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CORYNEBACTERIUM PSEUDOTUBERCULOSIS INFECTION IN GOATS I.

EVALUATION OF TWO SEROLOGICAL DIAGNOSTIC TESTS

By

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HOLSTAD, G.: *Corynebacterium pseudotuberculosis infection in goats I. Evaluation of two serological diagnostic tests.* Acta vet. scand. 1986, 27, 575—583. — The bacterial agglutination test (BAT) and the hemolysis inhibition test (HIT) were employed and evaluated.

The threshold value between positive and negative antibody titres in BAT and HIT was determined on the basis of titration results for sera from 25 adult goats infected with Corynebacterium pseudotuberculosis (positive), and sera from 25 adult goats from a herd in which caseous lymphadenitis did not occur (negative).

Antibody titres were expressed as $\log_{10}$ to the resiprocal value of the highest positive serum dilution in both tests. Positive titre (T) in BAT was stipulated as $T \geq 2.1$ and in HIT as $T > 0.6$. The sensitivity and specificity was 0.96 in both tests in the material used. Twenty-three of the 25 goats infected with C. pseudotuberculosis were positive in both BAT and HIT.

The reproducibility in both tests was described by the use of correlation coefficient and coefficient of variation. The values were estimated using duplicate determination of titres of 155 serum samples. Correlation coefficients were 0.87 for BAT and 0.95 for HIT. Coefficients of variation were 26.4 % for BAT and 14.1 % for HIT. The coefficient of variation was related to titres. It was highest for BAT at low titres.

It was concluded that both tests can be used for seroepidemiological investigations of C. pseudotuberculosis infection in goats.

sensitivity; specificity; reproducibility; bacterial agglutination test; hemolysis inhibition test.

Caseous lymphadenitis caused by Corynebacterium pseudotuberculosis (Syn. C. ovis) is a common disease in goats in many countries (*Addo & Eid* 1978, *Ashfaq & Campbell* 1979, *Burrell* 1981, *Hein & Cargill* 1981). In Norway, caseous lymphadenitis

is common in many dairy goat herds. The disease in characterized by abscesses in superficial lymph nodes. However, in some cases, abscesses can be found only in internal lymph nodes and organs (*Hein & Cargill* 1981). For this reason, several different serological tests have been applied for the diagnosis of infected animals. Many serological tests have been described (*Zaki & Abdel-Hamid* 1974, *Shigidi* 1979, *Burrell* 1980, *Barakat et al.* 1982, *Shen et al.* 1982). Due to the fact that the outer surface of C. pseudotuberculosis is rich in lipids, autoagglutination of the bacterial cells readily occurs. *Keskintepe* (1976) prevented autoagglutination by washing the cells in saline with 1 % Tween 80. Antigen prepared in this way was suitable for use in the agglutination test. *Lund et al.* (1982) prepared the antigen in a similar way and performed the agglutination test in microtitre plates. *Fraser* (1961) observed that erythrocytes from cow, sheep, goat and rabbit treated with metabolites from Corynebacterium equi and C. pseudotuberculosis readily hemolysed. *Knight* (1978) and *Lund et al.* (1982) established a hemolysis inhibition test in which this principle was applied.

The purpose of the present work was to study whether or not the bacterial agglutination test (BAT) and the hemolysis inhibition test (HIT) are suitable for use in the diagnosis of C. pseudotuberculosis infection. The threshold value between positive and negative antibody titres, and the sentitivity, specificity and reprodicibility in both tests were determined.

MATERIALS AND METHODS

Sera

In order to ascertain the prevalence of caseous lymphadenitis in 36 goat herds, clinical, bacteriological and serological examinations were carried out on 2428, 34 and 2458 animals, respectively (*Holstad* 1986a). A selection of this material was used in the present study. Sera from 25 goats infected with C. pseudotuberculosis were used as "positive" sera. Sera from 25 randomly selected goats from a herd with no history of caseous lymphadenitis were used as "negative" sera. No clinical sign of the disease was found in this herd. These "positive" and "negative" sera were used to detect the threshold value between positive and negative titres in the bacterial agglutination test and the hemolysis inhibition test. Sera from 155 animals from 3 infected herds were used to estimate the reproducibility in both tests.

