

# Synergistic Hemolysis-Inhibition Titers Associated with Caseous Lymphadenitis in a Slaughterhouse Survey of Goats and Sheep in Northeastern Brazil

Corrie C. Brown, Harvey J. Olander and Selmo F. Alves\*

## ABSTRACT

A survey of caseous lymphadenitis was conducted at a goat and sheep slaughterhouse in Northeastern Brazil. One hundred and fifty-eight goats and 43 sheep were examined for the presence of abscesses, with bacterial culturing of purulent material to define the etiological agent. Blood was collected simultaneously for determination of serological titer via the synergistic hemolysis-inhibition test which measures antibodies to an exotoxin of *Corynebacterium pseudotuberculosis*. Thirteen and nine-tenths percent of the goats had abscesses, with a high proportion having mediastinal or pulmonary lesions (9.5%). Two sheep had abscesses, both with internal organ involvement. *Corynebacterium pseudotuberculosis* was the most frequently isolated organism. Of 22 goats with abscesses, 20 were positive via the synergistic hemolysis-inhibition test. Both of the sheep with abscesses had positive synergistic hemolysis-inhibition titers. The proportion of serological reactors was greater than the proportion of animals with abscesses. The synergistic hemolysis-inhibition test may be detecting subclinically infected animals.

**Key words:** *Corynebacterium pseudotuberculosis*, caseous lymphadenitis, serology.

## RÉSUMÉ

Cette étude consistait à effectuer un relevé de la lymphadénite caséuse, dans un abattoir de moutons et de chèvres, au nord-est du Brésil. On y rechercha la présence d'abcès, chez 158 chèvres et 43 moutons et on en soumit le pus à un examen bactériologique, afin d'en préciser l'agent étiologique. On préleva aussi du sang, en vue de la détermination de la teneur sérique en anticorps, par l'épreuve synergique de l'inhibition de l'hémolyse qui permet de mesurer le titre d'anticorps à l'endroit d'un exotoxine de *Corynebacterium pseudotuberculosis*.

On décéla des abcès, chez 13,9 % des chèvres, et des lésions pleurales ou pulmonaires, chez 9,5 % d'entre elles. Deux des moutons arboraient des abcès, sur des organes internes. *Corynebacterium pseudotuberculosis* correspondait à la bactérie isolée le plus souvent des abcès précités. Vingt des 22 chèvres qui présentaient des abcès réagirent de façon positive à l'épreuve synergique de l'inhibition de l'hémolyse, tout comme les deux moutons. La proportion des réacteurs sérologiques se révéla plus élevée que celle des animaux qui arboraient des abcès; il se pourrait par conséquent que l'épreuve synergique de l'inhibition de l'hémolyse détecte des animaux atteints d'une infection subclinique.

**Mots clés:** *Corynebacterium pseudotuberculosis*, lymphadénite caséuse, sérologie, chèvres, moutons.

## INTRODUCTION

Caseous lymphadenitis (CLA), caused by *Corynebacterium pseudotuberculosis*, is a disease of worldwide importance in sheep and goats (1-3). It is characterized by abscessation of one or more lymph nodes, most commonly the large superficial nodes. The disease is insidious and there is increasing recognition of the involvement of internal nodes in the so-called visceral form of the disease.

Economic losses due to caseous lymphadenitis are most severe in the disseminated visceral form which can result in loss of the animal's value by wasting, death or carcass condemnation due to the extensive internal abscesses (4). Visceral CLA has been implicated as one of the major causes of ill-thrift, the "thin ewe syndrome", in the United States (5). Other occult losses can occur from a decrease in reproductive efficiency when internal abscesses are present (6). Economic effects of the superficial form of CLA are less well-documented. A decrease in milk production has been reported in a dairy goat herd with a high incidence of mammary *C. pseudotuberculosis* abscesses (1), and hide values can be drastically lowered by flaws due to superficial abscesses (7). Finally, concern about the spread of CLA can lower the market value of an animal with a superficial or skin abscess.

\*Department of Veterinary Pathology, University of California at Davis, Davis, California 95616. Present address of Dr. Brown: Department of Veterinary Pathology, School of Veterinary Medicine, Louisiana State University, Baton Rouge, Louisiana 70808.

Supported by Title XII Small Ruminant Collaborative Research Support Program (United States Agency for International Development).

Submitted November 29, 1985.

Control of CLA has been an area of major concern. Introduction of an infected animal into a previously uninfected herd can result in a surge of abscesses two to three years later (8,9). Once endemic, it is almost impossible to eradicate. Antibiotics are unable to penetrate the thick capsule of the abscess (8), organisms can survive and persist in soil and on fomites (10,11) and there are, as of yet, no commercially available vaccines in this country.

