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CORYNEBACTERIUM PSEUDOTUBERCULOSIS INFECTION IN GOATS V.

RELATIONSHIP BETWEEN THE INFECTION AND LESIONS RESULTING FROM VACCINATION AGAINST PARATUBERCULOSIS

By

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HOLSTAD, G.: *Corynebacterium pseudotuberculosis* infection in goats V. Relationship between the infection and lesions resulting from vaccination against paratuberculosis. Acta vet. scand. 1986, 27, 617—622. — In 2 goat herds, one infected with *Corynebacterium pseudotuberculosis* and one free from the infection, goats were examined for superficial swellings on the shoulder and chest. All animals in this study had been vaccinated against paratuberculosis before the age of 4 weeks. The vaccine had been applied subcutaneously behind the shoulder. Twenty-two of 40 (55 %) and 31 of 45 (69 %) goats had such lesions in the infected and non-infected herds, respectively. The difference between the herds was not significant, $P > 0.05$.

Swellings found behind the shoulder in 19 goat carcasses derived from 4 herds in which *C. pseudotuberculosis* infection occurred were examined bacteriologically. No bacteria could be isolated from such lesions in 15 animals, while *C. pseudotuberculosis* in pure culture was isolated from 3 carcasses, and a mixed bacterial flora from the remaining carcass. Bacteria could not be isolated from lesions situated behind the shoulder in 7 carcasses from 3 herds free from *C. pseudotuberculosis* infection.

It is concluded that most swellings on the shoulder and chest in goats were granulomas resulting from vaccination against paratuberculosis.

swellings; shoulder and chest; bacteria.

Superficial swellings on the shoulder and chest are common in goats in Norway. The lesions are considered to result from vaccination against paratuberculosis. This vaccine is applied subcutaneously behind the shoulder before the age of 4 weeks.

Fodstad (1980) reported that hazel-nut sized nodules could occur at the site of inoculation shortly after vaccination.

There is some uncertainty as to what extent *Corynebacterium pseudotuberculosis* infection contributes to the formation of lesions after vaccination against paratuberculosis. Nor is there any data concerning the relative frequency of such lesions among animals in herds with caseous lymphadenitis compared with herds free from the disease.

The purpose of the present study was to register and compare the prevalence of animals with superficial swellings on the shoulder and chest in 1 infected and 1 non-infected herd. In addition such lesions were subjected to bacteriological examination.

MATERIALS AND METHODS

Clinical examinations

Altogether 40 adult goats (1 year of age or older) in Herd A, and 37 adult goats and 8 kids (less than 1 year of age) in Herd B were examined. The oldest goats in the herds had given birth 6 times.

The animals were inspected and palpated and all superficial swellings were noted. Bacteriological examination of the lesions was not carried out.

H e r d A. Caseous lymphadenitis had been diagnosed by clinical and serological examinations of goats 2 years before the present study was carried out. The herd was a member of a goat "breeding circle"¹ together with other infected herds. The goats were placed in pens and all the animals had been vaccinated against paratuberculosis and pasteurellosis.

H e r d B. Clinical and serological examinations of animals in the herd, carried out about 3 years before the present study was initiated, had not revealed any evidence of caseous lymphadenitis. The farmer had not noticed any sign of the disease in subsequent years. This herd had no history of contact with animals from infected herds. The goats were placed in pens and all the animals had been vaccinated against paratuberculosis, pasteurellosis and enterotoxaemia.

¹ Breeding system practised in Norway in which several herds share the use of the same breeding males.

Serological examinations

Blood samples were collected from all animals examined clinically. Sera were prepared according to standard procedure and stored at -20°C until examination. The sera were examined for antibodies to *C. pseudotuberculosis* in both the bacterial agglutination test (BAT) and the hemolysis inhibition test (HIT) (Holstad 1986a).

Bacteriological examinations

Animals from 7 herds were examined. Caseous lymphadenitis had been diagnosed in 4 of these herds by clinical and serological examination of goats carried out in the same year as the present study. All these herds had a history of contact with animals from infected herds. In the remaining 3 herds, *C. pseudotuberculosis* infection had not been demonstrated by examinations carried out in the same year as the present investigation.

Lesions found behind the shoulder in 26 carcasses were examined. Nineteen of these carcasses came from the 4 herds with caseous lymphadenitis, and 7 from the 3 non-infected herds. The lesions were removed and stored at -20°C until bacteriological examinations were carried out 8–12 months later. All lesions were abscesses consisting of caseous pus and fibrotic tissue.

Bacteriological examination of pus was carried out by direct microscopy and by culture on blood agar. Blood agar plates were incubated in 10 % CO_2 atmosphere and anaerobically for 48 h at 37°C . Any bacteria isolated were identified according to standard bacteriological principles (Buchanan & Gibbons 1974).

Statistical analysis

Statistical calculations were carried out using Chi-square analysis.

RESULTS

Clinical and serological examinations

Superficial swellings on the shoulder and chest were detected in 22 (55 %) and 31 (69 %) animals in Herd A and B, respectively. The difference between the herds was not significant, $P > 0.05$. Swellings were demonstrated in animals of all ages. In Herd A superficial swellings localized on other parts of the

body were found in 11 animals. Most of these lesions were found in or close to superficial lymph nodes. Swellings on the shoulder and chest were demonstrated in 7 of these 11 animals. In contrast, swellings were only found on the shoulder and chest in animals from Herd B.

