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Caseous Lymphadenitis in Goats: The Pathogenesis, Incubation Period and Serological Response after Experimental Infection

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ABSTRACT

Twenty goats, in two groups of 10, were injected intradermally with *Corynebacterium pseudotuberculosis*. The doses of infection were 1×10^5 and 5×10^4 colony-forming units (cfu) for groups 1 and 2, respectively. Thereafter, a goat from each group was killed every 2–3 days and examined for gross and microscopic caseous lesions in the draining lymph nodes. Bands or zones of macrophages and polymorphonuclear granulocytes were observed microscopically on the second day of infection in both groups. Gross caseous lesions were observed from days 8 and 9 of infection, respectively. Positive bacterial agglutination test and haemolysis inhibition test titres were detected after 15–17 days and 20–25 days of infection, respectively. These results indicated that caseous lymphadenitis is a subacute disease with an incubation period of 8–9 days, but that it is not detectable serologically until after 15 days of infection.

Keywords: Corynebacterium pseudotuberculosis, diagnosis, goat, lymphadenitis, pathogenesis, serology

Abbreviations: BAT, bacterial agglutination test; CLA, caseous lymphadenitis; HIT, haemolysis inhibition test

INTRODUCTION

Caseous lymphadenitis (CLA) is reported to be a chronic disease of sheep and goats that causes economic losses due to loss of body condition and subsequent reproductive failure (Gates *et al.*, 1977; Stoops *et al.*, 1984; Batey, 1986a). The disease is characterized by encapsulated abscesses in superficial lymph nodes, although it frequently disseminates into the visceral lymph nodes and organs (Stoops *et al.*, 1984). Infection takes place through superficial skin wounds and abrasions. The organism, both free and phagocyte-borne, then moves into the regional draining lymph node (Batey, 1986b). Lesions arise from intracellular bacterial multiplication, causing rapid death of the host's cells (Jolly, 1965; Hard, 1969). The size of the lesion probably varies with the initial number of organisms, the rate of multiplication and the accessibility of

the lesion to the host's defence cells (Batey, 1986b). Histological changes preceding the development of lesions or the incubation period have not been reported previously. Knowledge of the incubation period would be important in the diagnosis of the disease and as an indicator of the length of the period from infection to the shedding of the organism into the environment (Holstad, 1986). In addition, the relationship between the incubation period and the serological response has not been determined. It is not known how soon after infection CLA can be serologically detected. This investigation determined the sequence of pathological changes leading to CLA lesions in goats, the length of the incubation period and the value of serological tests in early detection of the disease.

MATERIALS AND METHODS

Animals

Twenty male Small East African goats aged, by dentition, about 6 months were prescreened for CLA by physical and serological examination. They were randomly divided into two groups of 10. Each group was housed separately in a concrete pen with straw bedding. The goats were sprayed with an acaricide (Amitraz, 12.5% W/V, Kenya Swiss Co. Ltd, Nairobi, Kenya) once a week for the first two weeks. They were drenched twice, at a one-week interval, with albendazole (Valbazine, Kenya Swiss Co.) at a dose rate of 5 mg/kg. They were fed on a standard diet of hay and wheat bran throughout the study period. Water was provided *ad libitum*. They were allowed a two-week acclimatization period before the commencement of the experiment.

Infectious material

Caseous abscess material was produced in a rabbit by subcutaneous injection of 100 µl of a brain–heart infusion broth culture of *Corynebacterium pseudotuberculosis* emulsified in Freund's incomplete adjuvant. When the resultant abscess ripened, it was lanced and the abscess material was collected into sterile universal bottles. The concentration of bacteria in the abscess material was determined by a plate count method (Baker and Breach, 1980). The material was then diluted in sterile Ringer's solution to the required bacterial concentration.

Infection procedure

Each goat was injected in two sites. A volume of 100 µl of the infective material was injected intradermally approximately 5 cm caudal to each of the right prescapular and precrural lymph nodes. The doses of infection were 1×10^5 and 5×10^4 colony-forming units (cfu) for groups 1 and 2, respectively. The contralateral lymph nodes served as controls in each animal.

Post-mortem examination

A goat from each group was killed every 2–3 days starting from day 2 during the first 10 days of infection and randomly thereafter (Table I). Post-mortem examinations were then carried out. The test and control lymph nodes were excised, stripped of extracapsular tissues and weighed. They were then cut into thin slices and examined for abscesses. Lymph node samples for histopathology were fixed in 10% neutral buffered formalin solution, processed routinely, sectioned at a thickness of 5 μm and stained with haematoxylin and eosin (H&E).

