Acta vet. scand. 1989, *30,* 285–293.

Corynebacterium Pseudotuberculosis Infection in Goats IX.

The Effect of Vaccination against Natural Infection

By *Gudmund Holstad*

Department of Microbiology and Immunology, Norwegian College of Veterinary Medicine, Oslo, and the State Veterinary Laboratory for Northern Norway, Harstad, Norway.

Holstad G.: Corynebacterium pseudotuberculosis infection in goats IX. The effect of vaccination against natural infection. Acta vet. scand. 1989, 30, 285–293. – A vaccination trial was carried out in 10 infected herds. The trial included 247 female kids, the number of animals in each herd varying from 15 to 38. About half of the animals in each herd were vaccinated twice at 3 to 4 week intervals, the first vaccination being carried out before the age of 4 months. A combination of a crude filtrate of *C. pseudotuberculosis* toxoid with whole organisms, was used.
Overall, the prevalence of animals with superficial swellings was higher in the unvaccinated than in the vaccinated group during the first 1–2 years following immunization. However, in some herds superficial swellings were as common in vaccinated as in unvaccinated animals.
An antibody response following vaccination was demonstrated in the hemolysis inhibition test, but not in the bacterial agglutination test. Superficial swellings were more common in vaccinated animals which were negative than in animals which were positive in the hemolysis inhibition test at 1 1/2 months after vaccination.
The vaccine used in the present study, was not sufficiently efficacious to be recommended as the only protective measure against caseous lymphadenitis in Norwegian goat herds.

immunization; whole killed bacteria; crude filtrated toxoid; infected goat herds.

Introduction

Caseous lymphadenitis, a chronic disease caused by *Corynebacterium pseudotuberculosis,* is a worldwide problem in sheep and goats. The abscesses formed following the infection usually involve the superficial lymph nodes. In Norway caseous lymphadenitis is common in goat herds. Introduction of infected goats into non-infected herds can result in several cases of the disease in subsequent years (*Holstad* 1986a, *Holstad* 1986b). Once established, the disease is difficult to eradicate. The thick wall of the abscesses makes the lesions refractory to antibiotic treatment. The organisms are also able to survive and persist on common barnyard fomites (*Augustine & Renshaw* 1986). Effective environmental sanitation measures to decrease the incidence of the disease have, so far, not been reported.

Several experiments with inactivated vaccines against *C. pseudotuberculosis* infection in sheep and goats have been carried out (*Cameron* 1972, *Nairn et al.* 1977, *Burrell* 1978 & 1983, *Nairn* 1981, *Anderson & Nairn* 1984, *Brogden et al.* 1984, *Brown et al.* 1986, *LeaMaster et al.* 1987, *Holstad et al.* 1989). Some positive effects of vaccination have been demonstrated in these experiments, though the protection achieved has not been complete. In countries in which caseous lymphadenitis causes great econo-

mic losses by carcass condemnation, even a moderate effect of vaccination would be profitable. In Norwegian goat herds, however, caseous lymphadenitis is more a problem in the breeding system. Herds free from the disease in areas where it is endemic generally don't participate in "goat breeding circles"[1] in order to avoid infection. If highly effective vaccines were available, contact between animals from infected and non-infected herds would be feasible.

The main purpose of the present investigation was to evaluate the efficacy of an inactivated vaccine against natural *C. pseudotuberculosis* infection in Norwegian goat herds by the registration of superficial swellings in vaccinated and unvaccinated animals. Furthermore, the presence of antibodies against the organism was investigated and related to the clinical findings.

Materials and methods

Animals

Ten herds, with similar management conditions, in which caseous lymphadenitis had been diagnosed previously (*Holstad* 1986a), were selected for the study. Caseous lymphadenitis had been a problem in all these herds for several years, and clinical examinations carried out previously revealed superficial swellings in 25 % (mean value) of the adult animals, with herd variation from 11 to 36 %. When the vaccination trial started at the end of March, 247 female kids were included, the number of animals in each herd varying from 15 to 38. The mean age of the animals was then 67 days with a variation from 12 to 130 days. A previous investigation had indicated that immunoprophylactic measures against caseous lymphadenitis should be carried out before the animals reach the age of 4 months (*Holstad* 1986c). Only 1 kid in the present investigation had passed this age at the time of first vaccination. In each herd, the vaccinated and unvaccinated group were selected randomly, the age distribution being similar in the 2 groups. In each herd, about half the kids were vaccinated, while the remaining acted as controls. Whenever animals were placed in different pens, half of the kids in each pen were vaccinated, while the others served as controls.

