PARATUBERCULOSIS (JOHNE'S DISEASE) IN BIGHORN SHEEP (*Ovis canadensis*) AND ROCKY MOUNTAIN GOATS (*Oreamnos americanus*) IN COLORADO

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ABSTRACT

Between 1972 and February 1978, six cases of paratuberculosis have been diagnosed in a herd of free-ranging Rocky Mountain bighorn sheep (*Ovis canadensis*) and one case in a Rocky Mountain goat (*Oreamnos americanus*) on Mount Evans, Colorado. Sightings of 20 other sheep and four other goats showing clinical signs of the disease have also been reported during this time. Diagnosis of paratuberculosis was based on gross and histopathologic examination of the animals and by isolation of *Mycobacterium paratuberculosis* from three sheep and the goat. Clinical and pathologic changes seen in bighorn sheep resembled those described in domestic sheep and goats. A discussion of the importance of finding this disease in free-ranging bighorn sheep and Rocky Mountain goat herds is discussed.

INTRODUCTION

Paratuberculosis is a specific infectious enteritis of domestic livestock including cattle, sheep, goats, camel and reindeer (Thoen 1977, Katic 1961).

It is of great economic importance to the livestock industry in much of the United States (Kopicky 1973), Canada, Eastern and Central Europe. The disease has been recognized for over eighty years, having first been described in Germany by Johne and Frothingham in 1895. Paratuberculosis is caused by Mycobacterium paratuberculosis, a small (0.5ux - 1.2u) acid-fast bacillus. In ruminants this bacteria causes a chronic disease with involvement of the intestinal tract, particularly the distal small intestine, ileocecal valve, cecum, associated mesenteric lymph nodes and afferent lymphatics. Most information on paratuberculosis is based on studies of the disease in domestic livestock. Descriptions of the disease in wild species are incomplete and consist mainly of isolated case reports. Paratuberculosis in bighorn sheep has only been reported once in the literature (Thoen 1977) and reports of the disease have not previously been reported in Rocky Mountain goats. Other wild species in which paratuberculosis has been reported include white-tailed deer (Odocoileus virginianus) (Libke 1975), roe deer (Capreolus capreolus) (Hillermark 1966), European red deer (Cervus elaphus) (Vance 1961, Katic 1961), moose (Alces alces) (Soltys 1967), aoudad (Ammotragus lervia) (Boever 1977), mouflon (Ovis musimon) (Boever 1977, Katic 1961), camel (Camelus bactrianus) (Thoen 1977, Katic 1961), bighorn sheep (Ovis canadensis) (Thoen 1977), reindeer (Rangifer tarandus) (Katic 1961), Japanese sika deer (Pseudaxis sika) (Katic 1961), water buffalo (Bubalus bubalus) (Katic 1961), yak (Bos grunniens) (Katic 1961), gnu (Connochaetes alboujubatus) (Katic 1961), and Llama (Llama glama) (Appleby 1954).

M. paratuberculosis is relatively resistant to environmental con-

ditions. Viable organisms, capable of initiating infection in susceptable animals, have been recovered from contaminated pastures for one year and from barn walls for up to five years after the source of bacteria had been removed. The organism is relatively susceptable to sunlight (50-100 hours of direct sunlight will kill the organism), drying, high soil calcium, high soil pH, and continuous contact with feces and urine. The bacteria is fastidious and slow growing in culture requiring up to four months for identification. The paratuberculosis organism requires mycobactin, a product derived from Mycobacterium sp, in the culture media in order to grow.

The primary natural route of transmission of the disease is via ingestion of contaminated feces. Studies of paratuberculosis in domestic livestock indicate that animals become infected when they are very young. Experimental work with domestic lambs has shown that as few as 1,000 organisms will establish infection (Filmore 1976). Other modes of transmission, which have been documented in domestic livestock, but have not been examined in wild species, include intrauterine and transmammary infection.

