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A serological investigation of caseous lymphadenitis in four flocks of sheep

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A double antibody sandwich ELISA developed by ID-DLO, Lelystad to detect *Corynebacterium pseudotuberculosis* infection was used on 329 sheep from four pedigree Suffolk flocks in which clinical cases of caseous lymphadenitis (CLA) had occurred. At subsequent necropsy, typical CLA lesions were seen in 133 sheep, and the diagnosis was confirmed on culture. Lesions were most commonly seen in lungs (n=46), parotid lymph nodes (n=44), prescapular lymph nodes (n=38) and mediastinal lymph nodes (n=31). The sensitivity of the ELISA test for detecting culture-positive sheep was 0.88, while the specificity of the test was 0.55. The antibody ELISA detected 87.5 *per cent* of sheep that had CLA lesions restricted to internal organs only. It was concluded that the ELISA test has a valuable role in detecting sheep with both clinical and subclinical CLA.

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Introduction

Caseous lymphadenitis (CLA) is caused by the bacterium *Corynebacterium pseudotuberculosis*. Although it can affect a number of domestic species and man, it is as a disease of sheep that CLA is most important. *C. pseudotuberculosis* causes chronic abscessation in lymph nodes (LN) and viscera. The disease is present in many countries throughout the world and was first recorded in sheep in Northern Ireland in 1999, when the source of the infection was traced to imported Scottish sheep. CLA is a notifiable disease in both Northern Ireland and the Republic of Ireland, but restrictions have been removed in Great Britain since 1991.

Baird (2003) listed a number of factors that make the control of CLA difficult. CLA may be detected clinically only in superficial LN, whereas in a proportion of affected sheep the lesions may be present only in visceral LN or organs such as the lungs. CLA has a relatively long incubation period, of between two and four months, before it may be detected clinically. In addition, *C. pseudotuberculosis* may survive for long periods in the environment, thus providing a source of infection in the absence of clinical cases.

Dercksen *et al.* (2000) evaluated a double antibody sandwich ELISA test for the detection of CLA in healthy sheep from CLA-free flocks and in sheep with culture-confirmed CLA. They found that this ELISA test had a specificity of 99±1% and a sensitivity of 79±5%. The present study was designed to evaluate its use in four naturally infected flocks.

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Materials and methods

Flock histories

The study was undertaken in 329 sheep (63 male and 266 female) from four flocks, which were depopulated in 2001 and 2002 because of widespread CLA infection.

Flock A

CLA was first detected on this farm in July 2001 during a clinical examination of the flock that had been prompted by a backward trace of contacts from flock B. The flockowner reported seeing similar lesions in his sheep since December of the previous year, but did not realise that the cause was CLA. The source of the infection was considered to be two rams that were purchased from Scotland in August 2000. Subsequently, this flock was depopulated completely in batches until July 2002.

Flock B

CLA was first detected on this farm by a clinical examination of the flock in June 2001. The most probable source of infection was a ram purchased from farm A in late October 2000. Subsequently this flock was depopulated completely in batches until October 2001.

Flock C

CLA was first detected on this farm by a clinical examination of the flock in August 2001. The source of the infection was considered to be a ewe, purchased in November 1999, that had originated from the same flock in Scotland that was considered to have infected flock A. Subsequently, this flock was depopulated completely in batches until February 2002.

Flock D

CLA was first detected on this farm by a clinical examination of the flock in August 2001. The source of infection was traced to five ewes purchased from flock A in December 2000. Forty-one in-contact sheep were depopulated in batches until November 2001. On repeated clinical examinations, the remaining sheep, which were separately managed, were consistently found to be negative for CLA.

Serology

All sheep were blood sampled for the ELISA test prior to euthanasia and postmortem examination. Optical density (OD) readings for the ELISA had been determined from experience of using the test in The Netherlands (Daan Dercksen, personal communication). An OD value above 400 was considered positive, values between 200 and 400 were considered 'low positive', those between150 and 200 were inconclusive and those less than 150 were negative. A non-specific reaction was identified when the OD with the negative antigen was more than 50% of the maximum OD of the positive control.

Postmortem examination

All sheep were necropsied using a standard protocol. An external examination of the carcase was carried out, noting any sinus discharges or subcutaneous swellings. The superficial and visceral lymph nodes, lungs and liver were then examined for abscessation. Suspect lesions were cultured for *C. pseudotuberculosis* (Cowan and Steel, 1974).

Figure 1: *Corynebacterium pseudotuberculosis* abscessation in parotid lymph node.

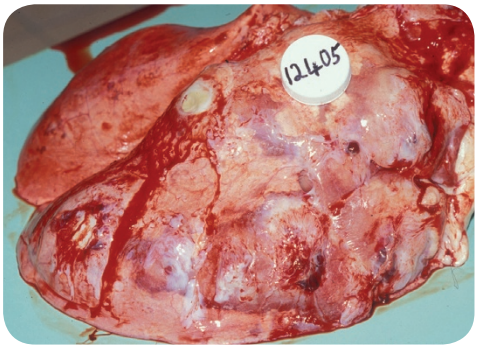

Figure 2: *Corynebacterium pseudotuberculosis* abscessation in sheep lungs.

