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ANTIBODIES TO CORYNEBACTERIUM PSEUDOTUBERCULOSIS IN ADULT GOATS FROM A NATURALLY INFECTED HERD*

By

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LUND, ARVE, TORBJØRN ALMLID, HANS JØRGEN LARSEN and TORSTEIN STEINE: *Antibodies to Corynebacterium pseudotuberculosis in adult goats from a naturally infected herd.* Acta vet. scand. 1982, *23*, 473—482. — Serum samples taken in 3 successive years (1977, 1978 and 1979) from adult dairy goats (Norwegian breed) originating from 1 herd were examined for antibodies to Corynebacterium pseudotuberculosis. Both bacterial agglutination test (BAT) and hemolysis inhibition test (HIT) were used. The proportion of seropositive goats increased 10—12 % during the investigation period. In 1979 all animals were seropositive to BAT and about 95 % had antihemolysins in their sera. Twenty-two of the 23 one-year old goats recruited to the herd in 1978 were seropositive. The average age-specific titres increased up to the age of 3 years, and subsequently decreased for goats aged 4—7 years. Caseous lymphadenitis is thus regarded as a chronic infection. The effect of age on the titre values was significant at the 5 % level in 1977 and 1978 when HIT was used and in 1978 when BAT was used. During the investigation period the same 36 and 40 goats were examined every year by BAT and HIT, respectively. Intermediate to high correlations between titre values for the same goats from year to year were found.

Both BAT and HIT are suitable for sero-epidemiological investigations concerning infection with C. pseudotuberculosis in goats.

caseous lymphadenitis; Corynebacterium pseudotuberculosis; goats; serological investigations.

Caseous lymphadenitis caused by Corynebacterium pseudotuberculosis (syn. C. ovis) is a common disease among goats in many countries (*Ashfaq & Campbell* 1979, *Burrell* 1981). In

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Norway caseous lymphadenitis is common in several dairy goat herds. The disease is characterized by caseous necrosis of both superficial and internal lymph nodes and may lead to decreased milk production, emaciation and even death. By clinical examination of 4013 goats of various ages *Ashfaq & Campbell* reported that 8.1 % of the animals had abscesses, 70 % of which were caused by C. pseudotuberculosis. *Burrell* (1981) examined serum samples collected from 2 dairy goat herds demonstrating C. pseudotuberculosis antibodies in 67 of 110 animals (61 %). Several different in vitro and in vivo tests have been applied for the serodiagnosis of caseous lymphadenitis (*Shigidi* 1979, *Zaki & Abdel-Hamid* 1974, *Burrell* 1980). *Keskintepe* (1976) prevented autoagglutination by washing the cells of C. pseudotuberculosis in saline with 1 % Tween 80. Antigen prepared in this way was suitable for use in the agglutination test. *Fraser* (1961) observed that erythrocytes pretreated with metabolites from C. equi were more easily hemolysed by C. pseudotuberculosis hemolysin than untreated blood cells. *Knight* (1978) established a hemolysis inhibition test by using this principle.

The purpose of the present work was to study the prevalence of C. pseudotuberculosis seropositive animals in 1 dairy goat herd during the years 1977, 1978 and 1979 using the agglutination and hemolysis inhibition tests.

MATERIALS AND METHODS

Animals

The dairy goat herd consisted of 80—90 milking goats of Norwegian breed. Number and age distribution is shown in Table 1. The kids were born in the period January to March. They were removed from the does 3 days after birth and reared in pens, receiving milk replacer or cow's milk during the first weeks of life. Lactating goats were placed either single in stalls or together in pens. During the months from June to August the herd stayed at mountain pastures. Each year 10 bucks were selected for breeding. Between 21 and 34 one-year old does were recruited to the herd while a similar number of adult goats were slaughtered each year.

Caseous lymphadenitis has been a problem for years among goats from this herd. Bacteriological examination of abscess material from these animals yielded pure culture of C. pseudotuberculosis.

Table 1. Number and age of goats examined during 1977, 1978 and 1979 in hemolysis inhibition test (HIT) and bacterial agglutination test (BAT).