In all the herds, animals were vaccinated against paratuberculosis before the age of 4 weeks. Vaccination against pasteurellosis and/or enterotoxaemia were also carried out in some of the herds.

In all herds, the kids were placed in separate pens until put out on pasture in June. In some herds, the kids were placed in the same room as adult goats, while in other herds, some of the kids were placed in the same room as adult animals, and some in separate rooms. During the summer, the kids grazed together with adult goats in most herds. At the end of the grazing season (September), they were housed for the winter, either in separate pens or in pens together with older animals.

Vaccination

The vaccine was prepared from a strain (NVH 2586*) of *C. pseudotuberculosis* isolated from goat. The vaccine consisted of 4 mg freeze-dried whole cells in an adjuvant described by *Fodstad* (1980), and crude filtrated toxoid suspended in equal parts of Freund's incomplete adjuvant. The vaccine was prepared as described by *Holstad et al.* (1989).

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

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

The animals were vaccinated twice, at intervals from 21 to 28 days in the different herds. The vaccine was applied subcutaneously behind the shoulder on both sides. The unvaccinated animals were given adjuvants only, at the same localization.

Clinical examinations

Clinical examinations were carried out in connection with the vaccinations (March and April), just before the animals were put out to pasture (June – 1 1/2 months after vaccination) and 4 times during the period they were housed for the winter (September – 5 months after vaccination, December – 7 1/2 months after vaccination, February – 9 1/2 months after vaccination and June – 13 1/2 months after vaccination). In addition an examination was carried out in December, 19 1/2 months after vaccination.

The animals were inspected and palpated, and superficial swellings recorded. Swellings on the shoulder and chest were, however, excluded as lesions at these sites were considered to be granulomas arising after vaccination against paratuberculosis.

Serological examinations

Blood samples were collected from most of the animals in connection with the clinical examinations. Sera were prepared according to standard procedures and stored at –20°C. The samples were examined for antibodies to *C. pseudotuberculosis* using the hemolysis inhibition test (HIT) and the bacterial agglutination test (BAT) (*Holstad* 1986d).

Statistical analysis

Statistical calculations were carried out using Chi-square analysis. $p \leq 0.05$ was taken to indicate statistical significance.

Results

Clinical examinations

No animals had superficial swellings when the vaccinations were carried out. The prevalence of animals with swellings at the different times of examination following vaccination, is presented in Fig. 1. Most of the lesions were localized in or close to the superficial lymph nodes. The prevalence of unvaccinated animals with superficial swellings increased to 29 % at 9 1/2 month after vaccination and remained almost constant on subsequent examinations. The prevalence of animals with superficial swellings in the vaccinated group was lower than in the unvaccinated at all the examinations from 5 months after vaccination. The difference, however, was significant at only 3 of the 5 examinations.

The prevalence of animals with superficial swellings in the groups varied between the herds. Indeed, in some herds, there were more vaccinated than unvaccinated animals with lesions at some of the examinations. Animals with superficial swellings were seen in both groups in 9 out of the 10 herds, while in one herd such lesions were found only in unvaccinated animals.

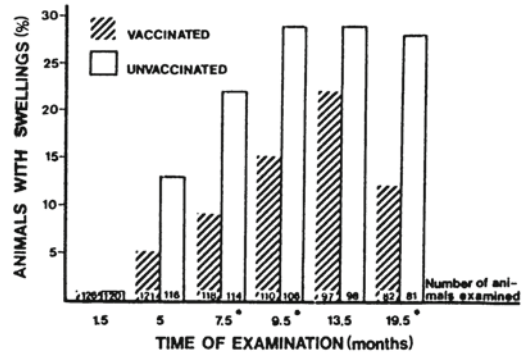

Figure 1. Percentage of vaccinated and unvaccinated animals with superficial swellings at different months after the last vaccination.

