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Corynebacterium Pseudotuberculosis Infection in Goats VI. Clinical and Pathological Changes after Intravenous Inoculation of the Organism

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Holstad, G. and J. Teige jr.: Corynebacterium pseudotuberculosis infection in goats VI. Clinical and pathological changes after intravenous inoculation of the organism. Acta vet. scand. 1988, 29, 281–286. – Twelve kids, 5 1/2 months old, were inoculated intravenously with about 1 million colony forming units of a *Corynebacterium pseudotuberculosis* strain isolated from goat. Nine of the animals had antibodies against the organism in the bacterial agglutination test and/or the hemolysis inhibition test before they were inoculated. Four kids developed acute toxemia and died 2–5 days after the inoculation. Three of these animals were negative in both the bacterial agglutination test and the hemolysis inhibition test, while the fourth was positive in the bacterial agglutination test only. Post mortem examination revealed severe icterus, anemia, hemoglobinuria and acute pneumonia with microabscess formation in 3 of the kids that died. Eight animals, all with antibodies against *C. pseudotuberculosis*, developed acute illness but survived the inoculation. These animals were sacrificed and examined post mortem 1 month after the experimental infection, and abscesses were demonstrated in internal organs in all cases.

It is concluded that intravenous inoculation with *C. pseudotuberculosis* is not a suitable challenge system to study the prophylactic efficacy of vaccines against caseous lymphadenitis in goats.

experimental infection; antibodies; icterus; anemia; pneumonia; abscesses.

Introduction

Corynebacterium pseudotuberculosis causes caseous lymphadenitis, a chronic disease in sheep and goats. Skin abrasions are reported to constitute the most important route by natural infection. Several routes of infection have however, been tested experimentally in sheep and goats, and the course of the disease seems to vary depending upon dose and route of inoculation.

In sheep several trials involving the intravenous inoculation of the organism have

been carried out. *Cameron et al.* (1972) found that 9 out of 14 non-immunized sheep given high doses of the bacterium intravenously, died, while 13 out of 14 immunized animals survived. *Brogden et al.* (1984a), who inoculated non-immunized lambs intravenously with different doses of *C. pseudotuberculosis*, demonstrated that doses containing 10^5 to 10^6 colony forming units could result in toxemia and death. *Hsu et al.* (1985) inoculated 3 gnotobiotic lambs and 1 kid intravenously with purified exo-

toxin from *C. pseudotuberculosis*, and observed that the animals developed a severe hemolytic anemia and succumbed within 18 h.

Studies on intravenous inoculation of live *C. pseudotuberculosis* in goats have not previously been carried out. The purpose of the present investigation was to study clinical symptoms and pathological changes in kids after this type of inoculum.

Materials and methods

Animals

The investigation included 12 kids, 7 females and 5 males, that were originally planned for use in a preliminary vaccination trial against *C. pseudotuberculosis* infection.

The animals came from a herd which had not been examined with regard to the possible occurrence of caseous lymphadenitis. According to informations from the farmer, the herd was thought to be free from the disease. However, serological examinations revealed that 5 and 4 kids had antibodies against *C. pseudotuberculosis* in the bacterial agglutination test (BAT) (Holstad 1986) and the hemolysis inhibition test (HIT) (Holstad 1986) respectively, at the age of 3 weeks.

The animals were placed in a thoroughly cleaned and disinfected pen at 3 weeks of age. However, goats with caseous lymphadenitis were housed in a nearby room. The kids were fed milk, hay and pelleted calf concentrates, as well as supplementary minerals and water.

Nine of the animals were immunized twice with inactivated *C. pseudotuberculosis* antigens when they were 2 1/2 and 3 1/2 months old, respectively. The antigens used were whole killed bacteria and crude toxoid. Three kids were immunized with whole killed bacteria only, 3 other animals were given only crude toxoid, while 3 kids were im-

munized with both whole killed bacteria and crude toxoid. All the kids were negative in BAT and HIT prior to the first immunization.

The animals were inspected weekly after immunization. A superficial abscess caused by *C. pseudotuberculosis* was registered in one of the immunized kids at the age of 4 1/2 months.

Serological examinations were also carried out prior to inoculation with *C. pseudotuberculosis*. Altogether 9 kids, 7 immunized and 2 non-immunized, were then seropositive, 4 of these being positive in both BAT and HIT, while 3 and 2 were positive only in BAT and HIT, respectively (Table 1).

Inoculation

The animals were inoculated at the age of 5 1/2 months with a *C. pseudotuberculosis* strain (NVH 2586*) isolated from goat. The strain was cultivated in brain heart infusion broth (Difco) (250 ml) for 2 days at 37°C on a shaker. Enumeration of the bacteria was carried out using the plate count method, 1 ml of the culture containing 1 million colony forming units. The bacteria in culture (50 ml) were pelleted by centrifugation, and then washed once in phosphate buffered saline (PBS). After a second centrifugation, the bacteria were resuspended in 50 ml PBS. Each animal was inoculated with 1 ml of this bacterial suspension in vv. jugularis.