Age (years)	HIT	BAT
1	78	76
2	55	54
3	41	41
4	23	23
5	27	27
6	16	15
7	11	10
8	3	2
9	1	1

Sera

Blood samples were collected from adult goats in the period April-May, i.e. 2—4 months post partum, during the years 1977, 1978 and 1979. Sera were prepared according to standard procedure and stored at — 20°C.

Serological tests

Bacterial agglutination test (BAT). Agglutination antigen was prepared by growing C. pseudotuberculosis (NVH 2586*) in brain heart infusion broth (Difco) with addition of 1 % Tween 80 for 18—20 h at 37°C. The cells were harvested and washed 3 times in phosphate buffered saline, pH = 7.2, (PBS) with addition of 1 % Tween 80. Thereafter the cells were diluted in PBS with 1 % Tween 80 to the appropriate concentration (absorption value of 0.65±0.05 at wavelength 535 nm). The cells were stored at +4°C for 14 days.

Serum samples were diluted in PBS in polystyrene microtiter plates and after addition of antigen the plates were incubated overnight at 37°C. Appropriate controls were included. The antibody titre was read as the $\log_{10}$ reciprocal value of the last serum dilution giving agglutination. Preliminary studies also including precolostral kid sera revealed that titres > 1.8 could be considered as positive.

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

Hemolysis inhibition test (HIT). C. pseudotuberculosis hemolysin was produced from a urease positive strain (ATCC 17410*) according to *Doty et al.* (1964). The culture was centrifuged and the supernatant filtered, titrated and stored at —20°C. Production of C. equi (NVH 3370) metabolites (sensitizer) was carried out as described by *Knight* (1978). Sensitized sheep erytrocytes (SE) were prepared by adding sensitizer to a 4 % suspension of washed red blood cells in PBS giving a 1 % final concentration of sensitizer. They were ready for use after 2 h incubation at room temperature and could be used the following 2 days when stored at +4°C.

Serum samples were diluted in PBS in microtiter plates. After addition of hemolysin (double the titer) the plates were preincubated for 1 h at room temperature. Thereafter sensitized SE were added and the plate was read after incubation overnight at 37°C. Appropriate controls were included. The antibody titre was given as the $\log_{10}$ reciprocal value of the last serum dilution without hemolysis. Preliminary studies also including precolostral kid sera revealed that titres ≥ 1.2 could be considered as positive.

Statistical analysis

Statistical calculations were carried out using student's t-test and least squares analysis (*Harvey* 1960).

RESULTS

The titres varied considerably between animals each year in both tests. The distribution of goats according to titre values showed approximately normal distribution curves for both methods every year. In the one-year old group of goats in 1978, 22 of 23 animals were seropositive in both tests.

The average titres of antibodies against C. pseudotuberculosis during the years 1977, 1978 and 1979, irrespective of age of the goats, are presented together with the percentages of seropositive animals each year in Table 2. No significant difference was detected between average titre values obtained each year using both HIT and BAT. The proportion of seropositive goats increased 10—12 % during the investigation period. In 1979 all goats exa-

* American Type Culture Collection.

mined were seropositive in the BAT, while 95 % were seropositive in the HIT.

The average titres of the animals calculated according to the age during the 3 years are presented in Fig. 1. The titre values

Table 2. Average titres ± s of antibodies to Corynebacterium pseudotuberculosis in goats in hemolysis inhibition test (HIT) and bacterial agglutination test (BAT) during the years 1977, 1978 and 1979, and percentage of seropositive animals each year (number of goats examined in brackets).

	HIT			BAT		
	1977	1978	1979	1977	1978	1979
Average titre*	1.68±0.59 (83)	1.93±0.55 (81)	1.74±0.54 (91)	2.92±0.61 (80)	3.00±0.61 (81)	3.01±0.59 (88)
% seropositive	82.8	91.6	94.7	90.9	97.6	100

* Titres are given as $\log_{10}$ reciprocal value of endpoint dilution.