Serology

A 10-ml sample of blood for serology was collected from each goat immediately before experimental infection and also at the time of slaughter. The haemolysis inhibition test (HIT) and the bacterial agglutination test (BAT) were performed as described by Lund and colleagues (1982) and by Kuria (1989). Titres were expressed as $\log_{10}$ of the reciprocal value of the highest dilution of serum that inhibited haemolysis (HIT) or agglutinated antigen (BAT). Titres $\geqslant 0.6$ and $\geqslant 2.1$ were considered positive for HIT and BAT, respectively (Lund *et al.*, 1982; Kuria, 1990).

Statistical analysis

Differences in weights between the test and control lymph nodes within groups (effect of infection) and between groups (effect of dose) were analysed by one-way analysis of variance.

RESULTS

Lymph node weights

The mean weights of the test and control lymph nodes are shown in Figure 1. The test lymph nodes were heavier than the control ones in both groups ($p < 0.05$). The higher dose of infection caused a greater increase in the weights of test lymph nodes than the lower dose ($p < 0.05$).

Pathology

The injection sites developed caseous abscesses measuring between 3 and 12 mm in diameter. Five goats, two from the high infection dose group, and three from the low infection dose group had only necrotic scars. The abscesses were similar in histological appearance in both groups.

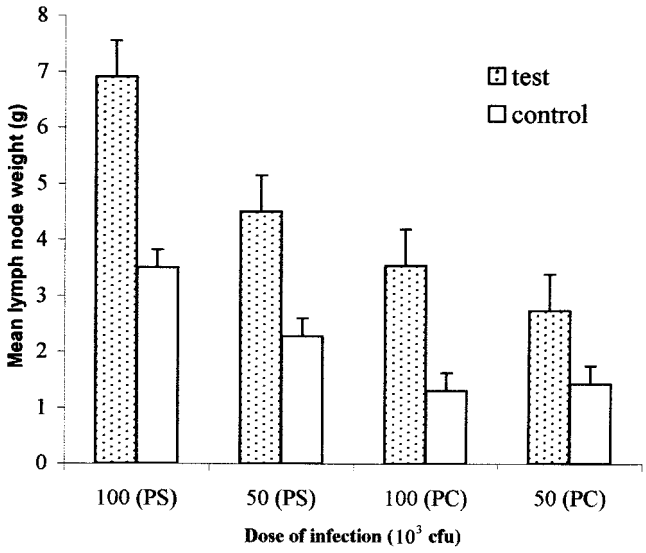
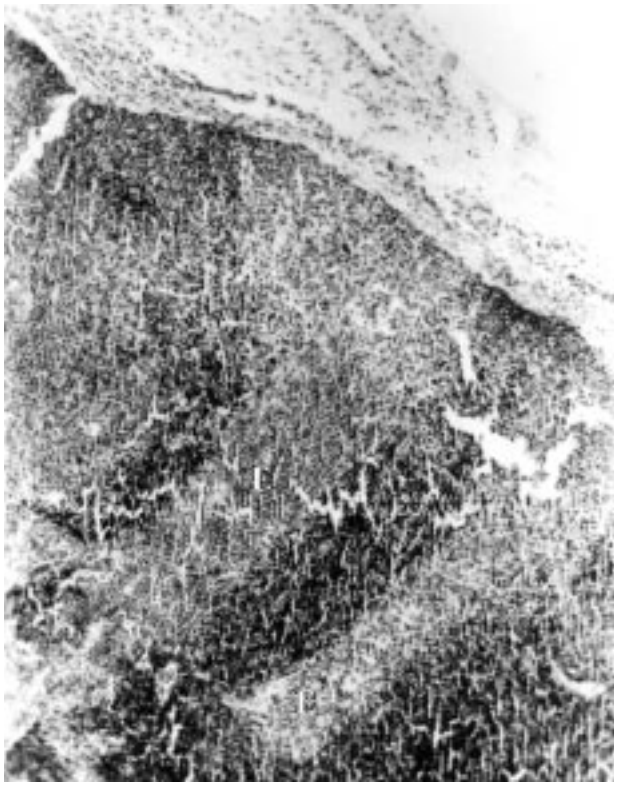


