Intestinal Spirochetosis in Wild Sika Deer (Cervus Nippon Yesoensis) Infected with Brachyspira Species

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(Received 23 March 2000/Accepted 17 May 2000)

ABSTRACT. Seven adult free-ranging sika deer (Cervus nippon yesoensis) were examined by histology, immunohistochemistry and electron microscopy for intestinal spirochetal infection. Histologically epithelial and goblet cell hyperplasia and edema of the lamina propria mucosa with macrophage and lymphocyte infiltration were observed in the cecum and colon in 6 of the 7 deer. Numerous argyrophilic spirochetes were present in the crypts and some had invaded epithelial and goblet cells and caused degeneration. Immunohistochemically the organisms stained positively with polyclonal antisera against Brachyspira (Seropula) hydysenteriae and B. pilosicoli. Ultrastructurally they were 6–14 μm long, 0.2–0.3 μm wide and had 4–6 coils and 13 axial filaments per cell; such features were closely similar to those in the Brachyspira species. These results showed that the spirochetes were capable of inducing enteritis in deer and this intestinal spirochete infection might already be prevalent among wild sika deer in Japan. There is a possibility that this spirochetal colitis is a new syndrome in sika deer and that the same and/or similar spirochetes have infected ruminants, including sika deer and cattle.

KEY WORDS: Brachyspira (Seropula), intestinal spirochete, intestine, sika deer (Cervus nippon yesoensis), spirochetosis.


Serpulina species were recently redesignated Brachyspira species [6, 7]. Moreover several reports of new diagnostic and typing methods of B. hydysenteriae, as well as on the classification of pathogenic and non-pathogenic intestinal spirochetes, have been published [1, 12, 13]. Intestinal spirochetal infection has been reported in human beings, pigs, dogs, monkeys, baboons, rats and various avian species [14]. However, there is little information on intestinal spirochetes in ruminants [4]. Recently bovine intestinal spirochetosis with dysentery has been reported in Hokkaido, Japan [11]. The disease is characterized by bloody diarrhea and thickened large intestinal mucosa with goblet cell hyperplasia, caused by intestinal spirochetes [11]. Considering the pathological findings and pathology of swine dysentery, this is the counterpart of swine dysentery, and can be named “bovine dysentery”. However, the source and route of infection are unknown. There are many free-ranging sika deer in Hokkaido. They frequently graze in pastures for cattle breeding and chase cattle, including the cow infected with intestinal spirochetes [11]. The prevalence and lesions associated with intestinal spirochetes in sika deer (Cervus nippon yesoensis) are not known. Likewise, little is known concerning the development and tissue distribution of intestinal spirochetes in ruminants that become infected via natural routes of transmission. We collected materials for pathological examination from wild sika deer and spirochetes were detected in the intestine. The purpose of this report is to describe the intestinal spirochetal infection in wild sika deer.

MATERIALS AND METHODS

Numerous free-ranging sika deer graze with cattle in pastures for cattle breeding in Hokkaido. Seven adults deer were killed with a shotgun as harmful animals in the deer hunting season. Tissue samples were collected within 30 min after death and fixed in 10% phosphate-buffered formalin. They were embedded in paraffin, and tissue sections (approximately 3 μm thick) were stained with hematoxylin and eosin, and Gram stain. Intestinal specimens were also stained by the Warthin-Starry technique. Serial histologic sections from the intestinal tissues were prepared for immunohistochemistry with streptavidin-biotin-alkaline phosphatase (BioGenex Laboratories, San Ramon, CA, U.S.A.). Endogenous peroxidase activity was blocked by methanol and 3% H2O2 (Sigma Chemical Company, St. Louis, MT, U.S.A.). The primary antibodies used were rabbit polyclonal antibodies against B. hydysenteriae and B. pilosicoli. Sections were lightly counterstained with Mayer’s hematoxylin and assessed under light microscopy. Negative controls were prepared by using nonimmune rabbit serum in place of the primary antibodies. Small blocks taken from 10% formalin-fixed cecum and colon tissues were post-fixed in 1% osmium tetroxide, embedded in epoxy resin, sectioned and stained with uranyl acetate and lead citrate. The sections were examined with a transmission electron microscope (TEM, JEM-1010, JEOL, Tokyo, Japan).

