Caprine Enteritis Associated with *Yersinia pseudotuberculosis* Infection

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**ABSTRACT.** Yersiniosis was prevalent among a caprine herd during the late autumn of 2003 in Iwate Prefecture, Japan. The disease affected 29 of about 100 lactating goats, but not dried or nonparous goats, mature male goats or kids. Four animals died within an epidemic period of 20 days. Affected animals developed decreased milk production with subsequent watery diarrhea, neutrophilia with increased band forms and multiple microabscesses characteristic of yersiniosis in the intestinal mucosa from the jejunum to cecum as well as in the mesenteric lymph nodes. *Y. pseudotuberculosis* serotype III was isolated from intestinal contents and mesenteric lymph nodes. The organism was also cultured from clinically normal dried animals. The outbreak might have been precipitated by multiple stress factors, such as lactation, cold weather, *Corynebacterium pseudotuberculosis* infection resulting in abscess formation and tape-worm and coccidium parasitisms.

**Key words:** enteritis, epidemic outbreak, goat, lactation stress, *Yersinia pseudotuberculosis* serotype III.

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The term yersiniosis refers to infections caused by either *Yersinia pseudotuberculosis* or *Y. enterocolitica* [12]. *Y. pseudotuberculosis*, a gram-negative and aerobic or facultatively anaerobic cocccobacillus, is an infrequently incriminated pathogen responsible for enteritis and septicemia in various species of mammals and birds with seasonal prevalence in the cooler months [12]. The lesions are characterized by microabscesses with bacterial colonies in the intestine and mesenteric lymph nodes, and often in visceral organs [1, 13, 15, 18]. The organism is also associated with mastitis [5, 9] and abortion [10, 21].

The organisms are divided into the serotypes I to VI based on the somatic O antigenic structure, and serotype III has been found to be associated with the disease in a wide range of ungulate species, such as sheep, goats, pigs [13, 15, 16] and less frequently cattle [4, 14]. Stress factors are generally thought to be necessary to precipitate the disease. The factors include cold, starvation, poor feeding, parasite infection, transport and pre-existing gastrointestinal mucosal lesions [2, 7, 12, 13]. There are few reports on epidemic yersiniosis in caprine herds in Japan, although generalized infection with *Y. pseudotuberculosis* serotype I was described in a goat [11].

This study describes the pathological and bacteriological findings of lactating goats suffering from enteritis associated with *Y. pseudotuberculosis* serotype III.

**MATERIALS AND METHODS**

An outbreak of the disease occurred in a caprine herd in Iwate Prefecture, a northeastern district of Japan, during the period from 30 October to 19 November in 2003. The herd comprised about 100 lactating Saanen goats aged 1 to 5 years and a total of 100 other animals, including dried and nonparous goats, mature male goats and kids. The lactating and 3 male animals were kept together in a shed with a loose housing system and the others in an adjacent shed. The lactating and male animals were fed hay, concentrates and corn silage, while the others given the same kinds of feed without concentrates. All animals had been treated with ivermectin once a year.

The disease affected only 29 lactating animals. Affected animals developed anorexia and reduction in milk production followed by watery diarrhea containing mucus and depression 1 or 2 days later. No fever was recorded in any animal. Four animals died after the disease course of 4 to 6 days. The total daily milk production in the herd decreased from 150 kg in the pre-epidemic period to 80 kg in the epidemic one. After the affected animals were separated from the others and treated with enrofloxacin for 3 days based on the isolates’ antibiogram, the symptoms resolved. The average lowest temperature was 1.3°C during the epidemic period, measured at the prefectural institute located about 500 m from the farm. Although the farm was located in a dairy-producing area, there was no evidence that the goats had immediate contact with cows or pigs. Feral birds had occasional access to the feed in the caprine sheds. Two nonparous goats had been introduced from another farm with no history of yersiniosis 7 days prior to the onset of the epidemic.

**Hematology:** Blood samples were obtained from 9 affected and 4 clinically normal lactating animals in the herd. Total and differential leukocyte and erythrocyte counts were obtained using standard methods.

**Pathology:** Three moribund animals (Nos. 1–3) with a disease course of about 10 days were necropsied following euthanasia by bleeding under anesthesia. The disease course was judged based on the observations of the owner. Tissue blocks collected from the whole body at necropsy were fixed in 10% neutral buffered formalin and embedded in paraffin wax. Sections of tissue were stained with hematoxylin and eosin (HE). Selected sections of the intestine,
mesenteric and submaxillary lymph nodes and subcutis with abscesses were stained with Gram staining (Brown and Brenn method) and phosphotungstic acid hematoxylin (PTAH).