Clinical and post mortem examinations

The animals were examined before inoculation, and on each day during the following week. The body temperature was also measured.

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

Animals dying as a result of inoculation were subjected to post mortem examination shortly after death. The other kids were killed one month after inoculation and post mortem examination immediately performed. Sections for histological examinations were collected from lungs, liver, kidney and abscesses. The sections were stained with hematoxylin and eosin.

Samples for bacteriological examination were taken from lungs, spleen, liver and kidney in the animals that died after inoculation, and from abscesses in the kids that survived the experimental infection. Cultivation was carried out on blood agar plates, the plates being incubated for 48 h at 37°C in 10% CO₂ atmosphere. Bacterial colonies suspected to be *C. pseudotuberculosis* were identified according to *Buchanan & Gibbons* (1974).

Results

Table 1 presents the relationship between positive/negative titres in BAT and HIT in the kids before inoculation, and survivals and deaths afterwards. In the following presentation, the animals are considered as 2 groups, i.e. those that died, and those which survived the inoculation, respectively.

Animals that died following inoculation

Four animals died, 3 of these being negative in both BAT and HIT, while the fourth was positive in BAT only (Table 1). All these kids showed high fever, respiratory distress, anorexia and rumen atonia. Three of the animals showed pale, yellow injected sclera before death, while one developed hematuria.

Post mortem examination of these 3 animals revealed severe icterus, anemia and hemoglobinuria. Microabscess formation and incipient inflammatory lesions in the lungs were seen on histological investigation. In

Table 1. Relationship between bacterial agglutination test (BAT) and hemolysis inhibition test (HIT) titres before i.v. inoculation with *C. pseudotuberculosis*, and the fate of the animals. S: survival. D: death.

Animal number	BAT	HIT	S/D (*)
1 i	pos.	pos.	S
2 i	pos.	pos.	S
3 i	pos.	pos.	S
4 i	pos.	pos.	S
5 i	pos.	neg.	S
6 ni	pos.	neg.	S
7 ni	pos.	neg.	D (5)
8 i	neg.	pos.	S
9 i	neg.	pos.	S
10 i	neg.	neg.	D (3)
11 i	neg.	neg.	D (4)
12 ni	neg.	neg.	D (2)

i) Immunized.

ni) Non-immunized.

*) Days from inoculation to death.

kid no. 7, the mucous membranes were just pale. The most significant pathological lesion in this kid was an acute pneumonia with alveolar epithelial proliferation.

C. pseudotuberculosis was isolated from the lungs in kids no. 7, 10 and 12, and from spleen in kid no. 12.

Animal that survived inoculation

The eight kids in question were all positive in BAT and/or HIT (Table 1). On the day after inoculation, all these animals showed respiratory distress, in most cases associated with high fever. Body temperature dropped, however, to normal values within 5 days.

The distribution of abscesses seen in these animals at post mortem is shown in Table 2. The abscesses seen in the lungs of kids no. 2 and 3 were relatively small (diameter 1–2 mm) and few in number. The lung abscesses in the other animals were, however, not only

Table 2. Post mortem findings in 8 kids killed 1 month after i.v. inoculation with *C. pseudotuberculosis*. Presence of abscesses signified by a +.

Localization of abscesses	Animal number							
	1i	2i	3i	4i	5i	6ni	8i	9i
Lnn. mandibulares	-	-	-	-	-	+	-	-
Lnn. retro-pharyngeales	-	-	-	-	-	+	-	-
Lungs	+	+	+	+	+	+	+	+
Lnn. bronchiales	-	-	+	-	-	+	-	-
Lnn. mediastinales	-	-	-	-	-	+	-	-
Heart	-	-	-	-	+	-	-	-
Liver	-	-	-	-	+	+	-	-
Kidneys	+	-	-	-	+	-	-	-
Bone	-	-	+	-	-	-	-	-
Subcutaneous*	+	-	-	+	+	-	+	+

i) Immunized.

ni) Non-immunized.

*) At/near site of inoculation (vv. jugularis).

more numerous but also more prominent, their diameter in general varying from 1 to 2 cm. Abscesses were also observed in the kidneys, liver and heart.