Serological tests

Bacterial agglutination test (BAT). A strain of C. pseudotuberculosis (NVH 3368*) isolated from goat was used as antigen. This strain had been passaged 22 times on blood agar. The antigen was prepared as described by *Lund* (1982). The cells were stored at +4°C and used for up to 14 days. Serum samples were diluted twofold in phosphate buffered saline, pH 7.2 (PBS) in polystyrene microtitre plates (type U, Greiner, Germany). After addition of equal volumes of antigen (50 µl) the plates were incubated overnight at 37°C. Appropriate controls were included. The antibody titre was expressed as $\log_{10}$ to the reciprocal value of the last serum dilution resulting in agglutination. The reproducibility was estimated using duplicate determination of titres of serum samples. Two antigen suspensions produced separately and stored for various periods of time, were used.

Hemolysis inhibition test (HIT). C. pseudotuberculosis metabolites (hemolysis) was produced according to *Doty et al.* (1964) from a strain (NVH 2586) isolated from goat. The culture was centrifuged and filtrated through a millipore filter (0.22 µm). Sodiumazid (1:10000) was added and the filtrate was stored at + 4°C. Production of C. equi (NVH 3370) metabolites (C. equi factor) was carried out as described by *Knight* (1978). The material was prepared and stored as the C. pseudotuberculosis hemolysin.

A suspension of 1 % washed sheep erythrocytes and 10 % C. equi factor in PBS was prepared. This suspension could be used for up to 3 days afterwards when stored at + 4°C.

The C. pseudotuberculosis hemolysin was titrated twofold in PBS in polystyrene microtitre plates (type V, Greiner, Germany). Equal volumes (50 µl) of the suspension of sheep erythrocytes and C. equi factor were added. After addition of PBS (50 µl), the plates were incubated for 22 h at 37°C. The hemolysin titre was read as the highest hemolysin dilution which gave complete hemolysis.

Serum samples were diluted in PBS in polystyrene microtitre plates. After addition of equal volumes (50 µl) of C. pseudotuberculosis hemolysin (4 × titre) the plates were preincubated for

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* Culture collection at the Department of Microbiology and Immunology, Norwegian College of Veterinary Medicine, Oslo.

1 h at room temperature. The suspension of sheep erythrocytes and C. equi factor (50 µl) were then added and the plates were read after incubation for 22 h at 37°C. Appropriate controls were included. The antibody titre was expressed as $\log_{10}$ to the reciprocal value of the last serum dilution without hemolysis. The reproducibility was calculated using duplicate determination of titres of serum samples. Two suspensions of sheep erythrocytes and C. equi factor produced separately were used. The same batch of C. pseudotuberculosis hemolysin and the same batch of C. equi factor were used in both experiments.

Statistical analysis

The reproducibility was described by the correlation coefficient and the coefficient of variation.

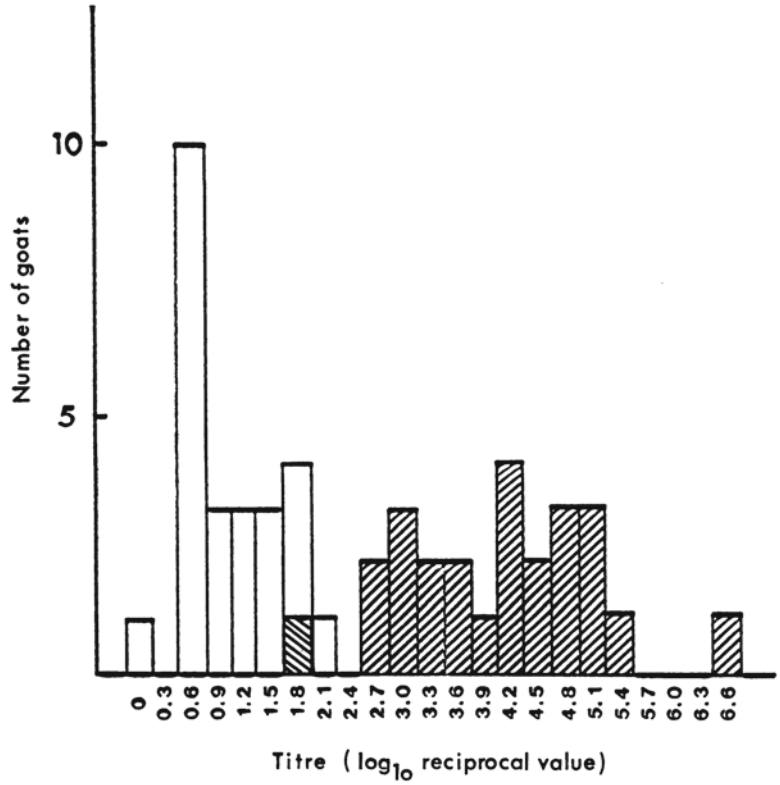

Figure 1. Distribution of titre values obtained in the bacterial agglutination test (BAT) with sera from 25 goats infected with Corynebacterium pseudotuberculosis ("positive") and 25 goats from a herd with no history of caseous lymphadenitis ("negative").
▨ "positive", □ "negative", ▩ includes "positive" and "negative".

