Intestinal Spirochetosis in a 21-Month-Old Thoroughbred Colt

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(Received 8 January 2002/Accepted 20 March 2002)

ABSTRACT. A 21-month-old Thoroughbred colt showed continuous diarrhea and developmental retardation for 7 months, and was thereafter subjected to euthanasia for necropsy and laboratory examinations. At necropsy, the cecal and colonic mucosae were diffusely rough and hyperemic. Histopathologically, the mucosa and submucosa were edematous and were infiltrated by numerous lymphocytes and macrophages. Meanwhile, three morphological types of Brachyspira antigen-containing spirochetes were found to be numerous in the crypts and in the mucous layer over the epithelium in the cecal and colonic lesions. They were frequently observed in intercellular gaps and in the cytoplasm of degenerative epithelial cells, and in the lamina propria, particularly in cavities around blood vessels. These invasive intestinal spirochetes might be one of pathogens inducing colitis and diarrhea in horses.

KEY WORDS: Brachyspira, equine, intestinal spirochete.

The existence of equine intestinal spirochetes was reported in 1964 [1]. They were observed in Gram-stained smears of cecal contents from the majority of samples from 51 horses and ponies in Scotland, and were considered part of the normal intestinal flora. In 1985, two morphological types of spirochetes were found in the equine cecal contents, and this again suggested that such organisms were not pathogenic [2]. Perhaps because of these findings, equine intestinal spirochetes have received little attention. The prevalence and pathology of equine intestinal spirochetosis are not known, and associated enteritis and diarrhea have not been previously reported. This study describes the histopathological characteristics of a Thoroughbred colt with invasive intestinal spirochetes in Japan.

On August 22, 2000, a 14-month-old Thoroughbred colt that had mild diarrhea (body weight 377 kg) was transferred to The Hidaka Yearling Training Farm of the Japan Racing Association (JRA) from a farm in Hokkaido. After moving onto the JRA farm, the colt was housed with 76 other horses in a facility consisting of two stables and a large livery yard with soil. At that time, the colt took pleasure in his food and drank much water, approximately 100 to 150 liters a day. The referring veterinarian tried antiparasitic therapy with ivermectin and bithionol, anti-inflammatory therapy with flunixin meglumine and anti-hyperperistaltic treatment with hyoscine-N-butylbromide (Buscopan), but no change occurred. While being treated, fecal samples were collected for microbiobiological examination on September 13, but no enteropathogenic organisms, such as Salmonella, Clostridium or rotavirus were identified. The horse showed weight loss of 44 kg (body weight 333 kg) by October 10, but slowly grew to 424 kg at the necropsy.

After a period of 7 months from the first diagnosis, the 21-month-old animal was subjected to euthanasia with anesthesia for necropsy and laboratory examinations on March 9, 2001. No clinical abnormality was found in any of the other horses.

Several tissue samples were taken from organs and fixed by immersion in 10% neutral buffered formalin. Tissue sections (approximately 3 µm thick) were obtained using routine histological techniques and stained with hematoxylin and eosin (H&E), Giemsa, Ziehl-Neelsen and Warthin-Starry stains. For immunohistochemistry, paraaffin wax-embedded sections were labeled by the streptavidin-biotin-peroxidase complex immunoperoxidase technique (SAB), with rabbit polyclonal antibodies to Brachyspira (formally Serpulina) hydysenteriae [5, 7] and an immunoperoxidase labeling system Histofine SAB-PO Kit (Nichirei Corp., Tokyo, Japan). Sections were lightly counterstained with Mayer’s hematoxylin and assessed by light microscopy. To assure the specificity of the staining, normal rabbit serum was used for staining control sections. For electron microscopy, small blocks taken from the 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 then examined with a JEOL JEM-1010 (JEOL Ltd., Tokyo Japan) transmission electron microscope (TEM).

Cultures for Brachyspira species, the cecal and colonic specimens with gross lesions and colonic contents were diluted in phosphate-buffered saline. They were plated on two types of modified BJ agar [3]: trypticase soy agar with 5% citrated equine blood, spiramycin (6.25 µg/ml), rifampin (12.5 µg/ml), vancomycin (6.25 µg/ml), colistin (6.25 µg/ml) and spectinomycin (25 µg/ml) or agar with 5% citrated equine blood, spiramycin (0.625 µg/ml), rifampin (1.25 µg/
mL), vancomycin (0.625 µg/mL), colistin (0.625 µg/mL) and spectinomycin (2.5 µg/mL). Plates were incubated at 37°C in an environment of 85% N2-10% H2-5% CO2 and were examined daily for up to 10 days for the presence of spirochetes. Suspected spirochete colonies were examined with Gram’s stain to determine cell morphology.
At necropsy, the large intestine was distended by large quantities of malodorous liquid feces, which contained a large amount of diphtheritic material. Significant intestinal changes were confined to the large intestines (Fig. 1). The cecal and colonic mucosae were diffusely rough and hyperemic. Careful examination of the cecum and colon showed edema of the mucosa, and thinning as regards the total thickness of the duct wall. Scattered patchy hemorrhagic lesions with several immature cyathostome (small strongyles) larvae were also found. The small intestine and rectum were only mildly affected. The mesenteric lymph nodes, especially the cecal and colonic lymph nodes, were enlarged and pale, with preservation of the cortical medullary zones. Thymic hyperplasia and bilateral small, undescended testes were also observed.