Diagnosis of CLA is straightforward when a mature superficial abscess is present. The abscess material is characteristically light green and slightly dry. The etiological agent, *C. pseudotuberculosis*, is readily cultivated on blood agar under normal aerobic conditions. However, prior to superficial abscess formation and in all cases of abscesses in internal nodes, the infection is not obvious on clinical examination. A reliable diagnostic assay is needed to detect animals with internal abscesses or in the sub-clinical phase of infection. Such a test would be of great benefit in protecting clean herds against introduction of infection and would allow for earlier diagnosis and earlier and more economic culling of those animals with visceral lesions.

The synergistic hemolysis-inhibition (SHI) test is a serological assay which measures antibodies to the exotoxin of *C. pseudotuberculosis* (12). Originally designed for use in horses, it has been applied to goats with caseous lymphadenitis in an experimental setting with promising results (13).

This study was undertaken to examine the reliability of the SHI test for detecting the presence of abscesses, both external and internal, in field cases of CLA in sheep and goats. In the process, a rough estimate was obtained of the prevalence of both serological reactivity and *C. pseudotuberculosis* abscesses in slaughterhouse specimens in one of the world's most concentrated goat and sheep producing areas.

## MATERIALS AND METHODS

A survey of 200 adult animals (158 goats, 43 sheep) was undertaken at the municipal slaughterhouse of Petrolina in Northeastern Brazil, in the center of one of the heaviest small ruminant

producing areas in South America (14). The survey was conducted during the month of August 1984. Of the 158 goats, there were 42 does and 116 bucks and castrated males. A wide range of ages was represented, with a mean of 2.1 years. All animals were of mixed breeding. The 43 sheep included 15 ewes and 28 rams and wethers. Ages ranged from one to six years, with the mean being 2.2 years. Most were mixed breed hair sheep.

All animals were inspected briefly antemortem for the presence of any enlarged superficial lymph nodes or obvious signs of disease.

Blood for serology was collected at the time of exsanguination. Superficial nodes were palpated prior to skinning and examined again when the hide was removed. Viscera were inspected, with special attention to spleen and the mesenteric, tracheobronchial, and mediastinal lymph nodes. Lumbar nodes were checked *in situ* within the carcass. Any enlarged nodes were cut open. With the exception of superficial abscesses that were already open to the skin surface, purulent material was collected from all abscesses for bacteriological culturing.

Samples for bacteriology were plated directly onto beef blood agar unless the collection interval was greater than 48 hours in which case the swabs were incubated in brain heart infusion broth (Difco Laboratories, Detroit, Michigan) for two hours prior to plating. Plates were incubated aerobically at 37°C and examined visually at 24, 48, and 72 hours. Identification was based on cultural and colonial characteristics, Gram stain reaction, catalase reaction, and presence of synergistic hemolysis with *Corynebacterium equi*. All *C. pseudotuberculosis* isolates were tested for ability to reduce nitrate.

Serum samples were processed via the SHI test (12). Briefly, this test detects antibodies to an exotoxin of *C. pseudotuberculosis* by inhibition of a synergistic hemolysis between the toxins of *C. pseudotuberculosis* and *C. equi*. Dilutions of serum incubated with a standardized aliquot of *C. pseudotuberculosis* crude toxin are adsorbed onto filter paper discs and arranged on a blood agar plate already containing the crude toxin of *C. equi*. After 24 hours at 37°C, a zone of

hemolysis surrounding a disc indicates the absence of antibodies at that dilution. A titer of 1:4 or greater was interpreted as positive on the basis of previous experimental work in which ten goats inoculated with *C. pseudotuberculosis* developed abscesses and titers of 1:4 or greater and five uninoculated controls remained disease-free with titers of 0 or 1:2 (13).

## RESULTS

Of the 158 goats, a total of 25 abscesses were found in 22 animals (13.9%). Sixteen goats had internal lesions only, five goats had external lesions only, and one animal had both external and internal abscesses. Body distribution and frequency of the 25 abscesses is summarized (Table I). The most frequently affected site was the mediastinal lymph node, with 13 animals (8.2%) having an abscess at this location. In two of these animals, the mediastinal abscess had extended to involve abscessation of the pulmonary parenchyma. Two other animals had abscesses in the tracheobronchial node, making a total of 15 goats (9.5%) with thoracic lesions. In all, 17 animals (10.7%) had internal abscesses.

