

# Case Report – Management of Paratuberculosis in a Dairy Goat Herd

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## Abstract

Adult, mixed-breed dairy goats were screened for paratuberculosis antibodies using ELISA and AGID methods licensed for bovine use. All does which had one or more parturitions were included in the serological screening tests conducted at six-month intervals over a two-year period. Test-positive animals were removed from the herd and, at the owners' discretion, were either euthanized and examined by necropsy, histopathology and culture, or sold for meat purposes. Management practices for control of paratuberculosis transmission were also implemented.

There was good agreement between ELISA and AGID test results ( $Kappa=0.625$ ). Of 41 seropositive goats submitted for necropsy, 39 (95%) were paratuberculosis-positive. Over a two-year period, herd seroprevalence declined from 9.83 to 3.59% among adult does.

Testing for *Mycobacterium avium* subspecies *paratuberculosis* and adopting management practices recommended for control of paratuberculosis in cattle appeared to reduce the seroprevalence of paratuberculosis in a goat herd.

## Résumé

Des chèvres laitières adultes de race croisée ont été soumises au dépistage d'anticorps de la paratuberculose avec les méthodes ELISA et AGID approuvées en pratique bovine. Toutes les chèvres femelles ayant eues au moins une mise-bas ont été soumises aux tests de dépistage sérologique menés à six mois d'intervalle sur une période de deux ans. Les animaux positifs ont été retirés du troupeau et, selon la volonté de l'éleveur, soit euthanasiés à des fins d'examen par la nécropsie, l'histopathologie et la culture ou soit vendus pour consommation. Des pratiques de régie pour

le contrôle de la transmission de la paratuberculose ont aussi été mises en place.

Il y avait un bon accord entre les résultats provenant des tests ELISA et AGID ( $Kappa = 0.625$ ). Parmi les 41 chèvres séropositives soumises à la nécropsie, un total de 39 (95%) testaient positives à la paratuberculose. Sur une période de deux ans, la séroprévalence au niveau du troupeau est passée de 9.83% à 3.59% parmi les chèvres adultes femelles.

L'utilisation d'un test pour le dépistage de *Mycobacterium avium* sous-espèce *paratuberculosis* et l'adoption de pratiques de régie recommandées pour le contrôle de la paratuberculose chez les bovins semblent avoir réduit la séroprévalence de la paratuberculose dans un troupeau de chèvre.

## Introduction

Paratuberculosis (Johne's disease) is a chronic, debilitating condition of cattle, sheep, goats and other ruminants. There are several strains of the etiologic agent, *Mycobacterium avium* subspecies *paratuberculosis* (MAP). All isolates from goats are IS900 (an insertion sequence defined as a repetitive stable DNA element unique to the MAP genome) positive. Using polymerase chain reaction (PCR) and DNA hybridization, most goat isolates show strain characteristics typical of cattle strains. Occasionally sheep strains or strains intermediate between cattle and sheep strains are identified in goats.<sup>9</sup>

The prevalence of paratuberculosis in goats in the United States (US) is largely unknown. Laboratory methods evaluated for diagnosis of paratuberculosis in goats include fecal culture, agar gel immunodiffusion (AGID) assay,<sup>4,6</sup> enzyme-linked immunosorbent assay (ELISA)<sup>1,4,5</sup> and histopathology.<sup>2,4</sup> Fecal culture detected 76 to 86% of clinical paratuberculosis cases in field studies in the US and the United Kingdom.<sup>3,11</sup> Fecal culture is consid-

ered to be highly specific for MAP infection. However, in a study of pygmy goats in a highly infected herd, three of 13 fecal culture-positive goats were MAP-negative when examined by histopathology or culture of tissues. The possibility of intestinal "pass-through" of MAP from a highly contaminated environment was suggested.<sup>4</sup> Antibodies were detectable by ELISA at about the same time subclinically infected goats were MAP-culture positive.<sup>10</sup> One study evaluated the use of ELISA in goats to detect MAP antibodies; apparent sensitivity was 54% and apparent specificity was 100%.<sup>1</sup> In another study where a commercial protoplasmic antigen was utilized, apparent sensitivity and specificity of ELISA were 86.2 and 95.2%, respectively.<sup>5</sup> Serologic response to MAP infection has been detected in goats as early as 15 weeks following experimental infection using the ELISA test,<sup>10</sup> although serologic response is preceded by positive culture status in most ruminants studied.