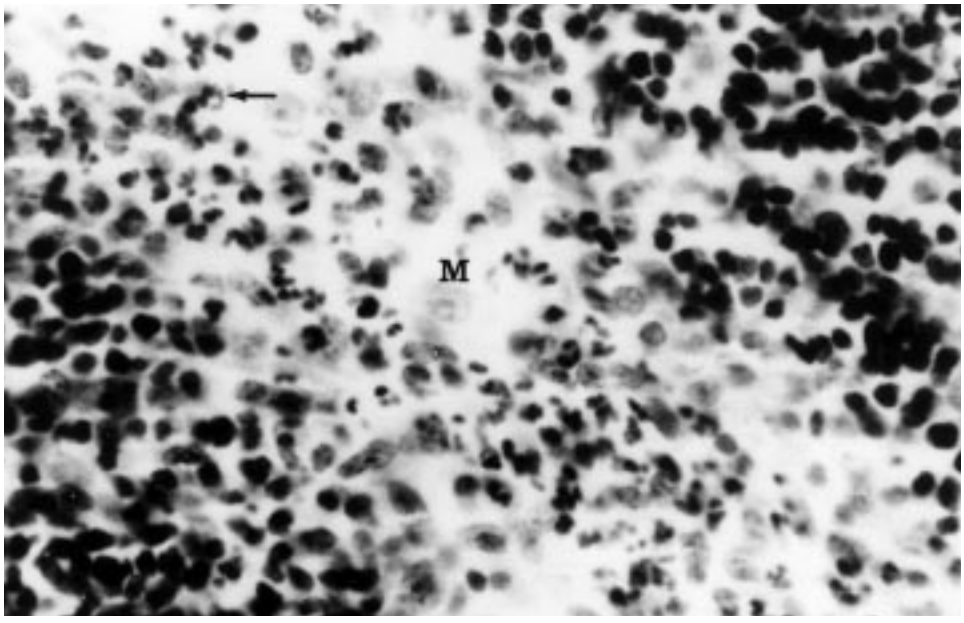
Figure 1. Effect of dose and site of infection of *C. pseudotuberculosis* on lymph node weights. Error bars represent the standard error of the mean. PS, prescapular lymph nodes; PC, precrural lymph nodes

The microscopic lymph node lesions seen between days 2 and 7 consisted of infiltrating bands or zones of macrophages and polymorphonuclear cells (polymorphs). The infiltration started from the cortical sinuses and extended towards the medulla, especially along trabecular sinuses (Figure 2). The cortical and the medullary sinuses were oedematous. Follicular hyperplasia was also observed.

With the exception of one goat in the low-dose group, all the goats killed 8 days or later after infection had gross lymph node lesions. The caseous abscesses ranged from about 0.25 to 3 mm in diameter. Histologically, the abscesses appeared as foci of pyogranulomatous lesions in different stages of development (Figure 3a). The lesions tended to be confined to the half of the cortex next to the afferent lymph vessels. Some foci had necrotic centres of nuclear debris, while others had macrophages and polymorphonuclear cells in various stages of degeneration. Immature epithelioid cells and large macrophages surrounded the necrotic centres of the foci (Figure 3b). The capsule in the affected areas of the lymph nodes was thickened by fibrosis. Test lymph nodes examined after 14 days of infection had one or more abscesses ranging from 1.5 to 11 mm in diameter. Histologically, the abscesses consisted of a necrotic centre of disintegrating macrophages, polymorphs and nuclear debris, surrounded by large macrophages, epithelioid, giant and plasma cells. They were in turn surrounded by a fibrous capsule. A microscopic granuloma was observed in one test lymph node of a goat without gross lesions. It was located close to the capsule and was surrounded by a zone of fibrosis.