Clinical signs described in domestic ruminants include a long incubation period, with most clinical cases being 2-6 years of age. Clinical signs include emaciation and often submandibular edema, which is related to low serum protein. Cattle characteristically have constant diarrhea, domestic sheep and goats may or may not have diarrhea, and in cases where it is present, diarrhea may be intermittent. Normally animals are alert and have good appetites until the terminal stage. The biggest economic

problem to the livestock industry is related to decreased production (milk, wool and weight gains). A second, less dramatic, but nevertheless important loss over a long period of time, is that up to 10 percent mortality can occur annually in infected herds (Larsen 1970).

At necropsy, domestic animals show classic signs of emaciation including serious atrophy of fat depots, submandibular edema and muscle atrophy. Subserosal edema and thickening of the wall of the distal jejunum, ileum and cecum are commonly found. The afferent lymphatics from these regions are also thickened, opaque, and sometimes torturous. The associated mesenteric lymph nodes are usually grossly enlarged (2 to 5 times normal), edematous, and in the case of domestic sheep and goats, may contain areas of necrosis, caseation and mineralization.

The classic histopathologic lesions in domestic ruminants include granulomatous enteritis, lymphangitis (afferent lymphatics) and lymphadenitis (mesenteric lymph nodes and occasionally pharengeal lymphnodes and tonsil). The granulomatous infiltrate is characterized by large epitheloid macrophages with large amounts of foamy eosinophilic cytoplasm, multinucleated giant cells and variable numbers of lymphocytes. With acid-fast stains, clumps of small bacilli are found intracellularly in the phagocytic cells. Caseation and mineralization is associated with the granulomatous reaction in domestic sheep and goats, and only very seldom in cattle.

Techniques for diagnosis of the disease have only been worked out for domestic species and at the present diagnosis of subclinical cases is quite difficult. Serological testing is of some value in cattle but

many false positives and negatives are found. Therefore, the test may be useful for indicating the presence of the disease in herds but is of little use for diagnosis of the disease in individual animals. Fecal culture presently is the most widely used technique for detection of paratuberculosis in domestic herds and for identification of individual infected animals. Problems associated with use of facalculture are that large numbers of bacteria are only shed relatively late in the disease and then may only be shed intermittently. The length of time required for a diagnosis and the difficulty of culturing the organism also are problems associated with fecal cultures. Promising data recently has been published applying the lymphocyte stimulation test to the diagnonis of paratuberculosis in cattle (Alhaji 1974). This test should be capable of detecting an animal sensitized to antigens of M. paratuberculosis within a short period after infection, thus detecting an infected animal long before any other test. The short period of time required for the test (two weeks) is therefore a definite advantage.

METHODS

Personnel of the Colorado Division of Wildlife submitted diseased animals to the Wild Animal Disease Center for thorough necropsy and evaluation. Some cases were presented alive and subsequently euthanized, others were shot or found dead in the field. A thorough post mortem examination was performed and representative tissue samples fixed in 10 percent formalin; mesenteric lymph nodes and sections of small intestine were frozen for later bacterial culture. Formalin fixed tissues were processed rountinely and stained with hematoxylin and eosin.

Appropriate tissues also were stained by the Kinyon acid-fast method. Bacteriology was performed by Dr. Charles Thoen, National Animal Disease Center, Ames, Iowa. The lymphocyte stimulation test was performed as described by Alhaji (1974) for bovine lymphocytes with the following modifications: (a) Heparin was used at 20 U/ml of venous blood; (b) ficoli-diatrizoate was adjusted to specific gravity of 1.077; (c) microtiter plates were used for cell culture; (d) 20 percent fetal calf serum was used in the culture media; (e) cultures were incubated for a total of five days; (f) concanavalin A (50 ug/ml) was used as a nonspecific mitogen; and (g) Otto Hiller automatic harvester was used to terminate cell cultures. A fecal smear of the mountain goat using the auramine-rhodamine stain to demonstrate the bacteria was performed.