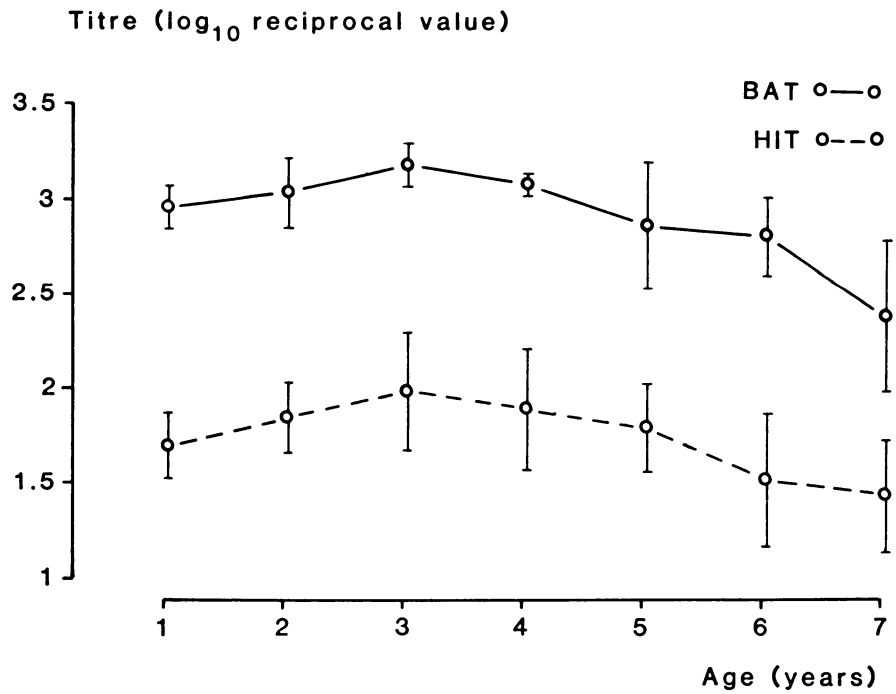

Figure 1. Titre of antibodies to Corynebacterium pseudotuberculosis by age of the goats calculated as averages of the values obtained in 1977, 1978 and 1979 by using bacterial agglutination test (BAT) and hemolysis inhibition test (HIT).

Table 3. Correlation coefficients for the titres of antibodies to Corynebacterium pseudotuberculosis in bacterial agglutination test (BAT) and hemolysis inhibition test (HIT) in serum samples from the same goats during the years 1977, 1978 and 1979 (number of goats examined in brackets).

Test year (36)		BAT		Test year (40)		HIT	
		1978	1979			1978	1979
BAT	1977	0.63**	0.62**	HIT	1977	0.82**	0.78**
	1978		0.77**		1978		0.91**

** Highly significant correlation ($P < 0.01$).

increased for goats aged 1—3 years, and subsequently decreased for goats aged 4—7 years. The effect of age on the antibody titre was significant at 5 % level in 1977 and 1978 when HIT was used, and in 1978 when BAT was used.

During the investigation period the same 36 and 40 goats were examined every year in BAT and HIT, respectively. Correlation coefficients between the titre values obtained are presented in Table 3. There is intermediate to high correlation between titres for the same goats from year to year.

DISCUSSION

The present study revealed that a majority of the goats from the herd under investigation had antibodies to C. pseudotuberculosis. Caseous lymphadenitis was a common disease among the goats examined, and this finding was therefore anticipated. The high proportion of seropositive goats indicates the extent to which C. pseudotuberculosis is transmitted between animals. Through spontaneous rupture of abscesses the purulent material contaminates the environment. *Seddon et al.* (1929) stated that the organisms can remain alive on wool after suppuration of lesions. *Bull & Dickinson* (1935) isolated C. pseudotuberculosis from soil at sheep assembly points in herds where caseous lymphadenitis was endemic. Thus, C. pseudotuberculosis most likely is part of the normal environmental bacterial flora in the herd examined.

During the investigation period the proportion of seropositive animals increased 10—12 %. This is difficult to explain. Possible change in pressure of infection and/or predisposing environ-

mental factors may be considered. During the investigation period the goats were routinely immunized and blood samples were collected at regular intervals during the first months of life. Possible contamination of syringe needles used for these purposes may have helped in spreading C. pseudotuberculosis. Experimentally, *Nagy* (1976) inoculated sheep subcutaneously with contaminated hypodermic needles. Abscesses were found in all the animals 35 days post inoculation.