C. pseudotuberculosis was isolated from abscesses in 7 of the 8 kids.

Discussion

In the present investigation, most kids with antibodies against *C. pseudotuberculosis* in BAT and/or HIT survived experimental intravenous infection with the bacterium, while animals without such antibodies died. Anemia, severe icterus, hemoglobinuria and acute pneumonia were observed in 3 of the animals that succumbed. *Cameron et al.* (1972) made similar observations after intravenous inoculation of non-immunized sheep, and concluded that the effect on the animals was due to the exotoxin. *Hsu et al.* (1985) showed that the hemolytic crisis following intravenous inoculation of the organism in lambs was due to the exotoxin. Acute toxemia and death has also been re-

ported in seronegative sheep after placing purulent material on fresh skin cuts (*Nairn et al.* 1977), and in seronegative goats after subcutaneous inoculation of high doses of a fresh broth bacterial culture (*Abdel-Hamid* 1973). *Carne & Onon* (1978) found that the exotoxin of *C. pseudotuberculosis* splits sphingomyelin in endothelial cells from sheep aorta, and sphingomyelin in cell membrane of erythrocytes from sheep and rabbits in vitro. In the present study, post mortem examination revealed hemolysis following inoculation of the organism in 3 of the animals. This may indicate that the exotoxin also has a toxic effect on the cell membrane of erythrocytes in goats in vivo. Antibodies against the exotoxin are detected in HIT (*Knight* 1978). The present investigation indicates that such antibodies may be of importance in protecting goats against the otherwise lethal effects following intravenous inoculation of large doses of the organism.

Abscesses were demonstrated in the lungs of all surviving animals, and occasionally also in other organs. Abscess formation in several internal organs after intravenous inoculation of *C. pseudotuberculosis* has been found in lambs (*Brogden et al.* 1984a). *Cameron et al.* (1972) and *Brogden et al.* (1984b) found that vaccination of sheep did not prevent abscess formation in lungs after intravenous challenge with the organism. These investigations revealed, however, that vaccinated animals had fewer abscesses than the unvaccinated. *Cameron et al.* (1972) found that this effect was dependent on the vaccine used. *Brogden et al.* (1984a) were further able to demonstrate that the number of abscesses in the lungs after intravenous infection correlated with the inoculation dose. In the present study, kids with antibodies against *C. pseudotuberculosis* were apparently protected against the lethal effects of

living bacteria injected intravenously. Nevertheless, it appeared that antibodies were unable to prevent the establishment of bacterial foci with subsequent abscess formation in lungs and other organs.

In the present study, caseous lymphadenitis was demonstrated in one animal before inoculation. Furthermore, the serological examinations indicated that other kids were also carrying natural infection with *C. pseudotuberculosis* prior to experimental infection. Some of the animals had maternal antibodies against *C. pseudotuberculosis* at purchase 3 weeks old, which indicates that they came from an infected herd. The kids could have been infected from animals in this herd, or perhaps from goats housed in a nearby room during the investigation period. *Jubb & Kennedy* (1970) reported that, in young animals, caseous lymphadenitis tends to be confined to the superficial lymph nodes, development of lesions in internal organs being most common in older animals. On this basis, abscesses seen in internal organs in the animals in the present investigation, most probably developed as a direct consequence to the intravenous inoculation of *C. pseudotuberculosis*.

Intravenous inoculation has been recommended as a suitable challenge system to study the effect of vaccines against *C. pseudotuberculosis* infection (*Cameron et al.* 1972, *Brogden et al.* 1984a). Intravenous infection is, however, not the normal route of infection under field conditions, and, moreover also seems, judged on the present results, to cause too drastic effects. It could thus be argued that more suitable challenge systems should be chosen to study the effect of vaccines against caseous lymphadenitis.

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Sammendrag

Corynebacterium pseudotuberculosis infeksjon hos geit VI. Kliniske og patologiske forandringer etter intravenøs inokulering av bakterier.

Tolv kje ble 5 1/2 mnd. gamle infisert intravenøst med ca. 1 million kolonidannende enheter av *Corynebacterium pseudotuberculosis*. Ni av dyrene hadde antistoffer mot *C. pseudotuberculosis* ved bakterieagglutinasjonstest og/eller antihemolysintest, før de ble eksperimentelt infisert. Fire kje ble akutt syke og døde 2-5 dager etter at de ble infisert. Tre av disse dyrene var negative både ved bakterieagglutinationstest og antihemolysintest

mens det fjerde var positivt ved bakterieagglutinationstest. Det ble påvist uttalt ikterus, anemi, hemoglobinuri og akutt pneumoni med mikroabscessdannelse hos 3 av dyrene. Åtte dyr som alle hadde antistoffer mot *C. pseudotuberculosis*, fikk akutte symptomer, men overlevde den eksperimentelle infeksjonen. Det ble påvist abscesser i indre organer hos alle disse kjeene ved obduksjon 1 måned etter inokulering.

Det konkluderes med at det er lite hensiktsmessig å benytte intravenøs belastning med *C. pseudotuberculosis* til å studere effektiviteten til vaksiner mot kaseøs lymfadenitt hos geit.

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