STUDY AREA

All clinical cases of bighorn sheep were from Mount Evans and Grant, Colorado. These areas are approximately 13 miles apart. The Mount Evans herd is estimated at 150 sheep and the Grant herd at 80 animals. Exchange of animals between these two herds has been documented by observation of movements of neck-banded individuals. The mountain goat was from the Lincoln Lake area on Mount Evans.

RESULTS

Six confirmed cases of paratuberculosis from the Mount Evans/Grant herds have been documented up to February, 1978 (Table 1). Descriptions of the clinical disease, gross, and histopathology in bighorn sheep are, however, based on five cases. Case #2, a mature ewe, was subclinical; she was not showing clinical signs and all systems

TABLE 1.

Cases of Paratuberculosis

Bighorn Sheep and Rocky Mountain Goats in Colorado

Species	Date	Age	Sex	Location
Bighorn	1972	4	male	Mt. Evans
Bighorn	1-26-77	4	female	Mt. Evans (Subclinical)
Bighorn	4-18-77	6	male	Grant, Colorado
Bighorn	5-20-77	4	male	Mt. Evans
Bighorn	4-30-77	10	male	Grant, Colorado
Bighorn	1-2-78	7½	female	Grant, Colorado
Rocky Mtn. Goat	2-21-78	2½	male	Mt. Evans

appeared grossly normal on examination.

Paratuberculosis in bighorn sheep.

Gross Pathology: All the sheep examined were emaciated, had dry, rough hair coats and diarrhea. Submandibular edema was noted in three of the sheep examined. Nonspecific lesions such as serious atrophy of fat depots, submandibular edema and generalized muscle atrophy were associated with the emaciated state of the animals. Marked subserosal edema was present in the jejunum and ileum. Afferent lymphatics from these regions were opaque, thickened and torturous. Mesenteric lymph nodes were greatly enlarged (2 to 5 times normal size) and edematous. The wall of the jejunum and ileum was thickened and in some cases corrugated.

Histopathology: Microscopic features consisted of granulomatous enteritis, lymphangitis, and lymphandenitis. The primary inflammatory cells were large epitheloid macrophages and, in three cases, multinucleated giant cells. Cellular infiltration disrupting normal architecture of the intestinal wall was present in the lamina propria and submucosa of the small intestine and cecum. In severe cases, cellular infiltrate also was present in the spiral colon. Necrosis or mineralization was not found. Lymphangitis was severe in most cases with many vessels nearly occluded by thickened irregular walls and debris within the lumen. Inflammatory cells were primarily epitheloid macrophages, and in two cases, giant cells were present in the mesentery and perilymphatic areas. Mild lymphocytic perilymphatic infiltration also was present. Granulomatous infiltrate in the lymph nodes occurred primarily in the subcapsular regions of the cortex. Again, the inflammatory infiltrate was mostly epitheloid

macrophages with occasional giant cells in three cases. In case #6, tonsils were examined histologically and found to have granulomatous foci present. Acid-fast stains of tissues from these animals revealed variable numbers of acid-fast bacilli present within the phagocytic inflammatory cells of intestine, lymphatics, lymph nodes and, in one case, tonsil.

Subclinical Case #2: On histopathologic examination of tissues mild lesions typical of paratuberculosis were found. Acid-fast stains demonstrated only a few bacteria within phagocytic cells of these tissues.

Paratuberculosis in Rocky Mountain Goat.

Gross Pathology: Clinical signs seen in this animal were similar to those described for bighorn sheep - emaciation, dry rough hair coat, and diarrhea. Gross examination, however, revealed somewhat different lesions. Subserosal edema was not present. Mesenteric nodes were massively enlarged and yellowish areas indicating the presence of necrosis were seen through the mesentery and on cut surface. The intestinal tract was diffusely hyperemic and a fibrino-necrotic membrane was present in the ileum. This was probably related to a secondary bacterial infection. The small intestine was greatly thickened. A 3 cm. abscess in the wall of the ileum and adhesions between ileum and peritoneal wall were present. The lymphatics were opaque but not thickened to the degree seen in bighorn sheep.