*) Statistical significant difference between the vaccinated and unvaccinated group.

The percentage of animals, in which superficial swellings were registered for the first time, varied between the different periods (Fig. 2). The percentage was higher in the

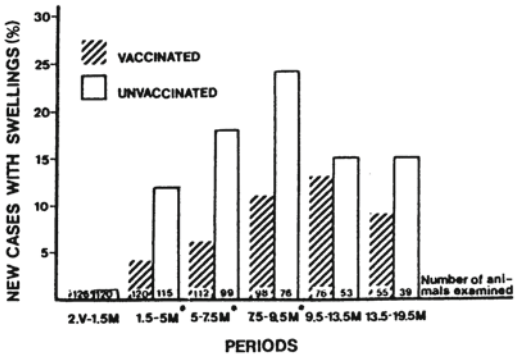

Figure 2. Percentage of vaccinated and unvaccinated animals that developed superficial swellings between the different examinations. All the animals included in the different periods had been examined earlier without demonstrating such lesions.

*) Statistical significant difference between the vaccinated and unvaccinated group. 2.V) The last vaccination M) Months after the last vaccination.

unvaccinated than in the vaccinated group during all the periods, the difference being most prominent the first 9 1/2 months after vaccination. In the unvaccinated animals, the percentage was highest between 7 1/2 and 9 1/2 months, while it was highest between 9 1/2 and 13 1/2 months in the vaccinated group.

Superficial swellings were demonstrated at one or more examinations in 31 % (30 out of 97) of the vaccinated and 54 % (53 out of 98) of the unvaccinated animals present at each and every examination during 13 1/2 months after vaccination. The difference was significant. Among these animals 43 % of the vaccinated and 49 % of the unvaccinated had lesions at 2 or more of the examinations, the lesions being often localized at the same part of the body at the different examinations. In some of the animals in both groups the swellings disappeared only to reappear on the same place several months later.

Serological examinations

The prevalence of animals positive in HIT and BAT at the different times of examination is presented in Fig. 3.

At all the examinations, the prevalence of HIT-positive animals was higher in the vaccinated than in the unvaccinated group, the difference being most prominent up to 7 1/2 months following vaccination. The prevalence of HIT-positive vaccinated animals increased up to 53 % at 1 1/2 months after vaccination, and continued to increase during the rest of the investigation period. In the unvaccinated group, the prevalence of HIT-positive animals decreased to 4 % at 1 1/2 months after vaccination, and then increased during the following examination period.

The prevalence of BAT-positive animals, both vaccinated and unvaccinated, showed a course similar to the prevalence of HIT-positive unvaccinated animals. The prevalence of unvaccinated BAT-positive animals was, however, higher than the prevalence of unvaccinated HIT-positive animals at most of the examinations. There was no difference in the prevalence of BAT-positive animals between the vaccinated and unvaccinated group until 7 1/2 months following vaccination. From then it was highest in the unvaccinated group.

Relation between clinical and serological examinations

Several animals in the vaccinated group had maternal antibodies when the first immunization was carried out (Fig. 3). However, superficial swellings were not more common in these animals than in vaccinated animals