Chronic weight loss, or wasting without diarrhea, is a common clinical sign of Johne's disease in goats. Age at onset is typically younger than in cattle,<sup>9</sup> most commonly at two to three years of age.<sup>8</sup> The differential diagnosis of weight loss in goats includes caprine arthritis-encephalitis, tuberculosis, caseous lymphadenitis, endoparasitism, ectoparasitism, melioidosis, nutritional deficiencies and imbalances, and paratuberculosis.<sup>8,9</sup>

This paper compares two serologic methods to post-mortem diagnostic methods, and correlates these findings with possible risk factors for paratuberculosis. There are few reports of attempted control or eradication of paratuberculosis in goat herds.<sup>3,8,9,11</sup>

### Herd History

In December 1999, owners of a multiple-breed dairy goat herd (about 230 mature animals) requested assistance with diagnosis and control of paratuberculosis. A doe from their herd developed clinical paratuberculosis subsequent to sale to another herd, prompting their concerns.

The case herd had previously purchased animals from at least four other goat herds. Prior to 1995, home-raised kids were fed unpasteurized milk from a nearby, paratuberculosis-positive cow herd or unpasteurized milk from the goat herd. Beginning in 1995, pasteurized goat or cow milk was fed. Goat milk was pasteurized by heating milk to 163° F (78° C) for a minimum of 15 seconds. Pasteurized cow milk was purchased from retail sources. During the 12 months preceding our initial farm visit, 22 does had been removed from the herd because of rapid weight loss. Most does culled because of weight loss were home-raised. Management practices in December 1999 and before included separation of kids from the dam at birth, raising kids in group pens

that permitted fence-line contact with mature does and purchase of replacement goats from several herds. To determine the cause of weight loss, one goat was submitted for necropsy and one was tested for MAP antibodies by AGID; both cases were positive for paratuberculosis.

During our first visit to the farm in January 2000, health history was collected, a paratuberculosis risk assessment adapted from dairy cattle use<sup>6</sup> was performed, and recommendations for diagnosis and control were made to the herd owners, including serologic sampling of all does that had freshened at least once, testing of serum samples by ELISA and AGID, repeated serological testing of the herd at six month intervals, fecal culture, and/or removal of all seropositive animals from the herd. The owners were encouraged to allow euthanasia and postmortem examination of all seropositive animals. Management recommendations included immediate separation of kids from dam at birth, before nursing; strict separation and segregation of kids from adult animals; continued use of pasteurized colostrum, followed by use of milk replacer; prevention of access of any goats to manure storage or manure runoff areas; and maintenance of a closed herd. The owners maintained individual animal identification and health records and made a diligent effort to implement the paratuberculosis control recommendations; however, seropositive animals were not confirmed by antemortem fecal culture. Fifty-four percent of seropositive goats were presented for euthanasia and necropsy.

All data were statistically analyzed to assess agreement between tests and odds ratio for test-positive status using EpiInfo<sup>a</sup> and SAS.<sup>b</sup>

### Diagnostic Methods

Laboratory submissions were analyzed using the following methods:

**ELISA.** Serum was evaluated for antibodies to MAP using an ELISA kit approved for use on cattle sera.<sup>c</sup> Testing was done following manufacturer's instructions, and using manufacturer-supplied bovine positive and negative controls. Sera from a known MAP positive and a known negative goat were run to assure consistency. Each individual serum was evaluated in a single well.

**AGID.** Serum was evaluated for antibodies to MAP using a commercially available AGID kit<sup>d</sup> approved for use in cattle. Testing was done following manufacturer's instructions. Both bovine and caprine positive and negative controls were used.

**Histology.** Animals were humanely euthanized and fresh tissues (ileocecal, mesenteric and mediastinal

lymph nodes, ileum, colon, liver and kidney samples were collected from all animals; additional tissues were collected from some animals, based on gross necropsy findings) were collected and fixed in 10% neutral-buffered formalin. Tissues were routinely processed, embedded in paraffin and stained with hematoxylin and eosin using standard histology techniques. Tissues were evaluated for histologic evidence of Johne's disease by a pathologist (DW). Histologic criteria for a positive diagnosis were based on previously reported classification of lesions in goats naturally infected with MAP.<sup>2</sup> Inflammation of the intestine resulting in lymphocytic or granulomatous enteritis was required for a positive histologic diagnosis. Acid-fast staining or immunohistochemistry (IHC) was used to demonstrate mycobacteria in cases with negative or inconclusive culture results.

**Culture.** At necropsy, fecal samples were collected and cultured in Herrold's egg yolk media using a standard protocol.<sup>12</sup> A 16-week incubation period was allowed before declaring any sample negative. An animal was considered infected if fecal culture was positive and/or histopathology characteristic of paratuberculosis was accompanied by positive acid-fast staining or IHC.