During the investigation period 21—34 does were recruited yearly to the herd while approximately the same number was slaughtered. Thus the average age of the animals in the herd was fairly constant from year to year. With this type of herd management the average antibody titre was not significantly changed from year to year. The majority of yearlings recruited to the herd were seropositive, although with titre values lower than for goats 2 and 3 years old. Thus it seems that the animals are infected with C. pseudotuberculosis during their first year of life. The increase in titre value from the first to the third year of age is probably the result of longstanding antigenic stimulation. Caseouc lymphadenitis is known to be a chronic disease (*Gates et al.* 1977, *Desiderio et al.* 1979). Decreasing titre values in goats aged 4—7 years may be related to lower immune response to C. pseudotuberculosis possibly because less antigen is presented to immunocompetent lymphocytes in immune animals.

Highly significant correlations exist between titre values obtained for the same goats examined from year to year. This indicates that goats having low or high titres one year, also have low or high titres, respectively, the successive years. Selection experiments with mice for high and low antibody response to complex antigens have shown that antibody production is under polygenic control. Further, the experiments demonstrated that the same group of genes regulates the antibody response to many complex antigens unrelated to those used during the selective breeding (*Biozzi et al.* 1979). Route of infection and antigen dose are also important when considering the magintude of antibody production. Selection experiments with goats concerning production of antibodies to several antigens showed considerable variation in immune response in different animals. Significant differences between high and low responder lines was demonstrated after 1 year of selection (*Almlid et al.* 1979). It remains however, to be seen how such high and low antibody production

affects course of infection. As regards caseous lymphadenitis it appears that goats with high production of antibodies may succeed better in neutralizing C. pseudotuberculosis toxin than low responding animals. Thus high production of antitoxin may mean better immunity against caseous lymphadenitis. Since C. pseudotuberculosis is an intracellular parasite it is assumed that resistance and course of infection also depend on cellular immunity.

For screening purposes both BAT and HIT are easy and quick to perform. In BAT there was some difficulty in reading the last dilution with positive reaction for a few batches of antigen whereas all HIT positive reactions were easy to read. In HIT it was a disadvantage that sensitized red blood cells had to be used within a couple of days after preparation. Although the tests detected antibodies to different antigens, almost the same proportion of seropositive animals were diagnosed.

In view of the high prevalence of goats that were seropositive to C. pseudotuberculosis in this herd, further studies should be carried out also including clinical and environmental observations from dairy goat herds in Norway.

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SAMMENDRAG

Antistoffer mot Corynebacterium pseudotuberculosis hos voksne geiter fra en naturlig smittet besetning.

Serumprøver fra voksne geiter (norsk rase) i én besetning ble undersøkt 3 år på rad (1977, -78 og -79) for antistoffer mot Corynebacterium pseudotuberculosis. Både bakterieagglutinasjonstest (BAT) og antihemolysintest (AHT) ble benyttet. I løpet av undersøkelsesperioden økte andelen av seropositive geiter med 10—12 %. I 1979 var samtlige dyr seropositive i BAT, mens ca. 95 % hadde antihemolysiner i serum. Blant 1-års gamle geiter som ble rekruttert til besetningen i 1978 var 22 av 23 seropositive. Gjennomsnittlig titerverdier i løpet av årene undersøkelsen varte, utregnet etter geitenes alder, økte opp til 3 års alder og avtok deretter hos 4—7 år gamle geiter. Kaseøs lymfadenitt betraktes således som en kronisk infeksjon. Effekten av alder på titerverdiene var signifikant ($0{,}01 < P < 0{,}05$) i 1977 og 1978 (AHT) og i 1978 (BAT).

En gruppe på ca. 40 geiter ble undersøkt årlig i 3 år med både BAT og AHT. Det ble påvist middels høge til høge korrelasjoner mellom titerverdiene hos de samme dyra fra år til år.

Både BAT og AHT er egnet for sero-epidemiologiske undersøkelser vedrørende kaseøs lmfadenitt hos geit.

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