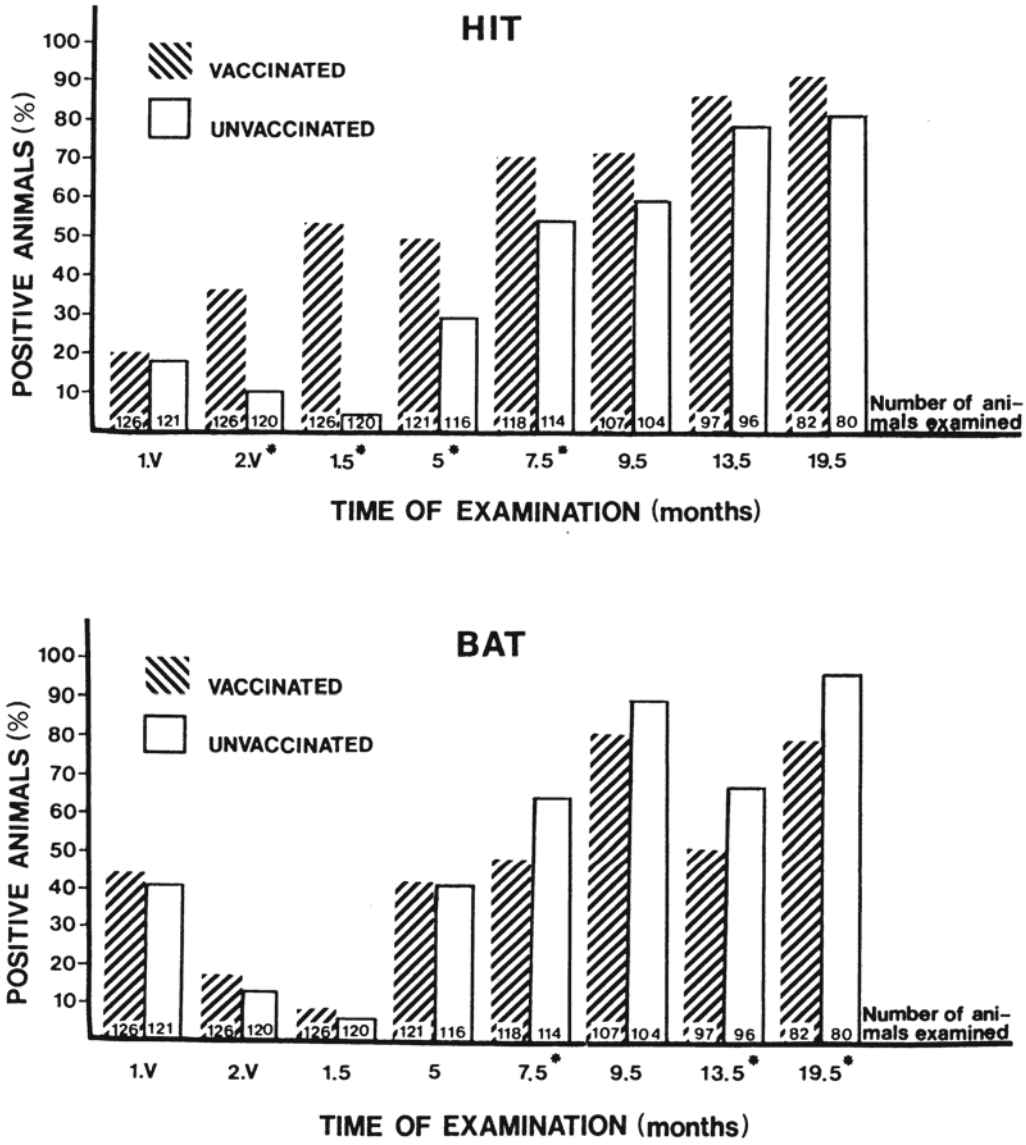

Figure 3. Percentage of vaccinated and unvaccinated animals positive in the hemolysis inhibition test (HIT) and the bacterial agglutination test (BAT) at the first (1.V) and the last (2.V) vaccination, and at different months after the last vaccination.

*) Statistical significant difference between the vaccinated and unvaccinated group.

without such antibodies at the first immunization.

At 13 1/2 months following immunization, 97 of the vaccinated animals were examined. Altogether 50 of these were positive, while 47 were negative in HIT at 1 1/2 months after vaccination. Of the HIT-positive animals superficial swellings were found in 18 % (9 out of 50) during the 13 1/2 months following vaccination, while the corresponding figure for HIT-negative animals was 40 % (19 out of 47). The difference was significant.

Most vaccinated and unvaccinated animals with superficial swellings at the different examinations were positive with high titres in BAT. Most vaccinated animals with such lesions were also positive in HIT. However, some of the unvaccinated animals with swellings were negative in this test. The tendency was most prominent at the first examinations following vaccination.