## Results

Sera from 368 goats, including all 234 adult goats in the herd at first sampling and additional goats that freshened or reached one year of age during the course of the control program, were tested for MAP antibodies. Goats were sampled every six months from January 2000 until January 2002, as long as they remained in the herd. All samples were tested by ELISA. Most samples were also tested with the AGID test. A total of 847 serum samples were tested by both ELISA and AGID tests. Using an S/P ratio of 0.25 or greater as the basis for determination of an ELISA result as positive, there was a high degree of agreement between ELISA and AGID results (Kappa = 0.625; Table 1). To minimize laboratory costs, serum samples taken after the first three herd samplings were tested only with ELISA, and

**Table 1.** ELISA and AGID agreement.

	ELISA positive	ELISA negative	Total
AGID positive	66	14	80
AGID negative	52	715	767
<b>Total</b>	<b>118</b>	<b>729</b>	<b>847</b>

Kappa=0.625 (the proportion of potential agreement beyond change)

sera from ELISA-positive goats were also tested by AGID. Thus, 1009 sera were tested by ELISA and 847 (84%) of these were also tested by AGID. Of 368 goats tested by ELISA and AGID, 69 (18.8%) were positive at one or more samplings by either or both tests. Any goat seropositive on either an ELISA or AGID test was considered presumptive-positive for paratuberculosis for purposes of the herd control effort.

All tested individuals within the herd were evaluated for factors that might increase their risk of testing positive relative to overall test-positive prevalence in the herd. Putative factors of increased risk were purchased animal source (source herds), consumption of unpasteurized colostrum, or having a dam that was test-positive. There were sufficient numbers of tested animals from two of the source herds, herds A and B, to allow determination of an odds-ratio for each of those herds when compared to other tested animals in the case herd. Animals purchased from herd B were at increased risk of being test-positive (odds ratio 6.18:1; Table 2). Daughters of test-positive dams were at higher risk of testing positive than daughters of test-negative dams (odds-ratio 2.90:1; Table 3).

Goats positive on a serologic test were removed from the herd, and 54% of these were submitted to the Pennsylvania Animal Disease Laboratory System, at Penn State University, for euthanasia and necropsy. Owners immediately removed test-positive non-lactating does, removed lactating does at the end of their lactation and elected to sell some seropositive goats for slaughter. During the early stages of the control program, the owners submitted all seropositive goats for necropsy. Because most of these were confirmed MAP-positive, the owners elected to sell seropositive goats found later in the control/eradication program for slaughter to capture some economic value. Goats submitted for necropsy were not different serologically from those sold for slaughter (necropsied goats: 83% ELISA positive, 58.3% AGID positive; slaughtered goats: 88.6% ELISA positive, 51.4% AGID positive). Of 76 goats positive on one or more serologic tests, 41 were submitted for necropsy, and 39 were confirmed positive for paratuberculosis by culture and/or histopathology (Table 4). Two goats were seropositive but not confirmed positive at necropsy. These animals had low-positive ELISA tests (0.322 and 0.376 OD), were negative on AGID and were younger (mean of 2.4 years vs. 4.6 years) than the 39 confirmed paratuberculosis cases. Use of positive results from both tests, AGID and ELISA, resulted in identifying more infected animals than using either test alone. Of 30 culture-positive goats, 15 were positive on both AGID and ELISA, nine by ELISA alone and six by AGID alone. Of 39 goats positive by histopathology, 17 were positive on both tests, while 15 were positive on ELISA alone and seven positive on AGID alone.

**Table 2.** Risk of paratuberculosis test-positive status, related to herd of origin (positive status = test-positive at any testing by ELISA and/or AGID).

		Serologic test status		Total	% pos.
		Pos	Neg		
Purchased from Herd A	Yes	14	64	78	17.9
	No	62	228	290	21.4
	Total	76	292	368	
OR=0.80 (95% CI=0.40 – 1.59)					
Purchased from Herd B	Yes	17	13	30	56.7
	No	59	279	338	17.5
	Total	76	292	368	
OR=6.18 (95% CI=2.68 – 14.38)					

**Table 3.** Paratuberculosis status of tested dam-daughter pairs (positive status = test-positive at any testing by ELISA and/or AGID).