Histopathology: On histopathologic examination, massive granulomatous enteritis was present in the jejunum and ileum. Less extensive lesions were present in the duodenum, cecum and spiral colon. Granulomatous lymphadenitis was also present. Inflammatory cells especially epitheloid macrophages and numerous large multinucleated giant cells were obvious

throughout. Necrosis was present in many areas of dense cellular infiltrate. Extremely large numbers of acid-fast organisms were demonstrated within the phagocytic cells. Fecal smears stained by both acid-fast stains and the auramine-rhodamine method indicate that the goat was shedding extremely large numbers of M. paratuberculosis in the feces.

Preliminary lymphocyte stimulation work using blood from the Grant sheep indicated approximately 30 percent infection rate (6 or 7 animals) in this herd.

DISCUSSION

From the description of the lesions found in the five clinical cases of paratuberculosis in bighorn sheep it can been seen that the disease appears to be similar to that described in cattle. The disease in Rocky Mountain goats appears to be more like that described for domestic sheep and goats. The extensive granulomatous reaction, the large numbers of bacteria present, and the relatively young age of this animal may indicate that mountain goats are more susceptible to this disease than are bighorn sheep.

The subclinical case of paratuberculosis is of interest in that she may have been a carrier of the organism and was more likely only at an early stage of the disease. The fact that subclinical cases exist, and animals may be shedding infective organisms is an important consideration to the epizootology of the disease. The massive numbers of bacteria being shed in the feces of the mountain goat is also important. More goat cases are needed to adequately describe the disease in this species.

Several important questions should be considered in regards to the

diagnosis of paratuberculosis within these two herds. First, how did the organism become established in this area? Paratuberculosis, while it is important in many areas of the United States, has not been diagnosed by the Colorado Veterinary Diagnostic Laboratory in animals in Colorado for twenty years. A possibility is that bighorn sheep and mountain goat populations contracted the disease from domestic species. A second possibility is that Rocky Mountain goats, transplanted into the Mount Evans area in early 1960, may have been infected and it has taken nearly 18 years for the disease to become established to the present degree. The extensive lesions present in the goat case gives support of this theory.

A second question is what other species may be or become infected with this strain of M. paratuberculosis? It is known that most strains readily infect domestic cattle, sheep and goats. Though this work has not yet been done, we strongly suspect that this bacteria will infect domestic ruminants as well as other wild ruminant species (mule deer, elk) where ranges overlap with infected sheep or goat herds. Cross transmission studies are needed to clarify this question and to determine the importance of this problem.

What steps should be taken to control or eradicate the disease in this area? Survey work to determine the rate of infection is necessary. More data on the epizootology and character of paratuberculosis in wild ruminants is necessary before rational steps can be taken. The lymphocyte stimulation test may be an important tool in this regard but it is still in the developmental stages. Fecal cultures presently are being run in an attempt to detect those animals shedding organisms. The lymphocyte

stimulation test together with fecal culture may be a good way of diagnosing paratuberculosis and giving information as to the preval nce of the disease in these herds.

Control of the disease is a difficult problem. Treatment for paratuberculosis is not presently available. Vaccination programs in Europe have had moderate success in decreasing the number of clinical cases. However, some vaccinated animals will still contract the disease and shed organisms in the feces. Detection of infected animals and their removal from the herd also may be a possibility. This is a method employed by domestic livestock farmers in controlling paratuberculosis in their herds.

Clearly, paratuberculosis is an important problem in this particular herd and maybe of significance to other bighorn sheep and Rocky Mountain goat herds or to other domestic and wild ruminants in the area. If the disease was in fact brought into the herds by way of infected Rocky Mountain goats, this becomes an added risk when considering transplanting animals into other ranges.

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