In all 40 (100 %) and 3 (7 %) animals were positive in BAT in Herd A and B, respectively. Positive animals in Herd B had low titres. In HIT 39 (98 %) animals were positive in Herd A, while no animal was positive in Herd B.

Bacteriological examinations

Examination of lesions from the 19 carcasses from herds with caseous lymphadenitis resulted in *C. pseudotuberculosis* being isolated in pure culture from 3 carcasses, and a mixed bacterial flora from 1. Bacteriological examination of the remaining 15 carcasses was negative. Bacteria were not isolated from lesions situated behind the shoulder in carcasses from herds in which clinical and serological examination had failed to reveal the presence of caseous lymphadenitis.

DISCUSSION

The present study indicates that superficial swellings on the shoulder and chest are common in goats in Norway. There was no difference in the occurrence of such lesions in a herd with caseous lymphadenitis compared with a herd which was free from the disease. *C. pseudotuberculosis* infection was demonstrated in Herd A. Most animals in this herd were positive in both BAT and HIT and superficial swellings were seen in many animals. Serological examinations revealed that Herd B was free from the disease.

The results indicate that superficial swellings on the shoulder and chest are granulomas resulting from vaccination against paratuberculosis, and that such lesions can exist for many years. The paratuberculosis vaccine consists of 2 attenuated bovine strains of *Mycobacterium paratuberculosis* suspended in olive oil, liquid paraffin and pumice powder (Fodstad 1980). Adjuvants consisting of mineral gels such as aluminium hydroxide and aluminium phosphate are employed in vaccines against pasteurellosis and enterotoxemia (Anon. 1983). Oil-based adjuvants produce a stronger reaction on the inoculation site than

mineral gels (Tizard 1982). Mycobacteria per se can however, cause granulomas (Buxton & Fraser 1977). No information concerning the actual site at which the vaccines against pasteurellosis and enterotoxemia were applied in animals in the herds included in the present study were available. The risk of granuloma formation at the site of inoculation associated with these vaccines is, however, less than with the vaccine against paratuberculosis.

The investigation indicates that swellings situated behind the shoulder in goats are usually bacteriologically negative. However, bacteriological examination for mycobacteria were not carried out in the present investigation. *C. pseudotuberculosis* was isolated from lesions from 3 out of 19 carcasses from herds with caseous lymphadenitis. Information from the farmers indicates that swellings arising after vaccination occasionally burst. Infection of open granulomas by *C. pseudotuberculosis* will probably occur in animals in infected herds. However, in animals in infected herds swellings on the shoulder and chest might also possibly be a result of primary infection with *C. pseudotuberculosis*. The examination of one lesion revealed a mixed bacterial flora, indicating the possibility that environmental bacteria may infect granulomas resulting from vaccination in some cases.

Freezing and thawing normally leads to bacterial death and the handling of the present material may have resulted in some originally infected granulomas becoming sterile.

REFERENCES

- Anon.: Vaccines and sera for use in animals. National Veterinary Institute, Oslo 1983, 53 pp.
- Buchanan, R. R. & N. E. Gibbons: Bergey's Manual of Determinative Bacteriology, 8th ed. Williams & Wilkins, Baltimore 1974, 1246 pp.
- Buxton, A. & G. Fraser: Animal Microbiology, Vol. 1. Blackwell Scientific Publications, Oxford 1977, p. 229—236.
- Fodstad, F. H.: Vaccination as a control measure for Johne's disease in goats. In: Fodstad, F. H.: Paratuberculosis (Johne's disease) and related mycobacterial diseases in Norway with special reference to diagnosis, epidemiology and control of paratuberculosis in goats. Thesis. Norwegian College of Veterinary Medicine. National Veterinary Institute, Oslo 1980, sep. pag.
- Holstad, G.: *Corynebacterium pseudotuberculosis* infection in goats I. Evaluation of two serological diagnostic tests. Acta vet. scand. 1986b, 27, 575—583.

Tizard, I.: An Introduction to Veterinary Immunology, 2nd ed. Saunders, Philadelphia 1982, p. 178—192.

SAMMENDRAG

Corynebacterium pseudotuberculosis infeksjon hos geit V.
Relasjon mellom denne infeksjon og prosesser som følge av vaksinasjon mot paratuberkulose.

En undersøkelse over forekomst av overfladiske hevelser på bog og bryst ble foretatt på geiter i 2 besetninger, en med og en uten kaseøs lymfadenitt. Alle dyrene i disse besetningene var vaksinert mot paratuberkulose. Vaksinen var blitt applisert bak bogen på dyrene før 4 ukers alder. Det ble påvist slike hevelser hos 22 av 40 (55 %) undersøkte dyr i en besetning med kaseøs lymfadenitt og hos 31 av 45 (69 %) undersøkte dyr i en besetning fri for sykdommen. Forskjellen mellom besetningene var ikke signifikant, $P > 0.05$.

Det ble foretatt bakteriologiske undersøkelser av prosesser lokalisert like bak bogen fra 19 slakt fra ialt 4 besetninger med kaseøs lymfadenitt. I prosesser fra 15 slakt ble bakterier ikke påvist. I prosesser fra 3 slakt ble *Corynebacterium pseudotuberculosis* isolert i renkultur, og i prosess fra ett slakt ble det påvist uspesifikk blandingsflora. Det ble ikke påvist bakterier ved undersøkelse av slike lesjoner fra 7 slakt fra 3 besetninger fri for kaseøs lymfadenitt.

Det konkluderes med at de fleste hevelser på bog og bryst var granulomer oppstått etter vaksinasjon mot paratuberkulose.

(Received September 8, 1986).

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