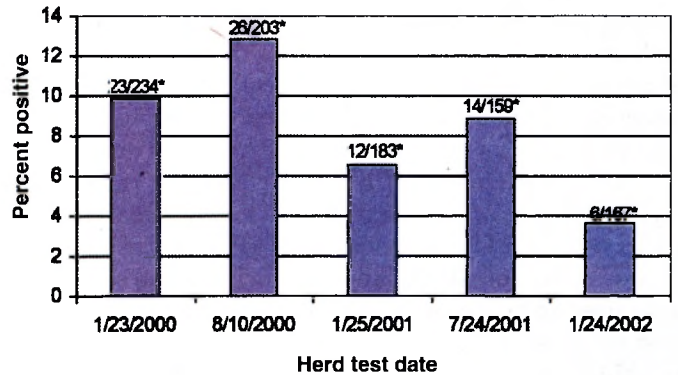
	Daughter pos	Daughter neg	Total	% pos.
Dam positive	13	29	42	31.0
Dam negative	21	136	157	13.4
Total	34	165	199	

OR=2.90 (95%CI= 1.21-6.93)

**Table 4.** Correlation between culture and histopathology results from seropositive does at necropsy.

	Culture positive	Culture negative	No culture	Total
Histopathology positive	30	4	5	39
Histopathology negative	0	0	2	2
Total	30	4	7	41

Number and percent of goats seropositive at each herd test declined over time during the testing period (Figure 1).



**Figure 1.** ELISA positive goats at whole herd test. \*Number positive / Number of goats tested

### Discussion

There was good agreement between the ELISA and AGID test results. An earlier report<sup>1</sup> of ELISA testing of goats utilized a test kit marketed by the same company, but reagents used in the earlier study were different than in the currently-marketed kit. Most seropositive goats in this herd that were necropsied were confirmed to be MAP-positive by both fecal culture and histopathology. Forty-six percent of test-positive goats were not presented for necropsy, but were sold for slaughter; thus, correlation of seropositive tests with confirmatory tests for this group is not known.

Diarrhea and loose feces were not reported in this herd. The herd owners observed a number of thin, poor-producing does prior to January 2000, but only a few animals with weight loss were noted after January 2000. Most ELISA and/or AGID test-positive goats had good body condition, and at necropsy abundant fat stores were seen in most of the goats. Perhaps access to diagnostic test results and heightened awareness of paratuberculosis by the owners prompted early removal of affected goats before weight loss, characteristic of late stages of clinical disease, occurred.

In this herd, risk factors for paratuberculosis included origin from herd B and being a daughter of a test-positive dam. Consumption of unpasteurized milk or colostrum was not a risk factor, but feeding of unpasteurized milk or colostrum was discontinued in 1995, five years before other management practices to control paratuberculosis were implemented.

Starting in January 2000, the herd owner implemented management practices that included separation of young kids from adults before nursing, feeding colostrum only from test-negative does, prevention of fecal contamination of the youngstock environment, separation of all goats from access to manure storage and manure-run-off areas, as well as test and cull measures. During a two-year period, antibody prevalence within the herd declined from 9.83 to 3.59%. As true prevalence declines in this herd, the value of fecal cultures for early detection will increase and the efficacy of serologic findings as a basis for removal from the herd will decrease.

Diagnostic methods commonly used in cattle, including ELISA, AGID, fecal culture and histopathology, all proved useful for diagnosis of MAP in this goat herd. To assess the sensitivity and specificity of the serologic tests used in this case study, the tests should be evaluated using sera from well-characterized MAP-positive and MAP-negative animals, including sera from animals exposed to antigens that might cross-react. Unfortunately, these studies were not possible in this commercial goat herd.

Caseous lymphadenitis (CLA) has been reported as a source of cross-reactions and possible false-positives when using the AGID test. The CLA status of the case herd was not determined, however, the owner had not observed swollen or abscessed lymph nodes. While the present case study demonstrates the use of currently available diagnostic tests in a field environment, controlled studies to assess test performance in goats in different geographic locations with different levels of infection are needed.

### Conclusions

Management practices based on accepted paratuberculosis control principles for cattle, coupled with test

and removal of positive animals, appears to have reduced the seroprevalence of MAP in this herd. The outcome in this herd suggests that ELISA testing may be comparable to AGID testing for herd screening, and that weight loss or loss of body condition may not be a sensitive indicator of paratuberculosis in a dairy goat herd on a high plane of nutrition. The use of fecal culturing on a herd-wide basis might have allowed detection of disease at an earlier stage of infection and may have affected the herd infection rate. It remains to be determined whether the diagnostic and control measures will prove adequate to ultimately eliminate MAP infection from this herd.

### Footnotes

<sup>a</sup>Epi Info™ v.6.1, Epidemiology Program Office, Centers for Disease Control, Atlanta, GA.

<sup>b</sup>SAS® v8.02, SAS Institute Inc., Cary, NC.

<sup>c</sup>HerdChek® *Mycobacterium paratuberculosis* Test Kits, IDEXX, Westbrook, ME.

<sup>d</sup>rjt™ Rapid Johne's Test, *Mycobacterium paratuberculosis* Antibody Test Kit, Immucell, Portland, ME.

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