A



B

Figure 2. Photomicrographs (A, original magnification ×100; B, original magnification ×630) of a draining lymph node section from a goat 2 days after intradermal infection with 1×10^5 cfu of *C. pseudotuberculosis*, showing bands (E) of inflammatory cells infiltrating from the cortex towards the medulla. The cells were composed of macrophages (M) and polymorphonuclear cells (arrowed)

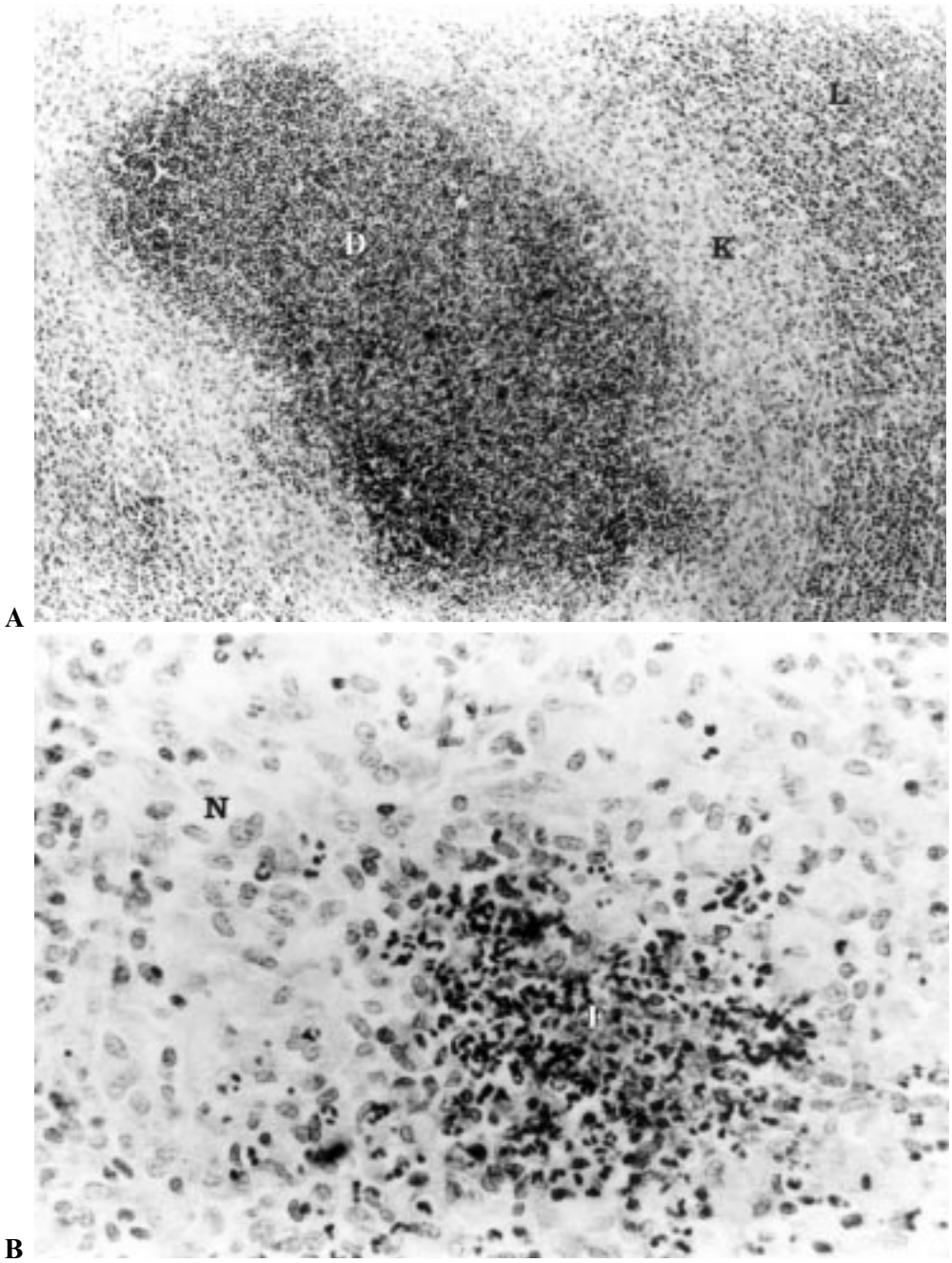


Figure 3. Photomicrographs (A, original magnification $\times 100$; B, original magnification $\times 400$) of draining lymph node sections from a goat 9 days after intradermal infection with 5×10^4 cfu of *C. pseudotuberculosis*: In (A), D is a pyogranulomatous lesion surrounded by a zone (K) of large macrophages compared with normal lymph node tissue (L). In (B), F is a necrotic focus surrounded by large macrophages and epithelioid cells (N)

Neither gross nor microscopic lesions were observed in the contralateral control lymph nodes, although follicular hyperplasia was occasionally seen.