The prevalence of animals positive in the serological tests after vaccination, and in which superficial swellings were not demonstrated on any examinations up to 13 1/2 months following, is presented in Fig. 4. Significantly more vaccinated than unvaccinated animals were positive in HIT at most of the examinations. The prevalence of HIT-positive animals increased to 80 % in the vaccinated, and to 52 % in the unvaccinated group at 13 1/2 months after vaccination. Many vaccinated and unvaccinated animals, in which superficial swellings were not demonstrated during the first 13 1/2 months following vaccination, were also positive in BAT (Fig. 4). However, many of these animals had low positive titres in this test, the tendency towards low positive titres being most prominent among the vaccinated animals up to 9 1/2 months after vaccination.

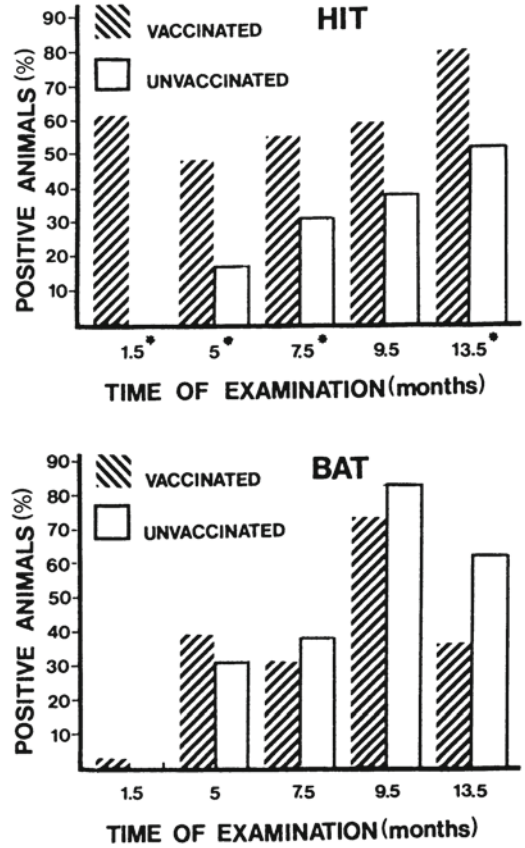

Figure 4. Percentage of animals positive in HIT and BAT at different months after the last vaccination; includes 64 vaccinated and 42 unvaccinated animals present at each and every occasion up to 13 1/2 months following vaccination and in which superficial swellings were not demonstrated on any examination.

*) Statistical significant difference between the vaccinated and unvaccinated group.

Discussion

In the present investigation, vaccination of kids with crude filtrated *C. pseudotuberculosis* toxoid in combination with whole killed cells, reduced the occurrence of superficial swellings during the first 1 to 2 years following immunization.

When the animals were about 1 year old (February), the prevalence of unvaccinated animals with superficial swellings had reached the same level (29 %) as found in 1 year old animals from infected herds in a previous investigation (*Holstad* 1986c). The prevalence of vaccinated animals with superficial swellings did not reach this level at any time in the present study.

The peak incidence of animals with superficial swellings was reached later in the investigation period in the vaccinated than in the unvaccinated group. This could have been due to a higher protective effect of the vaccine during the first months following vaccination.

The present investigation indicated, however, that there was no difference in the clinical course of caseous lymphadenitis in vaccinated and unvaccinated animals. Superficial lesions could persist for several months in animals in both the vaccinated and unvaccinated groups. The slightly lower percentage of vaccinated animals with lesions detected on 2 or more of the examinations was most probably due to the fact that only a few vaccinated animals had superficial swellings at the first examinations. The clinical investigations also indicated that apparently healed superficial lesions could reactivate several months later, an observation reported by several goat farmers.

In some herds vaccination had apparently no effect at all. The number of animals was, however, too small to draw conclusions about differences between herds with regard to the efficacy of vaccination. It should be noted that after the animals were housed for the winter, no measures were introduced to make the infection risk identical for the vaccinated and unvaccinated groups in the herds, for example by placing the same number of vaccinated and unvaccinated animals together in each pen. Some vaccinated animals might therefore have been exposed to a higher infection risk than unvaccinated, or vice versa, in some herds.

