Serodiagnosis of inapparent caseous lymphadenitis in goats and sheep, using the synergistic hemolysis-inhibition test

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SUMMARY

The synergistic hemolysis-inhibition (SHI) test, a serologic test for the detection of infection with Corynebacterium pseudotuberculosis, was applied to serum samples from 196 goats and 76 sheep, including animals both with and without C pseudotuberculosis abscesses. Fifty-one of 52 (98%) goats and 27 of 28 (96%) sheep with abscesses caused by C pseudotuberculosis had seropositive titers. Seropositivity continued on subsequent samplings, even after superficial lesions were completely healed. The SHI test may detect subclinically infected animals, as well as animals with clinically recognizable lesions. Of the animals without abscesses, 53 of 186 (28%) goats and 4 of 41 (10%) sheep were seropositive. Either the SHI test is lacking in specificity or these titers are a reflection of a past or a current infection without any grossly visible abscesses.

Caseous lymphadenitis, an important disease of goats and sheep, is characterized by obliteration of 1 or more lymph nodes by the formation of abscesses. Caused by Corynebacterium pseudotuberculosis, it is a chronic and recurring problem, with new abscesses appearing subsequent to rupture or surgical excision of others. Usually the superficial nodes are affected, but in a more serious visceral form of the disease, internal nodes are involved.

The disease may result in economic losses in a variety of ways. These include loss of the entire animal due to wasting or carcass condemnation when disseminated internal abscesses are present, a decrease in reproductive efficiency, production losses when abscessed nodes interfere with necessary functions such as milking, labor costs and drugs in treatment of abscesses, and devaluation in hides because of flaws.

Control of caseous lymphadenitis has been challenging. The organisms are liberated in massive numbers from ruptured abscesses and can survive and persist well in soil and on fomites, so attempts at environmental sanitation to decrease the frequency have been only marginally successful. Antibiotics are not capable of penetrating the thick wall of the abscess and consequently are of limited usefulness after an abscess has formed. Once the disease is established in a herd, it is difficult to eradicate. Various vaccine preparations, including a bacterin in South Africa and a formalized toxin in Australia, have been tested and are available for use in sheep in the field, but they are not available in the United States.

Diagnosis is straightforward when external abscesses are present, palpable, and accessible for bacterial culturing. But all too often, infection is occult and not detectable by clinical examination (e.g., during the long incubation period between bouts of abscess formation or when only internal abscesses are present). A dependable means of identifying these usually subclinically infected individuals could help to decrease the frequency by allowing earlier and more economic culling and preventing introduction of affected animals into clean herds.

Several serologic assays have been devised for the detection of animals with caseous lymphadenitis, including an anti-β-hemolysin-inhibition test, a double immunodiffusion test, enzyme-linked immunosorbent assays, using various bacterial components as antigens, and the synergistic hemolysis-inhibition test (SHI).

The purposes in the present study were to apply 1 of these serologic tests, the SHI test, to both clinically affected and nonaffected animals in a natural setting and to determine its value as an indicator of subclinical disease.

Materials and Methods

Selection of animals, observation, and sample collection—Experimental animals were selected from breeding herds at 2 state research stations in Northeastern Brazil.

At the Ceara state research station (EPACE), 45 adult animals were chosen on the basis of enlargement of 1 or more superficial lymph nodes. They included 26 goats (23 does and 3 bucks) of mixed breeding and 19 Morada Nova hair sheep (all ewes). All goats were 3 years old or older; the sheep were slightly younger, with an average age of 2.5 years. Clinical examinations and blood collections were made on each animal every 3 weeks for 27 weeks, and individual records were kept. As superficial abscesses developed and reached maturity, they were surgically drained and sampled for bacterial culture.
At the Paraiba state research station (EMEPA), 50 animals were chosen because they had mature superficial abscesses ready for excision. This group was composed of 34 goats (31 does and 3 bucks) of various breeds and 16 sheep (15 ewes, 1 ram) all of mixed breeding. The ages ranged from 1 to 5 years of age, with an average of 2.8 years. With these animals, surgical excision and bacteriologic sampling of the abscesses were done on day 0, and blood samples were obtained on days 0, 1, 7, 14, 21, and 28.

For controls, abscess-free animals were chosen from a group sent to slaughter at the municipal slaughterhouse of Petrolina, located in the state of Paraiba. Superficial nodules were palpated before animals were slaughtered; blood was obtained at the time of exsanguination, and both superficial and internal nodules were examined in the carcass. One hundred thirty-six goats and 41 sheep had no signs of either superficial or internal abscesses. All animals were of mixed breeding, and ages ranged from 1 to 6 years, with a mean of 2.1 years.