Intestinal contents were cultured to search for B. hydysenteriae on trypticase soy agar plates containing 5% defibrinated ovine blood and 400 μg/ml spectinomycin, at 37°C under anaerobic conditions for 10 days. Wet mount prepa-
Table 1. Distribution of intestinal spirochetes and lesions of wild sika deer

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*++ = no lesion; + = a few intestinal spirochetes; ++ = some intestinal spirochetes with lesions; +++ = many intestinal spirochetes with lesions.*

Fig. 1. Cecum. Epithelial and goblet cells have greatly proliferated, and the crypt is markedly elongated. HE. x 100.

Fig. 2. Cecum. Numerous curved spirochetes (arrows) are associated with and invading the crypt epithelial and goblet cells. Warthin-Starry stain. x 1,000.

...rations with physiologic saline and iodine were prepared. The formalin-ether concentration technique was used for the detection of helminth eggs and protozoan oocysts. Sheather's sugar flotation technique was used and cold acid-fast stained smears were prepared for the detection of Cryptosporidium spp. Trichrome-stained slides were prepared from soft-to-watery stool specimens whenever the presence of a pathogenic protozoan was suspected in one of the wet mount preparations.

RESULTS

Macrosopic lesions: All deer were clinically normal, but gross thickening and erosion of intestinal mucosa were detected throughout the cecum and colon in 6 of the 7 deer. Intraluminal contents were watery, greenish fluid with diphtheric material in the 6 cases. No gross lesions were detected in the other one.

Microscopic lesions: The distribution of intestinal spirochetes and intestinal lesions was similar in the 6 deer with gross lesions (Table 1). The lesions were associated with epithelia and lamina propria mucosa in the large intestine, and were characterized by severe erosion and abnormal proliferation of epithelial cells (Fig. 1). Increased cellularity with numerous macrophages and lymphocytes and edema were observed in the lamina propria mucosa. Numerous Gram-negative and argyrophilic spirochetes were present mainly in the crypts (Fig. 2). Some of the organisms were observed in the cytoplasm of epithelial and goblet cells. However, they did not adhere perpendicularly to the cell surface like B. pilosicoli. Small numbers of spirochetes were also detected in the ileum and rectum in spite of the absence of gross and histologic lesions in No. 2 (Table 1). No lesions or organisms were detected in the other one.
**Immunohistochemical detection of Brachyspira antigen:**
The spirochetes, which were stained with polyclonal antisera against *B. hyodysenteriae* and *B. pilosicoli*, were seen within goblet and epithelial cells and in the lamina propria mucosa (Fig. 3).

**Electron microscopic evidence:** The organisms were observed in goblet and epithelial cells or through the extracellular matrix in the lamina propria mucosa. Some of the organisms were observed in pits and cytoplasm of the epithelial cells (Fig. 4). They were loosely attached to the surface of crypt epithelium (Fig. 5). Glycocalyx was absent and microvilli were displaced by the organisms, which showed several complete convolutions, cone-shaped cell endings, a cell length of 6–14 μm, a cell diameter of 0.2–0.3 μm and had 4–6 coils and 13 axial filaments per cell (Fig. 6).

None of the spirochete-like organisms were isolated from the intestinal contents and no parasites were identified in the fecal samples.

**DISCUSSION**

Numerous spirochetes were present in the crypts of the cecum and colon in 6 of the 7 deer. Histopathological examination of the lesions using Warthin-Starry silver stain revealed silver-positive spiral bacteria invading the goblet cells and epithelial cells and their multiplication in these cells. The organisms were also observed in the lamina propria. The organisms found in this study were wider and not as tightly coiled as *Leptospira* spp. or *Treponema* spp. Furthermore, they were shorter, more slender, and not as loosely coiled as *Borrelia* spp. *B. hyodysenteriae* is 0.3–0.4 μm in width, 6–8 μm in length, and loosely coiled with 2–4 coils and tapered ends [9]. Ultrastructural considerations favor the inclusion of the microorganisms in the genus *Brachyspira* rather than *Treponema, Leptospira* or *Borrelia*.