TABLE I. Body Location and Frequency of 25 Abscesses Discovered at Slaughter in 22 Goats

Internal abscesses	
Mediastinal lymph node	13
Tracheobronchial lymph node	2
Lung	2
Spleen	1
Lumbar lymph node	1
Superficial abscesses	
Parotid lymph node	2
Mandibular lymph node	1
Prescapular lymph node	1
Prefemoral lymph node	1
Subcutaneous tissue	1

In the sheep, abscesses were found in only two animals. One animal had a single tracheobronchial node abscess. The other displayed multiple lesions including an extensive subcutaneous pectoral abscess and series of abscesses in the mediastinal nodes, tracheobronchial nodes and liver.

Of 21 abscesses cultured, three were from the sheep, and *C. pseudotuberculosis* was isolated from each. Of the 18 swabs from the goat abscesses, *C. pseudotuberculosis* was isolated from four in pure culture and from another three in combination with either

*Staphylococcus* sp. or Gram-negative rods. One abscess yielded only *Moraxella bovis*. Gram-negative rods were cultured from all of the remaining ten abscesses. Isolates included *Escherichia coli*, *Enterobacter* sp., *Pseudomonas* sp., and others which were not identified. All *C. pseudotuberculosis* strains were negative for nitrate reduction activity.

Serological results are presented (Tables II and III). Of the 22 goats with abscesses, 20 (90.9%) had positive titers. Eighty-three of 136 (61%) goats without abscesses were seronegative. Both sheep with *C. pseudotuberculosis* abscesses had positive titers and 37 of 41 (90.2%) sheep without abscesses were seronegative.

## DISCUSSION

A striking finding in this study was the high prevalence of abscesses (13.9%) in apparently healthy goats. The majority of these abscesses were internal. Of 22 abscessed goats, 17 (77.2%) had internal lesions, and in all but one case, these were in the absence of external abscesses. Intrathoracic locations predominated, with mediastinal node responsible for 52% and tracheobronchial nodes and pulmonary parenchyma constituting 16% of all abscesses seen in goats. This preponderance of internal, primarily intrathoracic, lesions in the absence of external abscesses raises questions about the means of transmission. Although methods of transmission of CLA have been the subject of some speculation, there is widespread support for abraded skin or oral mucous membranes as most probable portals of entry (15,16). Thoracic lesions have been produced by intradermal or subcutaneous inoculation of cultures and so hematogenous origin of abscesses in pulmonary nodes or parenchyma has been proposed (17). Alternatively, it is possible that organisms reach the pulmonary parenchyma directly through inhalation. Although intranasal inoculation of broth culture was not successful in establishing infection experimentally (13), it may be that this area of Brazil which is hot, dry and dusty throughout the year provides optimal conditions for generating a bacteria-laden dust aerosol which could be carried past the defenses of the upper respiratory tract and into the lung.

TABLE II. Serological Response and Presence of Abscesses in 158 Goats

	Animals with Abscesses (22)	Animals without Abscesses (136)
Animals with positive SHI titer (73)	20	53
Animals with negative SHI titer (85)	2	83

TABLE III. Serological Response and Presence of Abscesses in 43 Goats

	Animals with Abscesses (2)	Animals without Abscesses (41)
Animals with positive SHI titer (6)	2	4
Animals with negative SHI titer (37)	0	37

The recovery rate of *C. pseudotuberculosis* from goat abscesses was disappointingly low. In all cases, the abscess material had the light green slightly dry appearance which is characteristic of the organism. Although samples were handled in as sterile a manner as possible many of the primary plates were overgrown with contaminants, making it very difficult to pick out the desired colony. This contamination is believed responsible for the inability to isolate *C. pseudotuberculosis* from many of the abscesses and might have been reduced by the use of selective media. Another reason may be sampling from a sterile portion of the abscess, since histologically caseous lymphadenitis abscesses consist of a large amorphous mass of necrotic material within which relatively few organisms are distributed in clusters.

Previous work in an experimental setting had shown the SHI test to have 100% sensitivity and specificity (13). In the present study, SHI titers were examined in field cases of the disease. Of the 22 goats with abscesses, 20 were serologically positive, making the sensitivity of the SHI test under field conditions 90.9%. However, of the 136 animals without abscesses, 83 were seronegative, for a specificity of 61%. The percentage of serological reactors was much greater than the percentage

of animals with abscesses. Consequently, the predictive value of the test, i.e. the probability that an animal with a positive titer will actually have an abscess, is 27.4% (20/73). However, since the prevalence of abscesses is quite high (13.9%), it may be that many animals become infected but only relatively few show abscesses at any given time. This is in agreement with another study in which a group of 26 abscessed goats was followed for 27 weeks (18). Antibody levels remained high even after complete healing of the superficial lesion. Caseous lymphadenitis is noted for its chronic, recurring nature and it may be that animals once infected continue to harbor the organism and so retain seropositivity.