Antibody response following vaccination was detected in HIT, but not in BAT, and this was in accordance with *Holstad et al.* (1989). During the first year of life, the prevalence of BAT- and HIT-positive unvaccinated animals showed a similar course as that described in a previous investigation (*Holstad* 1986c). The fact that the prevalence of BAT-positive unvaccinated animals were higher than the prevalence of HIT-positive animals in this group, indicated further that the antibody response following infection can be detected earlier in BAT than in HIT. This observation was in accordance with (*Holstad* 1986b) and *Holstad et al.* (1989). The prevalence of BAT-positive animals was higher in the unvaccinated than in the vaccinated group from 7 1/2 months following vaccination. This was probably due to the fact that more unvaccinated animals developed lesions.

Superficial swellings were more common in vaccinated animals which were negative than in vaccinated animals which were positive in HIT at 1 1/2 months after vaccination. This might indicate that the antitoxin had been of importance in providing protection against development of lesions.

The serological examinations of the animals with superficial swellings indicated that most of the lesions were caused by *C. pseudotuberculosis,* an observation in accordance with *Holstad* (1986a). The serological examinations of the animals, in which superficial swellings were not demonstrated, indicated that many of these animals also became infected during the investigation period. Some of these, both unvaccinated and vaccinated, probably had non-registered superficial swellings or internal lesions caused by *C. pseudotuberculosis.* Abscesses can be

situated in internal lymph nodes and organs only (*Hein & Cargill* 1981, *Anderson & Nairn* 1985, *Batey et al.* 1986), being inaccessible for examination in live animals. It could, however, not be excluded that some animals in the present study might have shown an immune response without developing lesions following infection. The fact that many vaccinated animals without superficial swellings had low BAT-titres, might give reason to believe that some of them had become infected without developing abscesses. However, the possibility that some animals with low positive BAT-titres might have been false positive in the test cannot be excluded.

Only superficial swellings can be recorded by clinical examination of goat herds, making the efficacy of vaccines against caseous lymphadenitis more difficult to evaluate under field conditions than in experiments where necropsy can be performed. However, assuming that vaccination has no influence on the localization of abcesses in the body, vaccines preventing formation of superficial swellings, should be regarded as effective. The vaccine used in the present study were chosen in the light of the possibility that both the exotoxin and cell wall antigens may be important for development of immunity (*Burrell* 1983). Although the vaccine to some extent reduced the occurrence of superficial swellings in the animals, it gave no effective protection. Use of this type of vaccine should not, on the basis of the results obtained in the present study, be recommended as the sole protective measure against caseous lymphadenitis in Norwegian goat herds.

Acknowledgement

The investigation was supported by grants from the Agricultural Research Council of Norway.

References

Anderson VM, Nairn ME: Control of caseous lymphadenitis in goats by vaccination. In: Les maladies de la chevre. Niort 1984, Les Colloques de l'INRA, No. 28, 605–609.

Anderson VM, Nairn ME: An abattoir survey of the prevalence of caseous lymphadenitis in feral goats in Western Australia. Aust. vet. J. 1985, *62,* 385–386.

Augustine JL, Renshaw HW: Survival of Corynebacterium pseudotuberculosis in axenic purulent exudate on common barnyard fomites. Amer. J. vet. Res. 1986, *47,* 713–715.

Batey RG, Speed CM, Kobes CJ: Prevalence and distribution of caseous lymphadenitis in feral gots. Aust. vet. J. 1986, *63,* 33–36.

Brogden KA, Cutlip RC, Lehmkuhl HD: Comparison of protection induced in lambs by Corynebacterium pseudotuberculosis whole cell and cell wall vaccines. Amer. J. vet. Res. 1984, *45,* 2393–2395.

Brown CC, Olander HJ, Biberstein EL, Morse SM: Use of a toxoid vaccine to protect goats against intradermal challenge exposure to Corynebacterium pseudotuberculosis. Amer. J. vet. Res. 1986, *47,* 1116–1119.

Burrell DH: Vaccination against caseous lymphadenitis in sheep. 55th Ann. Conf. Aust. vet. Ass. Proc. 1978, 79–81.