**Bacteriology:** Intestinal contents, mesenteric lymph nodes, liver, spleen, kidney, heart, lung, brain and abscess contents from several lymph nodes and subcutis were obtained at necropsy. The materials were cultured aerobically on MacConkey agar at 30°C for 48 hr, aerobically on 5% sheep blood agar and anaerobically on GAM agar supplemented with 5% sheep blood at 37°C for 48 hr. Fecal samples were collected from 10 clinically normal dried and 2 introduced animals in the unaffected shed. They were suspended in a 0.067 M phosphate buffer solution (pH 7.6) to a concentration of about 10%, incubated at 4°C for 3 weeks for enrichment and then subcultured for Yersinia sp on modified Irgasan-novobiocin (IN) selective agar [6] at 30°C for 48 hr.

Gram-negative, oxidase-negative and lactose-nonfermenting colonies were identified using a commercial system (API 20E, bioMerieux, France). Slide agglutination tests were conducted for serotyping isolates of *Y. pseudotuberculosis* with rabbit hyperimmune sera against serotypes I, II, III, IV, V and VI. Isolates suspected to be *Corynebacterium* were identified with a commercial system (API Coryne, bioMerieux, France). Antibiotic sensitivity tests were carried out by the disc method on Mueller Hinton agar with discs containing penicillin, ampicillin, cefazolin, cefotaxime, kanamycin, gentamicin, streptomycin, tetracycline, oxytetracycline, enrofloxacin, colistin and fosfomycin.

**RESULTS**

**Hematological findings:** The average values with standard deviation of total leukocyte, neutrophil and neutrophil band form counts were 21.2 ± 6.7, 14.9 ± 6.1 and 3.2 ± 2.5 × 10³/mm³ in the affected animals and 7.4 ± 1.1, 3.8 ± 1.1 and 0.02 ± 0.04 × 10³/mm³ in the clinically normal ones, respectively. Those of erythrocyte counts were 20.7 ± 7.9 and 13.7 ± 2.5 × 10⁶/mm³ in the same order.

**Pathological findings:** Macroscopically, the small and large intestine contained moderate amounts of fluid content mingled with mucus. The mucosa was edematous with several whitish lesions measuring 1 to 2 mm in diameter (Fig. 1). *Moniezia expansa* was present in the jejunum and ileum of 1 case (No. 2). Mesenteric lymph nodes were enlarged and edematous. There were multiple mucosal erosions sized 2 to 3 mm in diameter with adherent bile-stained fibrin in the gallbladder of 1 case (No. 1). Abscesses encapsulated by fibrous tissue, sized 15 to 25 mm, were found in the submaxillary lymph nodes and adjacent subcutis of 3 cases. Similar abscesses sized 50 mm were also present in the subcutis and the medial iliac lymph node of 1 case (No. 1). The abscess contents were uniformly pasty without lamellated structure.

Histologically, the intestinal lesions were characterized by multiple microabscesses comprising colonies of gram-negative coccobacilli surrounded by infiltrated neutrophils in the superficial lamina propria of extensive mucosa from the jejunum to caecum, predominantly in the ileum (Fig. 2). Desquamation was often found in the luminal and intestinal glandular epithelial cells adjacent to the microabscesses. There was hyperplasia of intestinal glands accompanied by lymphocyte infiltration in the deeper lamina propria of the jejunal and ileal mucosa (Fig. 3). *Coccidium* gametocytes and oocysts were present in the glandular epithelial cells and lumina in the jejunum and ileum. There were multiple foci of neutrophils with fibrinous exudate in the sinus and lymphatic tissues of mesenteric lymph nodes. Colonies of gram-negative coccobacilli were sometimes present in the purulent lesions.