*B. hyodysenteriae* has 16 to 18 axial filaments per cell (8 to 9 axial filaments inserted at each end) and *B. innocens* isolates B256 and 4/71 have 13 to 14 and 9 to 14 axial filaments per cell, respectively [10]. In another report, *B. pilosicoli* was found to have 8 to 12 axial filaments per cell (4 to 6 axial filaments inserted at each end) [14]. Under culture conditions, it was not possible to distinguish cells of *B. hyodysenteriae*, *B. intermedia* and *B. murdochii* based on cell dimensions, cell morphology and numbers of axial filaments (22 to 28 axial filaments per cell, 11 to 14 axial filaments inserted at each end) [12]. In the same report, *B. pilosicoli* cells were generally found to be shorter (length 5.3 to 7.3 μm) and thinner (width 0.25 to 0.3 μm) than the cells of the other *Brachyspira* species and had (8 to 12) fewer axial filaments per cell (4 to 6 axial filaments inserted at each end) [12, 14]. Therefore, ultrastructural similarity between the spirochetes in the present study and *Brachyspira* species rather than *B. pilosicoli* was observed. Immunohistochemically the organisms were stained positively by polyclonal antisera against *B. hyodysenteriae* and *B. pilosicoli*. However, because of the low specificity of those antisera and the possibility of mixed infections by several *Brachyspira* species, immunohistochemical examination can only be used to identify *Brachyspira* species. Morphological and immunohistopathological findings in deer and
cattle [11] suggest that the same or similar Brachyspira species could infect these ruminants.

As shown in Fig. 3, certain Brachyspira-like spirochetes were capable of inducing enteritis in deer. The lesions of deer intestinal spirochetosis are to some extent similar to those of other mammalian spirochete diseases such as B. hyodysenteriae infections in pigs [8]. Different virulence mechanisms had been shown to contribute to the pathogenesis of the B. hyodysenteriae infections: motility, adherence, and production of hemolysin and lipopolysaccharide [9]. It is not known whether such virulence factors also occur in deer spirochetes. The electron microscopic findings for the affected deer, however, suggested invasion of the spirochetes into the epithelium with pits. The lesions on the internal organs of the deer infected with the spirochetes in the intestinal tract may indicate toxic activity [13]. Among Brachyspira spp., B. hyodysenteriae, B. alvinipulli and B. canis are thought to be pathogenic [1, 13]. B. innocens, B. intermedia and B. murdochii are thought to be non-pathogenic [2, 3, 13]. However, inoculation of gnotobiotic pigs with several strains of B. innocens causes mucoid diarrhea and mild colitis [5]. Since our attempts to isolate spirochetes from the deer failed, their exact identity is still unknown. Further studies with different media and culture conditions will be necessary to try and isolate the spirochetes. An association with spirochetes was demonstrated on the basis of histological examination of tissue sections stained with silver and immunohistochemistry with polyclonal rabbit serum produced against B. hyodysenteriae and B. pilosicoli. Additional confirmation was obtained by TEM examination of affected intestinal tissue. Although Koch’s postulates were not fulfilled, this article provides evidence of a pathogenic role of the Brachyspira-like spirochetes in deer. There is a possibility that this spirochetal colitis is a new syndrome in sika deer and that the same and/or similar spirochetes infect ruminants, including sika deer and cattle. This is the first report suggesting a role for spirochetes in colitis of sika deer. It is suggested that intestinal spirochete infection might already be prevalent among wild sika deer and cattle in Japan.

ACKNOWLEDGEMENTS. We would like to thank Dr. Y. Ando and Mr. T. Fujisawa for preparation of photomicrographs.

REFERENCES