The SHI test was positive for two of two (100%) sheep with *C. pseudotuberculosis* abscesses and negative in 37 of 41 (90%) animals without abscesses. As with the goats, there were more serological reactors than there were animals with abscesses. The predictive value of the SHI test for sheep was only 33% (2/6), similar to the value found for goats. However, the number of sheep involved in this study, in particular, the low number of animals with abscesses, is too small to make any definitive statements concerning the value of the SHI test in this species.

## REFERENCES

1. **BURRELL DH.** Caseous lymphadenitis in goats. *Aust Vet J* 1980; 57:105-110.
  2. **MADDY KT.** Caseous lymphadenitis in sheep. *J Am Vet Med Assoc* 1953; 122:257-259.
  3. **NAIRN ME, ROBERTSON JP, McQUADE NC.** The control of caseous lymphadenitis in sheep by vaccination. *Proc Ann Conf Aust Vet Assoc* 1977; 54:159-161.
  4. **STOOPS SG, RENSCHAW HW, THILSTED JP.** Ovine caseous lymphadenitis: Disease prevalence, lesion distribution, and thoracic manifestations in a population of mature culled sheep from western United States. *Am J Vet Res* 1984; 45:557-561.
  5. **RENSHAW HW, GRAFF VP, GATES NL.** Visceral caseous lymphadenitis in thin ewe syndrome: Isolation of *Corynebacterium*, *Staphylococcus* and *Moraxella* spp. from internal abscesses in emaciated ewes. *Am J Vet Res* 1979; 40:1110-1114.
  6. **GATES NL, EVERSON DO, HULET CV.** Effects of thin ewe syndrome on reproductive efficiency. *J Am Vet Med Assoc* 1977; 171:1266-1267.
  7. **FIGUEIREDO EAP, SHELTON M, PANT KP.** Goat skins. *Proc Int Conf Goat Prod Dis* 1982; 3:488-490.
  8. **ASHFAG MK, CAMPBELL SG.** A survey of caseous lymphadenitis and its etiology in goats in the United States. *Vet Med Small Anim Clin* 1979; 74:1161-1165.
  9. **AYERS JL.** Caseous lymphadenitis in goats and sheep: A review of diagnosis, pathogenesis, and immunity. *J Am Vet Med Assoc* 1977; 171:1251-1254.
  10. **AUGUSTINE JL, RENSCHAW HW.** *Corynebacterium pseudotuberculosis* survival in soil samples amended with water. *Proc Int Conf Goat Prod Dis* 1982; 3:526.
  11. **AUGUSTINE JL, RENSCHAW HW.** Survival of *Corynebacterium pseudotuberculosis* on common backyard fomites. *Proc Int Conf Goat Prod Dis* 1982; 3:525.
  12. **KNIGHT HD.** A serologic method for the detection of *Corynebacterium pseudotuberculosis* infections in horses. *Cornell Vet* 1978; 68:220-237.
  13. **BROWN CC, OLANDER HJ, BIBERSTEIN EL, MORENO D.** Serologic response and lesions in goats experimentally infected with *Corynebacterium pseudotuberculosis* of caprine and equine origins. *Am J Vet Res* 1985; 46:2322-2326.
  14. **GUTIERREZ NF, DeBOER J.** Marketing and price formation for meat goats, hair sheep and their products in Ceara state, Northeast Brazil. *Proc Int Conf Goat Prod Dis* 1982; 3:50-54.
  15. **NAGY G.** Caseous lymphadenitis in sheep — methods of infection. *J S Afr Vet Assoc* 1976; 47:197-199.
  16. **ASHFAG MK, CAMPBELL SG.** Experimentally induced caseous lymphadenitis in goats. *Am J Vet Res* 1980; 41:1789-1792.
  17. **NAIRN ME, ROBERTSON JP.** *Corynebacterium pseudotuberculosis* infection of sheep: role of skin lesions and dipping fluids. *Aust Vet J* 1974; 50: 537-542.
  18. **BROWN CC, OLANDER HJ, ZOMETA C, ALVES SF.** Serodiagnosis of inapparent caseous lymphadenitis in goats and sheep, using the synergistic hemolysis-inhibition test. *Am J Vet Res* 1986; 47:1461-1463.
-