Laboratory processing of samples—All serum samples were used in the SHI test. This test detects antibodies to an exotoxin of C. pseudotuberculosis by inhibition of a synergic hemolysis between the toxins of C. pseudotuberculosis and C. equi. Dilutions of serum incubated with a standardized aliquot of C. pseudotuberculosis adjusted to a specific hemolytic activity were adsorbed onto filter paper disks and arranged on a blood agar plate already containing the toxin of C. equi. After 24 hours' incubation (37 °C), a zone of hemolysis surrounding a disk indicated that the antibodies were absent at that dilution. A titer of 1:4 or greater was considered positive on the basis of previous work in a controlled experimental setting.

Swabs of abscess material were streaked directly onto bovine blood agar plates and incubated aerobically. If the interval after collection were greater than 48 hours, swabs were incubated in 1 ml of brain-heart infusion broth for 2 hours before they were streaked onto plates to enhance the probability of producing positive cultures. Identification of isolates was based on standard methods.

Results

Results are presented (Table 1).

In the long-term study at EPACE, all 26 goats developed mature abscesses at some time during the 27 weeks, with C. pseudotuberculosis being isolated from 22 animals. Of the remainder, 3 had mature abscesses which were opened by the herdsman and not available for bacteriologic cultural examination. One doe had an abscess which yielded Staphylococcus sp and gram-positive aerobic rods, but no C. pseudotuberculosis. All EPACE goats had seropositive SHI titers at every sampling.

In the 1-month EMEPA study, C. pseudotuberculosis was cultured from 30 of 34 goats. Abscesses in the remaining 4 animals yielded C. pyogenes, Staphylococcus sp, and gram-negative aerobic rods. Twenty-nine of the 30 C. pseudotuberculosis culture-positive goats were seropositive on all samplings. Of the 4 goats from which organisms other than C. pseudotuberculosis were cultured, all were seropositive.

All 19 ewes in the long-term EPACE study developed mature abscesses. Corynebacterium pseudotuberculosis was isolated from 14 animals. Four abscesses, having been opened by the herdsman, were unavailable for cultural examination, and 1 ewe had 3 external abscesses which yielded Staphylococcus sp, Streptococcus sp, and C. pyogenes. Thirteen of the 14 C. pseudotuberculosis culture-positive animals were consistently seropositive. The other was seronegative on 7 of 9 samplings and only weakly seropositive (1:4) on the other 2. The remaining 5 animals were consistently seropositive.

In the 1-month EMEPA study, C. pseudotuberculosis was cultured from 14 of 16 sheep. Abscesses in the other 2 animals yielded a Staphylococcus sp and unidentified gram-positive aerobic rod. All 14 C. pseudotuberculosis culture-positive animals were seropositive on all samplings, as was the animal infected with gram-positive rods. The sheep from which the Staphylococcus sp was recovered was consistently seronegative.

Of the nonabscessed control animals, 53 of 136 goats and 4 of 41 sheep were seropositive, with the remainder being seronegative.

Discussion

Combining results from both short- and long-term studies, 51 of 52 (98%) goats with abscesses from which C. pseudotuberculosis was isolated had positive SHI titers. Titers remained positive, even after the lesions were completely healed. The remaining 8 goats with abscesses, which either were not culturally examined or were associated with organisms other than C. pseudotuberculosis, were all SHI-test positive, as were 53 of 136 (38%) of the nonabscessed control goats.

Similar data were obtained for the combined groups of sheep. Twenty-seven of 28 (96%) with abscesses from which C. pseudotuberculosis was isolated had positive titers via the SHI test. Titers remained positive even after all external evidence of abscesses had disappeared. Of the other 7 sheep with abscesses that either were not cultured or yielded organisms other than C. pseudotuberculosis, 6 were seropositive, as were 4 of 41 (10%) control sheep without abscesses.

In this limited survey, SHI titers remained positive, even after complete resolution of superficial, palpable C. pseudotuberculosis lesions was observed. Consequently, the SHI test may be useful for detecting subclinically affected animals (i.e., infected carriers).

The high percentage of seropositive animals among animals without abscesses and animals with abscesses which yielded organisms other than C. pseudotuberculosis indicates that the SHI test lacks specificity. However, under the conditions of the present experiment, it is possible that the positive serologic tests in these animals were a response to a past infection or to a current infection without a grossly visible lesion.

References

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