Gastroenteritis associated with *Helicobacter*-like organisms and rotavirus in a reindeer (*Rangifer tarandus*)

**SHORT CONTRIBUTION**

**R**otavirus diarrhoea or enteritis is recognised in infant children, calves, mice, lambs, pigs, dogs, foals, birds and other animals.1 After the discovery of *Helicobacter pylori* in diseased gastric tissue of humans in 1982, at least 16 and probably 18 species of *Helicobacter* have been isolated and identified from the stomachs and intestines of various animals, including dogs, cats, ferrets, minks, pigs, monkeys, sheep, mice, rats, hamsters, cheetahs and birds.2 Bacteria that resembled *Helicobacter*-like organisms were positively stained with polyclonal antisera against *H. pylori* (Rabbit anti *H. pylori*; DAKO). Sections were lightly counterstained with haematoxylin and assessed by light microscopy. Negative controls were prepared by using nonimmune rabbit serum in place of the primary antibodies. Small blocks taken from 10% formalin-fixed abomasal tissues were post-fixed in 1% osmium tetroxide, embedded in epoxy resin, and sectioned and stained with uranyl acetate and lead citrate and examined by transmission electron microscopy. The faecal sample was positive for group A rotavirus antigens by a commercially available latex agglutination test (Rotalex Dry; Orion Diagnostica, Finland).

At necropsy, the intraluminal contents of the jejunum, ileum and large intestine were liquid and grey. Conspicuous gross lesions consisted of extensive and multifocal congestion and erosion in the fundic and pyloric regions of the abomasum, thinning of the small intestinal wall and atrophy of the thymus.

Histologically, severe focal to diffuse infiltration with lymphocytes and macrophages was present in the lamina propria in the submucosal region of the mucosa in the fundic and pyloric region of the abomasum (Figure 1). A large number of lymphoid nodules were present in the pyloric gland region. There were many silver-positive, Gram-negative, spiral-shaped organisms in the lumen of the gastric glands and they were mainly present in the upper half of the gastric mucosa. Many were attached to the epithelial cells of the gastric glands and some were found in groups and large clusters on the surface of the gastric mucosa, in the gastric pits, or deep in the glandular lumina. Numerous neutrophils had infiltrated into the lumen of the gastric gland, the gastric pits and the lamina propria. There was a correlation between the number of organisms observed and the degree of gastric inflammation. The lesions of the proximal small intestine, especially the jejunum, showed advanced degeneration and desquamation or shedding of the villous epithelial cells (Figure 2). Also identified were oedema and proliferation of lymphocytes and macrophages in the lamina propria and decrease of lymphocytes in the lymphatic follicles. The spiral-shaped organisms were also present in the intestinal mucosal surface, adhering to the degenerative epithelium. In the other organs, there were decreased lymphocytes and atrophy of lymphatic follicles in the spleen, lymph nodes and thymus.

Immunohistochemically, the silver-positive, spiral-shaped organisms were positively stained with polyclonal antibodies against *H. pylori* in the abomasum and small intestine (Figure 3). Rotavirus antigens were identified in the degenerative and desquamative epithelial cells lining the apical halves of villi in the jejunum (Figure 4). These two antigens were present in the same small intestinal lesions. No coronavirus antigen was found in either the abomasum or the small intestine. Transmission electron microscopy revealed loosely spiral-shaped organisms, 5 to 9 μm in length, 0.4 to 0.5 μm in diameter, with no periplasmic fibers and no sheathed flagella at the end of the cells (Figure 5). The organisms were often found in close contact with the cell membrane of the gastric epithelial cells especially the intercellular canalculus of parietal cells in the gastric glands. Some organisms also were observed in the cytoplasm of infiltrating neutrophils in the lumen of the gastric glands.
Figure 1. Severe focal to diffuse infiltration with lymphocytes and macrophages in pyloric region of the abomasum. Haematoxylin and eosin, x200.

Figure 2. Degeneration of the epithelial cells at the tops of the villi in the jejunum. HE, x100.

Figure 3. H pylori antigens (arrow) are clearly visible in the degenerative epithelium in the jejunum. Anti-H pylori immunoperoxidase, x400.

Figure 4. Rotaviral antigens are clearly visible in the epithelium in the jejunum. Anti-rotavirus immunoperoxidase, x630.
Degeneration of the gastric glands and cellular infiltration in the gastric lamina propria were observed in the present study. Three morphologic forms of these organisms in dogs have been reported. All three are now known to be Helicobacter on the basis of 16S rRNA sequence analysis. The organism in this study could not be strictly classified according to Lockard typing methods based on morphologic criteria, including the length, width, coils, periplasmic fibers and sheathed flagella. They were stained by polyclonal antisera against H pylori, and the location of these organisms was similar to H pylori of humans, therefore, they seem to be one of the pathogenic Helicobacter species. However, we could not check the specificity of the polyclonal antisera with all other morphologically similar bacteria such as Arcobacter spp and Campylobacter spp. Therefore, the term Helicobacter-like organisms is appropriate in this study. In unweaned beef calves H pylori was not visualised or cultured from any of the abomasal tissue samples. On the other hand, one report indicates the presence of serum antibodies in 6 of 22 calves, which reacted with epitopes from H pylori. Moreover, spiral-shaped bacteria, including H pylori, may be found frequently in the bovine abomasum. However, the relationship between those organisms and gastrointestinal lesions in ruminants is still unclear. Our results indicate that the inflammatory lesions and erosions found in the gastrointestinal tracts in the reindeer were associated with Helicobacter-like organisms.

The spiral-shaped organisms in the present study were located in the intercellular canalculus of parietal cells in gastric glands.

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Figure 5. Phagocytosed bacteria (arrows) in the cytoplasm of a neutrophil in the lumen of a gastric gland. Uranyl acetate and lead citrate, x9800.