Histopathologically, the mucosa and submucosa of the cecum and colon were edematous and were infiltrated by numerous leukocytes, mainly lymphocytes and macrophages (Fig. 2). Furthermore, a large number of Brachyspira antigen-containing, argyrophilic spirochetes were seen in the crypts and in the mucus layer over the epithelium (Fig. 3). They were frequently observed in intercellular gaps and in the cytoplasm of degenerative epithelial cells (Fig. 4), and in the lamina propria, particularly in cavities around blood vessels. Despite careful examination of the sections, there was no visible accumulation of spirochetes attached end-on to the surface epithelium, unlike B. pilosicoli. Eosinophils and epithelioid cells were observed, especially in the patchy hemorrhagic lesions with small strongyle larvae. In the lymphoid organs, including the mesenteric lymph nodes and thymus, there was marked germinal center formation. An increase in the number of macrophages and edema in the

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Fig. 5. Cecum. Three types of spirochetes with different morphologies (length, width and axial filaments) were identified in the cytoplasm and in intercellular gaps of epithelial cells. Large arrows, small arrows and arrowheads indicate Types 1, 2 and 3, respectively. TEM. Bar=1.22 µm.

Fig. 6. Cecum. Intestinal spirochete (Type 2) has overlapping rows of 9 axial filaments under the outer membrane. TEM. Bar=0.06 µm.
paracortical area were evident.

Ultrastructurally, three morphological types of spirochetes were found in the cecal and colonic lesions (Fig. 5). Type 1 showed a cell diameter of 0.28–0.33 µm, cell length of 6–11 µm and had 9 axial filaments and 4–6 coils. Type 2, the most frequently occurring, was a loose spiral with a cell diameter of 0.16–0.24 µm, cell length of 5–10 µm, 9 axial filaments and 4–6 coils. Type 3 had a cell diameter of 0.09–0.12 µm, cell length of 4–7 µm, 4 axial filaments and 4–6 coils. No bacteriophage-like particles were observed in association with these organisms. No other pathogenic organisms such as *Rhodococcus*, *Salmonella*, rotavirus or coccidian parasites were identified pathologically, and no pathogenic organisms, including spirochetes, were isolated from the intestinal samples microbiologically.

Swine dysentery, an intestinal spirochetal infection, is one of the most significant production-limiting porcine infections, and causes serious economical damage. Intestinal spirochetes in cattle and deer have been reported to be pathogenic [5–7], whereas those in the horse are considered to be part of the normal intestinal flora and to be nonpathogenic [1, 2]. Since invasive intestinal spirochetes were demonstrated in the intestinal lesions of the present animal by immunohistochemical and ultrastructural methods, they should be taken into account as causative agents in equine intestinal disorders.

In our case, the histological changes were similar to those in swine dysentery and those caused by porcine colonic spirochetes, with the exception of severe hemorrhagic changes and the end-on attachment of *B. pilosicoli* to the epithelial surface [9, 10]. In swine dysentery, the lesions are restricted to the colon, cecum and rectum, and are most constant and severe in the spiral colon. In bovine dysentery, the lesions are in the cecum and proximal colon [7]. The cecal and colonic mucosa were severely affected, with numerous invasive spirochetes in the present colt, and cellular infiltrates were more prominent than those in the porcine and ruminant cases. Such differences in the distribution of lesions and the intensity of inflammatory reaction may be partly attributable to interspecies differences in both hosts and organisms.

We found three ultrastructurally different types of spirochetes. Because attempts to isolate these organisms failed, their exact identification was impossible. The ultrastructural features of nonpathogenic spirochetes in the equine intestine have been briefly described, and they are classified into two types, *Treponema* species and *Borrelia* species [2]. The three types of organisms in our case resembled *Brachyspira* morphologically and immunohistochemically [4], and were apparently distinct from the previously reported equine spirochetes. Thus, various spirochetes are present in the large intestine in horses, and some invasive ones might be capable of inducing colitis and diarrhea in immature or immunocompromised horses. The cells of *B. pilosicoli* generally are shorter and thinner than those of the other *Brachyspira* species and have fewer axial filaments (4 to 6 axial filaments inserted at each end) [8, 11]. It is difficult to distinguish cells of *B. hyodysenteriae*, *B. intermedia* and *B. murdochii* based on cell dimensions, cell morphology and numbers of axial filaments under culture conditions [8]. In the present study, the Type 1 and 2 spirochetes could belong to *Brachyspira* species other than *B. pilosicoli*, and Type 3 to *B. pilosicoli* or a closely related species.

ACKNOWLEDGMENTS. We would like to thank Dr. T. Ohy for his advice, and we also acknowledge Dr. Y. Ando and Mr. T. Fujisawa for preparation of the photomicrographs, and Mr. M. Kim Barrymore for his critical reading of the manuscript.

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