RESULTS

Fig. 1 presents the distribution of titre values (T) obtained in the bacterial agglutination test with sera from 25 "positive" and 25 "negative" goats. One "positive" goat had $T = 1.8$ while the remaining "positive" animals had $T \geq 2.7$. One "negative" goat had $T = 2.1$, the remaining "negative" animals having $T \leq 1.8$. On this basis, positive titre in BAT was stipulated as $T \geq 2.1$.

Fig. 2 presents the distribution of titre values (T) obtained in the hemolysis inhibition test with sera from 25 "positive" and

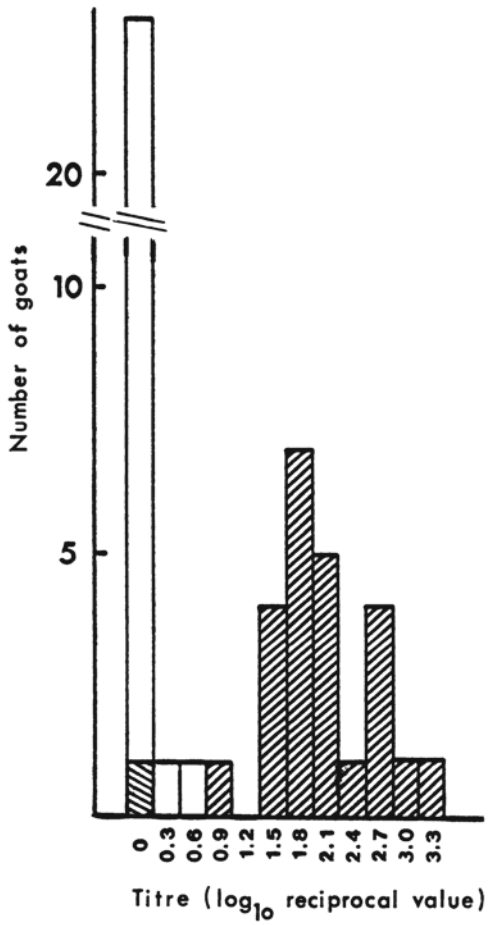

Figure 2. Distribution of titre values obtained in the hemolysis inhibition test (HIT) with sera from 25 goats infected with Corynebacterium pseudotuberculosis ("positive") and 25 goats from a herd with no history of caseous lymphadenitis ("negative").
▨ "positive", □ "negative", ▧ includes "positive" and "negative",

25 "negative" goats. One "positive" goat had $T = 0$ while the remaining "positive" animals had $T \geq 0.9$. One "negative" goat had $T = 0.6$, the remaining "negative" animals having $T \leq 0.3$. On this basis, positive titre in HIT was stipulated as $T \geq 0.6$.

Applying these threshold values, sensitivity and specificity was 0.96 in both tests in this material.

Twenty-three of 25 goats infected with C. pseudotuberculosis were positive in both BAT and HIT. No "positive" animal was negative in both tests. Twenty-three of 25 "negative" goats were negative in both tests. No "negative" animal was positive in both tests.

The correlation coefficient was 0.87 for BAT and 0.95 for HIT, the corresponding figures for the coefficient of variation being 26.4 % and 14.1 %, respectively. The coefficient of variation decreased with increasing titre values in both tests and was highest for BAT at low titres.

DISCUSSION

The present study indicated that both the bacterial agglutination test and the hemolysis inhibition test can be used to diagnose C. pseudotuberculosis infection in goats. Both tests are easy to perform. The present study reveals correlation between the tests. Most animals infected with the bacterium were positive in both BAT and HIT.

The threshold value between positive and negative antibody titres was chosen on the basis of the titration results for sera from goats infected with C. pseudotuberculosis and sera from goats from a herd in which caseous lymphadenitis did not occur. Positive titre (T) in BAT was stipulated as $T \geq 2.1$ and in HIT as $T \geq 0.6$.

