado Division of Wildlife, Fort Collins. 7 pp.
- Hole, N.H. and M.H. Maclay. 1959. I. The diagnosis of Johne's disease in cattle and the identification of M. johnei infection. Vet. Rec. 71:1145-1149.
- Jensen, M.H. 1956. A complement-fixation test for Johne's disease in cattle. Nord. Vet. Med. 8:357-367.
- Jessup, D.A., B. Abbas, D. Behymer and P. Grogan. 1981. Paratuberculosis in tule elk in California. J. Am. Vet. Med. Assoc. 179:1252-1254.
- Loessberg, H.W. Jr. 1972. 1972 census of <u>Ovis canadensis canadensis</u> and <u>Oreamnos americanus</u> in the Mount Evans summer range. Unpublished manuscript. Colorado Division of Wildlife, Fort Collins. 15 pp.
- Lucas, cited by Thiery, J.P. 1953. La Maladie de Johne. Bull. Off. Int. Epiz. 40:129-135.

- Martin, L.M. and D.W. Stewart. 1977. 1977 summer census of the Mount Evans bighorn sheep and Rocky Mountain goat populations. Unpublished manuscript. Colorado Division of Wildlife, Fort Collins. 33 pp.
- McEwen, A. D. 1939. Investigations of Johne's disease of sheep. J. Comp. Pathol. 52:69-87.
- Merkal, R.S. 1970. Diagnostic methods for detection of paratuberculosis (Johne's disease). Proc. Annu. Meet. U.S. Animal Health Assoc. 74:620-623.
- Richards, W.D. and C.O. Thoen. 1977. Effect of freezing on the viability of <a href="Mycobacterium">Mycobacterium</a> paratuberculosis in bovine feces. J. Clin. Microbiol. 6:392-395.
- Riemann, H., M.R. Zaman, R. Ruppanner, O. Aalund, J.B. Jorgensen, H. Warsaae and D. Behymer. 1979. Paratuberculosis in cattle and free-living exotic deer. J. Am. Vet. Med. Assoc. 174:184-843.
- Rogan, W.J. and B. Gladen. 1978. Estimating prevalence from the results of a screening test. Am. J. Epidemiol. 107:71-76.
- Streeter, R.G. 1969. Demography of two Rocky Mountain bighorn sheep populations in Colorado. Pn.D. dissertation. Colorado State University., Fort Collins. 96 pp.
- Wilks, C.R., T.K. Taylor and E.G. Russell. 1981. Isolation of mycobacteria inducing cross-reactions in the complement fixation test for Johne's disease. Res. Vet. Sci. 30:323-327.
- Williams, E.S. 1981. Spontaneous and experimental infection of wild ruminants with <u>Mycobacterium paratuberculosis</u>. PhD dissertation. Colorado State University, Fort Collins. 362 pp.
- Williams, E.S. and T.R. Spraker. 1979. Paratuberculosis in free-ranging bighorn sheep and a Rocky Mountain goat with a brief review of the disease in wild species. Pages 122-125 in Proc. Am. Assoc. Zoo Vet. Denver, CO.
- Williams, E.S., T.R. Spraker and G.G. Schoonveld. 1979. Paratuberculosis (Johne's disease) in bighorn sheep and a Rocky Mountain goat in Colorado. J. Wildl. Dis. 15:221-227.

## CONFERENCE DISCUSSION

- Q. I gather that it is likely that the sheep have had this for a long time, presumably since at one time they were in contact with livestock. Can you tell us if there has been a declining trend in sheep and goats on Mt. Evans over the last 20 years?
- ANS We don't know when the infection got into the herd, but it's likely that it came in with domestic animals. Streeter, in work on Mt. Evans, indicated that he saw animals that were emaciated and had diarrhea as early as about 1965-1967, so the disease has likely been there since then.
- Q. I just wondered, has the Mt. Evans sheep herd showed a decline over these years?
- ANS Surveys suggest that the herd is relatively stable though good data are lacking.
- Q. As I understand it, it's been decided that this infection in the herd at Grant, on the south side of Mt. Evans, is a threat to other wild ungulates, particularly deer and elk on that range, and that the herd of sheep should be reduced or eliminated to reduce the possibility of that infection getting into the deer and elk populations. A couple of things make me wonder about that. (1) Do we know whether or not the disease is already in the elk and deer? It's in the goats which are nearby. (2) Do we need to control goats also if we are going to continue this philosophy? (3) You said the disease lasts for 3 years on the ground. Would you comment about this approach to handling the disease, that is reducing or eliminating that herd of sheep.
- ANS I think Gene might be able to say something more about the management decision that was made. Paratuberculosis is a very difficult disease to control. You mentioned some of the big problems with trying to control it. There is not a good diagnostic test. You can't go in, as they do with some types of diseases in domestic animals, and test and slaughter. If paratuberculosis appears in a cattle ranch, in Colorado, the ranch is put under a modified quarantine. In other words, they are not supposed to sell their animals and it becomes a considerable economic hardship to the rancher. The domestic-animal people have been working for a long, long time, trying to control the disease in livestock, and they haven't been very successful. The options available are not very satisfying. In a few situations, paratuberculosis has been managed by slaughtering, the animals. This approach has been used with the less valuable stock. Vaccination could be another way to approach the problem. There is a vaccine that protects to some extent in domestic sheep and has been shown to greatly reduce the infection rate in a very large field study in Iceland. Another option is to live with the paratuberculosis problem and risk the chance of the infection getting into the other ungulate populations

on Mount Evans. I have some evidence, though not definitive, that the disease is already in the elk and deer. Certainly elk are quite susceptible and so are deer, as shown by my experimental work and also by some recent reports in the literature. We know that the mountain goats on Mount Evans have paratuberculosis, and I believe these animals must be considered in any management plan.

The fact that the organisms are viable on the ground for a long time greatly complicates management. If it were possible to remove the infected populations, ruminants should not be put back on that range for three years, to be safe, because those animals could potentially become infected. My understanding is that the Division of Wildlife has taken the approach that it would be better to give up on the sheep and to try to decrease the chances for paratuberculosis to become a problem in the valuable elk herd in the area. I'm not satisfied with the management decision but there are problems with all the other options.

- Q. Sheep from the Mt. Evans herd were transplanted over near Dinosaur National Park. Do you happen to know if any symptoms have shown up in that population?
- ANS I haven't heard of any. I doubt if they have been watched closely enough to find out.