Burrell DH: Caseous lymphadenitis vaccine. New South Wales Vet. Proc. 1983, *19,* 53–57.

Cameron CM: Immunity to Corynebacterium pseudotuberculosis. J. S. Afr. vet. med. Ass. 1972, *43,* 343–349.

Fodstad FH: Vaccination as a control measure for Johne's disease in goats. In: Fodstad FH: 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.

Hein WR, Cargill CF: An abattoir survey of diseases of feral goats. Aust. vet. J. 1981, *57,* 498–503.

Holstad G: Corynebacterium pseudotuberculosis infection in goats II. The prevalence of caseous

lymphadenitis in 36 goat herds in northern Norway. Acta vet. scand. 1986a, *27*, 584–597.

Holstad G: Corynebacterium pseudotuberculosis infection in goats IV. Course of the infection in two recently infected goat herds. Acta vet. scand. 1986b, *27*, 609–616.

Holstad G: Corynebacterium pseudotuberculosis infection in goats III. The influence of age. Acta vet. scand. 1986c, *27*, 598–608.

Holstad G: Corynebacterium pseudotuberculosis infection in goats I. Evaluation of two serological diagnostic tests. Acta vet. scand. 1986d, *27*, 575–583.

Holstad G, Teige J jr.: Corynebacterium pseudotuberculosis infection in goats VII. Clinical, pathological, serological and hematological changes after subcutaneous inoculation of the organism. Acta vet. scand. 1988, *29*, 287–294.

Holstad G, Teige J jr., Larsen HJ: Corynebacterium pseudotuberculosis infection in goats VIII. The effect of inactivated vaccines against experimental infection. Acta vet. scand. 1989, *30*, 275–283.

LeaMaster BR, Shen DT, Gorham JR, Leathers CW, Wells HD: Efficacy of Corynebacterium pseudotuberculosis bacterin for immunologic protection of sheep against development of caseous lymphadenitis. Amer. J. vet. Res. 1987, *48*, 869–872.

Nairn ME, Robertson JP, McQuade NC: The control of caseous lymphadenitis in sheep by vaccination. Proc. 54th Ann. Conf. Aust. vet. Ass., Perth 1977, pp. 159–161.

Nairn ME: The control of caseous lymphadenitis in sheep. Refresher course on sheep. University of Sydney, Post-graduate committee on veterinary science. Proc. 58, 1981, 309–312.

Sammendrag

Corynebacterium pseudotuberculosis infeksjon hos geit IX. Effekten av vaksinasjon mot naturlig infeksjon.

Ett vaksinasjonsforsøk ble utført i 10 infiserte besetninger. Det var ialt 247 dyr med i forsøket, og det var fra 15 til 38 dyr i hver besetning. Omlag halvparten av dyrene i hver besetning ble vaksinert 2 ganger med 3–4 ukers mellomrom. Første vaksinasjon ble utført før dyrene var 4 måneder gamle. Dyrene ble vaksinert med filtrert urenset toksoid og hele drepte bakterier av *C. pseudotuberculosis.*

Prevalensen av dyr med overfladiske hevelser var høyere i den uvaksinerte enn i den vaksinerte gruppa ved undersøkelser foretatt i løpet av de første 1–2 årene etter immunisering. Overfladiske hevelser var imidlertid like vanlig hos vaksinerte som hos uvaksinerte dyr i noen besetninger.

Etter vaksinering ble det påvist antistoffrespons ved antihemolysintest, men ikke ved bakterieagglutinasjonstest. Overfladiske hevelser forekom vanligere hos dyr som var negative enn hos dyr som var positive ved antihemolysintest 1 1/2 måned etter vaksinering.

Den vaksinen som ble benyttet i foreliggende undersøkelse gav for dårlig beskyttelse til å kunne anbefales som eneste beskyttelsestiltak mot kaseøs lymfadenitt i norske geitebesetninger.

(Received June 12, 1988; accepted October 17, 1988).

Reprints may be requested from: Gudmund Holstad, Department of Microbiology and Immunology, Norwegian College of Veterinary Medicine, P. O. Box 8146, Dep., N-0033 Oslo 1, Norway.