Seroepidemiological Evidence that Shearing Wounds are Mainly Responsible for *Corynebacterium pseudotuberculosis* Infection in Sheep

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ABSTRACT. Anti-*Corynebacterium pseudotuberculosis* toxin titers were examined by an enzyme-linked immunosorbent assay (ELISA) at 3 to 5 month intervals in sheep of three age groups (lambs, 1- and 2-year-old sheep). A significant increase in the ELISA-positive ratio was found only in July, 3 months after shearing, in 1- and 2-year-old sheep. A decrease in the positive ratio was found in the same month in lambs, possibly due to a decrease in maternal antibodies. Since the 1- and 2-year-old sheep had been sheared in April, it was considered that the increase in the positive ratio in July might be closely related to the shearing in April—KEY WORDS: *Corynebacterium pseudotuberculosis* infection, ELISA-positive ratio, shearing wound.

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In most cases of caseous lymphadenitis (CLA) in sheep caused by *Corynebacterium pseudotuberculosis* it has been assumed that the infection is introduced through superficial skin wounds from shearing, tagging, docking, castration and common wounds [1, 4–5]. Among those wounds, shearing wounds are presumed to be of prime importance [1], based on the results of experimental infection of sheep with organisms through shearing cuts, which resulted in production of CLA lesions [6–7]. No concrete evidence is, however, available to support the relation between infection and shearing.

Recently an ELISA, a sensitive serodiagnostic procedure, has been used in seroepidemiological surveys of CLA to study the seroconversion period in sheep. Paton *et al.* [8] found an increase in ELISA-positives in 1- and 2-year-old sheep 5 to 7 months after shearing. Chikamatsu *et al.* [2] also observed a high ELISA-positive ratio of 51.3% in sheep aged 1 year, but not in sheep less than 1-year-old. The seroconversion might have been due to shearing. However, the 5–7 month period of Paton *et al.* [8] and the 1-year time frame used by Chikamatsu *et al.* [2] are considered to be too long to observe the seroconversion.

We have continued the investigation of Chikamatsu *et al.* [2] by periodic examination of anti-*C. pseudotuberculosis* toxin ELISA-titers in sheep of three age groups (less than 1 year and 1- and 2-year-old) and verified the assumption that the seroconversion was related to shearing.

Suffolk female sheep bred in the Takikawa Animal Husbandry Experiment Station were used for this study from April 1989 to April 1990. They consisted of three age groups: 98 lambs born in 1989, the year the survey started, 74 1-year-old sheep born in 1988, and 70 2-year-old sheep born in 1987. All the sheep were born in February to March, and grazed with their dams from May. They were weaned in June, finished grazing at the end of October, and were housed during winter. The first shearing was

done in the following spring. The 2-year-old sheep had lambed in February to March 1989. None showed clinical signs of CLA during the experiment.

All the sheep except lambs were sheared commercially in every April. During the experiment, the 1-year-old sheep were sheared once in April 18–21, 1989, and the 2-year-old sheep were sheared twice, in April 18–22, 1988, and in April 18–21, 1989. As a result, most of them had several shearing cuts.

Serum samples were collected from the lambs and sheep four times: April 12–13, July 17–19 (during grazing), and November 6–7 (1 week after grazing was finished), in 1989; and April 9–11 in 1990. These corresponded to ages of 1, 4, 8 and 13 months in lambs; 13, 16, 20 and 25 months in 1-year-old sheep; and 25, 28 and 32 months in 2-year-old sheep. The 4th sampling was not done in 2-year-old sheep. Sera were stored at –20°C until used.

The ELISA procedure previously described by Chikamatsu *et al.* [2] was used to detect antibodies to the toxin antigen of *C. pseudotuberculosis* ATCC 19410. Sera were absorbed preliminarily with *Rhodococcus equi* ATCC 33702 (Prescott's serotype 2) to remove non-specific reaction. The optical density (OD) of the contents of each well was measured using an Immuno Reader (MPR-A4; Toyo Soda Industry Co., Tokyo) at a wave length of 405 nm. The cut-off point of OD (0.384) was determined as the mean of the sera from 235 sheep with no CLA lesions plus 3 times the standard deviation according to Chikamatsu *et al.* [2]. The results were analysed by the χ^2 test.

The seasonal ELISA-positive ratios in the three age groups of sheep are shown in Table 1. A significant increase in the positive ratio was found in 1- and 2-year-old sheep in July 1989, 3 months after shearing. No significant increase in the ratio was found in these groups in other seasons. In the lambs, which had not been sheared since birth, the positive ratio decreased to 0% in July 1989, but slightly increased by the following April.

Paton *et al.* [8] reported that the ELISA-positive ratio in 1- and 2-year-old sheep increased significantly 5–7 months after shearing, referring to that period as the most susceptible time period for the infection. According to our results, the most susceptible period may be July, 3 months

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Table 1. ELISA-positive ratios in sheep of three age groups

Age group	Born in	ELISA-positives in				Significant difference between seasons
	Feb.-March in	1989			1990	
		April ^A	July ^B	Nov. ^C	April ^D	
1 year old	1988	10/74 ^{a)} (13.5%)	31/74 (41.9%)	33/74 (44.6%)	33/74 (44.6%)	A and B (P<0.01)
2 years old	1987	27/70 (38.6%)	46/70 (65.7%)	46/70 (65.7%)	ND ^{b)}	A and B (P<0.01)
Lambs	1989	24/98 (24.5%)	0/98 (0%)	3/98 (3.1%)	10/98 (10.2%)	A and B (P<0.01) and C and D (P<0.05)

a) No. of positive/No. examined. Percentages are shown in parentheses.

b) Not done.

after shearing. In addition, 5–7 months may be too long to accept a conclusion that the seroconversion was due to shearing.

In lambs, the ELISA-positive ratio was 24.5% in April, but it decreased to 0% in July. This was possibly due to the declining levels of maternal antibodies. In goat, Holstad [3] had also observed a decrease in the antibody-positive ratio at 3–4 months old. The ELISA-positive ratio in lambs then increased through the next April. The ratio, however, remained much smaller than in 1- and 2-year-old sheep in July. Since the lambs had not been sheared after birth, the increase may be due to non-shearing wounds.

These findings indicate that shearing wounds might be more susceptible to infection by CLA than other wounds, suggesting that CLA may be controlled by proper treatment of wounds immediately after shearing. Studies are in progress to prove this hypothesis.

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