The abscesses in the lymph nodes and subcutis were characterized by a large necrotic center surrounded by a thick layer of collagen fibers with infiltration of lymphocytes, histiocytes and neutrophils. There were large amounts of degenerated neutrophils and macrophages with gram-positive coccobacilli in their cytoplasm, and various degrees of calcification in the necrotic area. In the other organs and tissues, occasional foci of several neutrophils were seen in the hepatic sinusoid of 3 cases and fibrinonecrotic cholecystitis with colonies of gram-negative coccobacilli in 1 case (No. 1). The thymus was atrophic, and lymphocytes were moderately or mildly decreased in the lymphatic tissues throughout the body, such as the spleen, tonsil, Peyer’s patches and lymph nodes, in 3 cases.

**Bacterial isolation:** *Y. pseudotuberculosis* was isolated from the mesenteric lymph nodes and intestinal contents of 3 necropsied cases. The organism was also obtained from 2 fecal samples of 10 dried animals but not from the fecal samples of 2 introduced animals. The isolates were serologically typed in serotype III. *Corynebacterium pseudotuberculosis* was cultured from the abscess contents of the 3 examined cases. The isolates of *Y. pseudotuberculosis* were

![Fig. 1. The mucosa is edematous with several whitish lesions (arrowheads) in the ileum of case No. 1.](image-url)

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sensitive to enrofloxacin, ceftiofur, gentamicin, tetracycline, oxytetracycline, colistin and fosfomycin, but resistant to the other antibiotics. Those of *C. pseudotuberculosis* were sensitive to antibiotics other than colistin and fosfomycin.

**DISCUSSION**

The disease associated with decreased milk production and watery diarrhea was prevalent among a caprine herd in the cooler months. Affected animals developed dehydration, neutrophilia with increased band forms and microabscesses characteristic of yersiniosis in the intestinal mucosa and mesenteric lymph nodes, and *Y. pseudotuberculosis* serotype III was isolated from affected animals. These results suggest that the epidemic disease was associated with this organism.

The present outbreak might have been precipitated by multiple stress factors, such as lactation, cold weather, *C. pseudotuberculosis* infection resulting in abscess formation and tapeworm and coccidium parasitisms. The disease affected only lactating animals, although the organism was also cultured from the fecal samples of non-lactating animals. *C. pseudotuberculosis* is well known as the specific cause of caseous lymphadenitis in sheep and goats, a disease which is characterized by abscess formation of peripheral lymph nodes and which results in a chronic debilitating condition when accompanied by the involvement of visceral organs [3, 17]. No visceral lesions associated with *C. pseudotuberculosis* were found in the examined animals with the exception that 1 animal possessed an abscess in the medial iliac lymph node. It seems likely that the non-lactating animals would also have been exposed to tapeworms and coccidia, since no effective anthelmintics for either parasite had been administered to any animals in the farm. These facts suggest that lactation might have played a most important role in the present stress factors.

The lesions associated with *Y. pseudotuberculosis* in the examined animals, which had been affected for about 10 days, were restricted to the intestine and mesenteric lymph nodes without involvement of the visceral organs, except the gallbladder in 1 animal. The intestinal lesions consisted of multiple microabscesses and intestinal glandular hyperplasia. The lesional distribution resembled the previously reported finding [15] that infection by this organism in sheep and goats rarely produced characteristic microabscesses in the visceral organs. Yersiniosis takes a variety of clinicopathological forms: fulminant, subacute and chronic. Fulminant Yersinia infection causes fibrinous or fibrinonecrotic enteritis [1, 8, 20], the subacute infection produces microabscesses in the intestinal mucosa, and the chronic infection is accompanied by villous atrophy and intestinal glandular hyperplasia [13]. The present intestinal lesions seem to have the characteristics of subacute to chronic infections.

A decrease of lymphocytes was found in the lymphatic tissues throughout the body of the examined animals. The condition might have been associated with several of the stress factors described above, and might have facilitated the infection and colonization of the bacteria in the intestinal mucosa.

It seems unlikely that the infection in the farm originated in the goats introduced prior to the epidemic, since the animals were obtained from a source with no history of yersiniosis and the organism was not isolated from the feces of the introduced goats. Cattle and pigs are thought to be reser-
voirs, particularly of *Y. pseudotuberculosis* serotype III [14, 19]. No animals in the farm had immediate contact with cows or pigs. Although the exact origin of the present infection remains unknown, there might have been indirect contacts between the goats and cows in the dairy-producing area via feral birds or other animals. Alternatively, reservoirs maintaining infection might have been present in the caprine herd, as pointed out previously [13, 15]. Further investigations on the origin of the infection should be performed for the purpose of prophylaxis of the disease.

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REFERENCES