Results

On postmortem examination, 133 of 329 sheep had lesions typical of CLA **(Figures 1 and 2)**; these were confirmed on culture (**Table 1**). The lesions were present in either single or multiple sites (**Table 2**: 221 sites in 133 sheep). Lesions in superficial lymph nodes (LN) were most frequently present in the parotid LN (n=44) and the prescapular LN (n=38). Internal lesions were most frequently present in the lungs (n=46) and mediastinal LN (n=31).

Table 1: Prevalence of caseous lymphadenitis (CLA) lesions in the four flocks of sheep

Flock	No.of sheep examined	No.of sheep culture-positive for CLA	Prevalence of CLA in flocks (%)
A	98	35	35.7
B	95	37	38.9
C	100	55	55.0
D	36	6	16.7
All four flocks	329	133	40.4

Lesions were present in internal organs only in 32 (24.1 *per cent*) of the 133 sheep (**Table 3**); of these 28 (87.5 *per cent*) were ELISA-positive.

Three hundred and eight sheep were defined as either ELISA-positive or ELISA-negative, 18 sheep gave inconclusive tests and three gave non-specific results. When inconclusive and non-specific results were treated as negative, the overall sensitivity of the ELISA for detecting CLA lesions was 0.88 and the specificity was 0.55 (**Table 4**). Sensitivity varied in the different flocks from 0.83 to 1.0 and specificity varied from 0.31 to 0.87 (**Table 5**).

Table 2: Distribution of caseous lymphadenitis (CLA) lesions in 133 sheep

Site	Number (%) of sheep with CLA lesions	Site	Number (%) of sheep with CLA lesions
Lungs	46 (35%)	Bronchial LN	9 (7%)
Parotid LN	44 (33%)	Submandibular LN	8 (6%)
Prescapular LN	38 (29%)	Liver	5 (4%)
Mediastinal LN	31 (23%)	Supermammary LN	4 (3%)
Retropharyngeal LN	11 (8%)	Mesenteric LN	4 (3%)
Prefemoral LN	10 (8%)	Other sites	11 (8%)

LN: lymph node.

Table 3: Distribution of caseous lymphadenitis (CLA) lesions in 133 sheep

Flock	A	B	C	D	All 4 flocks (%)
Number of sheep with CLA lesions	35	37	55	6	133 (40.4%)
Number of sheep with superficial LN and cutaneous lesions only	13	16	37	3	69 (21.0%)
Number of sheep with internal lesions only (lungs and visceral LN)	12	11	8	1	32 (9.7%)
Number of sheep with both internal and external CLA lesions	10	10	10	2	32 (9.7%)
Total number of sheep examined	98	95	100	36	329

LN: lymph node; CLA: caseous lymphadenitis.

Table 4: Sensitivity and specificity of the ELISA test for caseous lympadenitis (CLA)

CLA lesion	positive	negative	total	
Detected	117	16	133	Sensitivity 0.88
Not detected	88	108	196	Specificity 0.55
Total	205	124	329	

Table 5: Sensitivity and specificity of ELISA test in individual flocks

Flock	Sensitivity	Specificity
Flock A (n=98)	0.83	0.59
Flock B (n=95)	0.86	0.53
Flock C (n=100)	0.91	0.31
Flock D (n=36)	1.00	0.87
All 4 flocks (n=329)	0.88	0.55

Discussion

Brown and Olander (1987) reported that the introduction of a single CLA-abscessed animal into a naïve flock results in a high incidence of abscesses within two to three years. In the present study CLA abscesses (**Table 1**) were detected in 133 animals in these four flocks between seven and 21 months after the introduction of CLA-affected sheep (flock A: 11 months; flock B: seven months; flock C: 21 months; flock D: eight months). Intensive husbandry methods in these flocks may have contributed to the relatively rapid spread of infection.

The present study demonstrated that CLA lesions were present only in the viscera (lungs and visceral LN) in approximately 24 *per cent* of clinical cases. Consequently, clinical examination alone will not detect all infected sheep in a flock. Forty-six of the 133 CLA-positive sheep had lung abscesses. These lesions may lead to more rapid spread of the disease when sheep are closely confined, as for shearing and routine treatments, or when housed.

In this study, the ELISA test was relatively sensitive at detecting CLA-infected sheep. However, the specificity of the test in detecting animals with lesions was low (**Table 3**). This contrasts with the experience of Dercksen *et al* (2000), who found that the ELISA test had a specificity of 99±1% and a sensitivity of 79±5%. In their study, the sensitivity and specificity were determined in sera from sheep with clinical abscesses from which *C. pseudotuberculosis* was isolated and in sera from healthy sheep in flocks without a record of CLA. The present study differs in that both CLA-positive and CLA-negative sheep were drawn from infected flocks.

Dercksen *et al* (2000) suggested that cross-reactions may occur due to infections with other bacteria (for example, other *Corynebacterium* species, *Listeria monocytogenes* and *Mycobacterium avium* subspecies *paratuberculosis*). Evidence of these bacterial infections was not found in the four flocks examined. Batey (1986) suggested that many CLA lesions undergo resolution during the early stages of development. In the present study, it is more likely that seropositive, culture-negative sheep resulted from sheep in these flocks being exposed to infection, but either subsequently not developing the disease or eliminating the infection. The wide variation in specificity between flocks may be attributed to differences in time exposed to *C. pseudotuberculosis*.

Twenty-eight of the 32 sheep with internal lesions only were ELISA-positive. These results indicate that ELISA testing could play a valuable role in CLA control by supplementing clinical detection of superficial lesions. It is anticipated that such ELISAs will become commercially available in the near future.

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