Lund (1980) determined the threshold value between positive and negative titres in BAT and HIT on the basis of the titration results for precolostral kid sera and for serum samples from various non-infected animals and man. Positive titre was stipulated in BAT as $T \geq 1.8$ and in HIT as $T \geq 1.2$. These values cannot be compared with the threshold values chosen in the present study because the tests were not performed in the same way and different sera were used.

Burrell (1978) concluded that precolostral lamb sera contained a clumping factor of non-antibody character acting on bacterial cells. *Holstad* (unpublished) examined 28 precolostral

kid sera in BAT and HIT. The titre values varied from 0.9 to 2.7 in BAT while all the sera had T = 0 in HIT. Altogether 7 of these precolostral sera had titre values from 2.1 to 2.7 in BAT. Sera from 3 kids were examined for IgG, the result being negative in all cases. This investigation indicated that precolostral kid sera cannot be used to stipulate the threshold value between positive and negative titres in BAT.

The threshold values chosen in the present study gave 1 false-positive (4 %) and 1 false-negative (4 %) animal in each test. *Awad* (1960) examined serum samples from sheep and found that 3.5 % of the infected animals reacted negatively in the agglutination test.

There is some uncertainty as to the extent to which serologic false-positive reactions may be a result of cross reactions between other microbes and C. pseudotuberculosis. *Shigidi* (1974) did not demonstrate any cross reaction between C. pseudotuberculosis and C. pyogenes in the agglutination test and immunodiffusion test. The same study, however, revealed a cross reaction between C. pseudotuberculosis and C. renale.

As regards the present investigation, it does not seem very likely that any "negative" animal was infected with C. pseudotuberculosis. Information from the farmer indicated that caseous lymphadenitis did not occur, and the herd had no contact with animals from herds considered to be infected. Clinical examination of goats carried out on the same day as serum samples were collected, revealed no evidence of the disease.

The correlation coefficient was highest for HIT. The coefficient of variation was highest for BAT at low titres. These results indicate that the risk for false-positive and false-negative reactions due to test variability is highest for BAT. This test thus seem to be less suitable than HIT, if only a single serological test is to be employed for the examination of individual animals.

Due to the presence of a clumping factor in precolostral serum samples and sera from young animals, *Burrell* (1978) recommended that antitoxin tests should be used to survey immunity in such sera. This clumping factor could give rise to non-specific agglutination in the bacterial agglutination test.

Shigidi (1979) compared 5 different serological tests for the diagnosis of experimental C. pseudotuberculosis infection in sheep and concluded that serodiagnosis should be based on results from different tests.

The present investigation indicates that most infected animals can be identified using either BAT or HIT.

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SAMMENDRAG

Corynebacterium pseudotuberculosis infeksjon hos geit I. Anvendbarhet av to serologiske tester ved diagnostikk.

Bakterieagglutinasjonstest (BAT) og antihemolysintest (AHT) ble etablert og anvendbarheten av testene vurdert.

Serumprøver fra 25 voksne geiter som med sikkerhet var infisert med Corynebacterium pseudotuberculosis ble benyttet som „sikre" positive sera, og serumprøver fra 25 voksne geiter fra en besetning antatt fri for kaseøs lymfadenitt ble benyttet som „sikre" negative sera for å fastsette grensen mellom positive og negative titer ved testene. Titeret ble angitt som $\log_{10}$ til den resiproke verdi av høyeste serumfortynning som ga agglutinasjon ved BAT og høyeste serumfortynning uten hemolyse ved AHT. Titer lik 2.1 eller høyere ved BAT og lik 0.6 eller høyere ved AHT ble fastsatt som positive.

Sensitiviteten og spesifisiteten ved begge testene var lik 0.96 i det benyttede materialet.

Av 25 dyr hvorfra Corynebacterium pseudotuberculosis ble påvist var 23 positive ved både BAT og AHT.

Som mål på reproduserbarheten til testene ble korrelasjonskoeffisienten og variasjonskoeffisienten benyttet. Parvise målinger av titeret i 155 sera ble foretatt. Korrelasjonskoeffisienten var 0.87 for BAT og 0.95 for AHT. Variasjonskoeffisienten var 26.4 % for BAT og 14.1 % for AHT. Variasjonskoeffisienten varierte med titeret. Den var høyest for BAT ved lave titer.

Det konkluderes med at begge testene kan brukes ved seroepidemiologiske undersøkelser av kaseøs lymfadenitt hos geit.

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