Serology

Bacterial agglutination titres were negative (<2.1 units) between days 2 and 14 and positive on and after day 17 of infection, while HIT titres were negative (<0.6 units) between days 2 and 17 and positive on and after day 20 (Table I).

DISCUSSION

Serial slaughter of the infected animals allowed observation of the progressive development of CLA lesions. Microscopic lesions in draining lymph nodes were observed two days after intradermal infection. The microorganism is believed to reach the draining lymph nodes via the lymphatics, either free or within phagocytes (Batey, 1986b). The reaction started as accumulations of macrophages and polymorphonuclear cells in the cortical sinuses and extended to the trabecular sinuses. The reaction then progressed to form several foci of pyogranulomatous lesions that coalesced to form one or more granulomas, which then became encapsulated and contained.

In this study, the incubation period for the development of gross lesions of CLA was 8–9 days after intradermal infection. This has not been documented previously. After subcutaneous, intradermal or submucosal infection in goats, Ashfaq and Campbell (1980) observed abscesses 45–147 days post-infection. This study demonstrated that abscesses form long before that.

The appearance of lesions of CLA did not coincide with seropositivity in either BAT or HIT. Although macroscopic caseous lesions appeared by days 8–9, seropositivity was not observed until between days 15 and 17 in BAT and days 20 and 25 in HIT. In naturally infected animals, it has been observed that some animals with lesions of CLA have no detectable antibody titre, even when the causative agent can be removed from the lesions (Brown *et al.*, 1986; Kuria, 1989). This might be explained by the fact that the BAT and HIT titre only became positive one week and two weeks, respectively, after the development of lesions. The overall implication is that serology is not effective in detecting cases of CLA that are less than 1–2 weeks old.

Caseous lymphadenitis is usually introduced into disease-free herds by newly acquired animals (Holstad, 1986). In Kenya, goat improvement centres provide facilities for hiring out breeding males to goat farms. Such males can introduce CLA either from the farms to the centres or vice versa.

Our study highlights the characteristics of *C. pseudotuberculosis* infection in the initial subacute phase of the disease. Knowledge of the incubation period will facilitate tracing the source of infection in a previously disease-free herd. An isolation period of at least 20 days, followed by a BAT test, is recommended before introduction of new animals into a clean herd.

TABLE I
Relationship of pathological changes to antibody titres in goats following experimental infection with *Corynebacterium pseudotuberculosis*

Dose of infection	Goat no.	Day of slaughter	Reaction at injection[a]				Antibody titre[b]			
			Site 1		Site 2		BAT		HIT	
			Ln	Skin	Ln	Slin	0	T	0	T
1×10^5 cfu	33	2	–	+[c]	–	+	1.2	1.2	<0.3	<0.3
	34	3	–	+	–	+	1.2	1.2	<0.3	<0.3
	35	4	–	+	–	+	1.8	1.5	<0.3	<0.3
	36	6	–	+	–	+	1.5	1.5	<0.3	<0.3
	73	8	+	+	+	+	1.2	1.5	<0.3	<0.3
	38	11	+	+	+	+	1.2	1.5	<0.3	<0.3
	39	17	+	+	–	+	1.5	3.0	<0.3	0.3
	40	25	–	+	+	+	1.8	3.0	<0.3	0.6
	37	30	+	H[c]	+	H	1.2	3.3	<0.3	1.2
	41	34	+	H	+	H	1.2	3.0	<0.3	2.4
5×10^4 cfu	43	–	+	+	+	1.2	1.2	<0.3	<0.3	
	49	5	–	+	+	+	1.5	1.5	<0.3	<0.3
	44	7	–	+	+	+	1.5	1.5	<0.3	<0.3
	46	9	+	+	+	+	1.2	1.2	<0.3	<0.3
	47	11	+	+	+	+	1.5	1.5	<0.3	<0.3
	48	14	–	+	+	+	1.2	1.8	<0.3	<0.3
	50	17	+	Nec[c]	+	Nec	1.5	2.7	<0.3	<0.3
	51	20	–	Nec	–	Nec	1.2	3.6	<0.3	1.2
	52	27	+	+	+	+	1.8	3.6	<0.3	0.6
	42	30	+	+	+	H	1.8	2.7	<0.3	0.9

[a]Site 1, prescapular; site 2, precrural; Ln, lymph node; [b]0, pre-experimental sera; T, sera at slaughter; [c]+, abscessed; H, healed; Nec, necrosis

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