SHORT CONTRIBUTION

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

T SHIBAHARA — Hokkaido Research Station, National Institute of Animal Health, 4 Hitsujigaoka, Toyohira, Sapporo 062-0045 Japan
Email: tshiba@affrc.go.jp

Y WADA — Rumoi Livestock Hygiene Service Centre, 11 Miyazono, Horonobe 098-3207 Japan

H TSUNEMITSU — Shichinohe Research Unit, National Institute of Animal Health, 31 Uminai, Shichinohe, Kamikita, Aomori 039-2586 Japan

M KUBO — National Institute of Animal Health, 3-1-1 Kannondai, Tsukuba, Ibaraki 305-0856 Japan

Y ISHIKAWA
K KADOTA — Hokkaido Research Station, National Institute of Animal Health, 4 Hitsujigaoka, Toyohira, Sapporo, 062-0045 Japan

Rotavirus 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 *H pylori* also have been found frequently in the bovine abomasum.[3] To date neither rotavirus nor *Helicobacter* spp have been documented in reindeer (*Rangifer tarandus*). In the present paper, the association between rotavirus and *Helicobacter*-like organisms in diarrhoea or enteritis in a reindeer calf is described.

A 5-month-old female reindeer on a reindeer farm showed depression, loss of appetite and yellowish watery diarrhoea, which soon led to severe dehydration and weight loss, and was humanely killed. The organs were fixed in 10% phosphate buffered formalin and embedded in paraffin. Tissue sections (approximately 3 μm thick) were obtained using routine histological techniques. The sections were stained with haematoxylin and eosin, Gram and Giemsa for histological examination. The gastrointestinal specimens were also stained by the Warthin-Starry technique.

Serial histological sections from the fundic and pyloric regions of the abomasum and the small intestinal tissues were prepared for immunohistochemical staining with streptavidin-biotin-alkaline phosphatase (Histofine SAB-PO Kit; Nichirei Corporation, Japan). Endogenous peroxidase activity was blocked by methanol and 3% H_2O_2. Primary antibodies were rabbit polyclonal antibodies against bovine group A rotavirus 22R strain (G6 P5), bovine coronavirus Mebus strain and *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 rotaviral 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 subglandular 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 antisera 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 canaliculus 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.

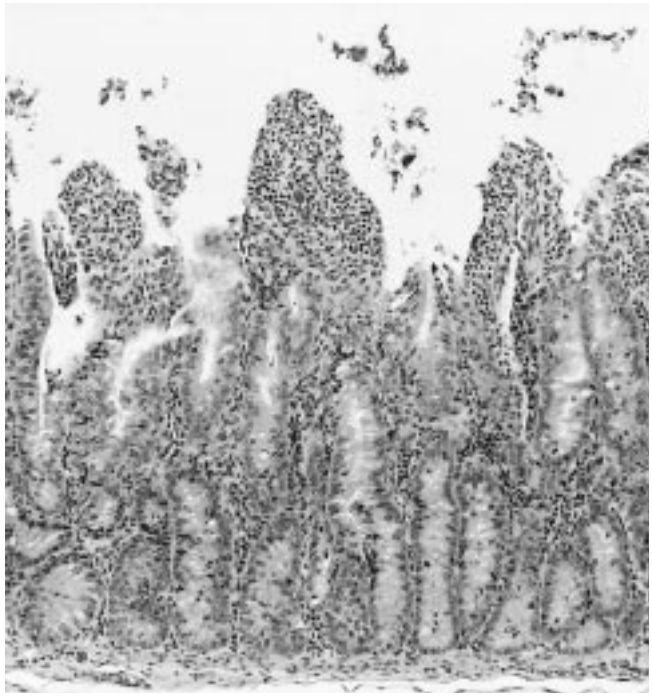

Figure 2. Degeneration of the epithelial cells at the tops of the villi in the jejunum. HE, x100.

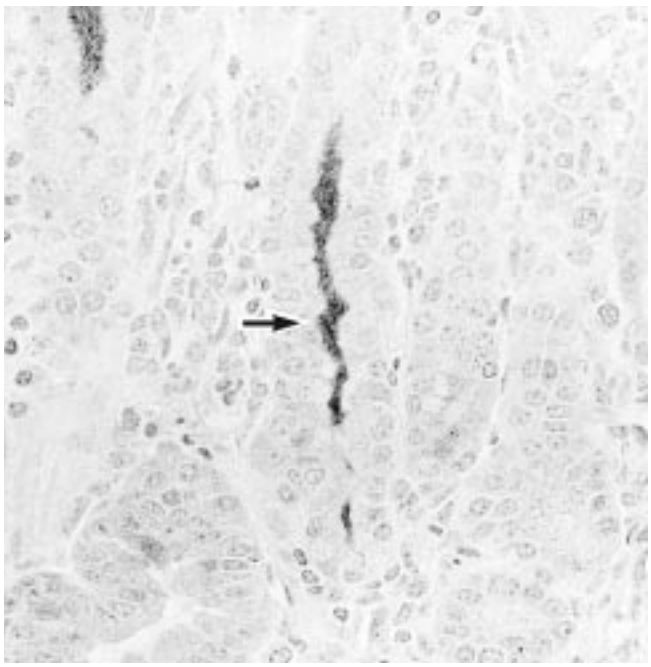

Figure 3. *H pylori* antigens (arrow) are clearly visible in the degenerative epithelium in the jejunum. Anti-*H pylori* immunoperoxidase, x400.

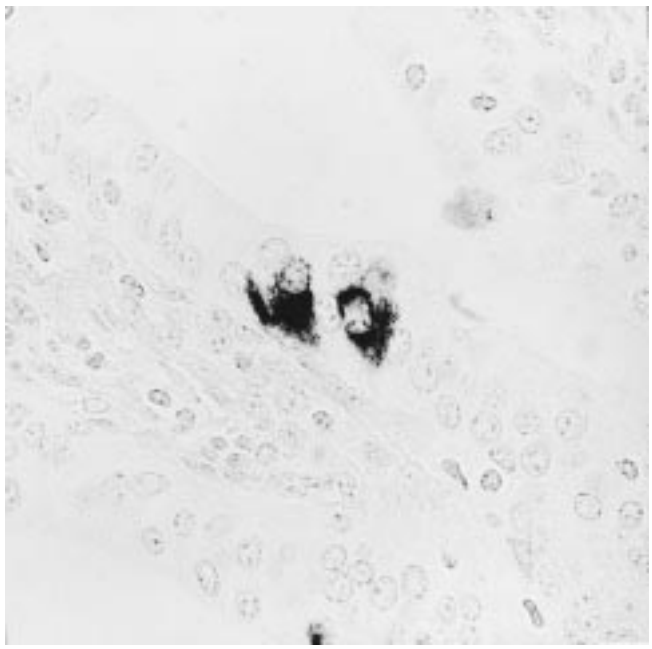

Figure 4. Rotaviral antigens are clearly visible in the epithelium in the jejunum. Anti-rotavirus immunoperoxidase, x630.

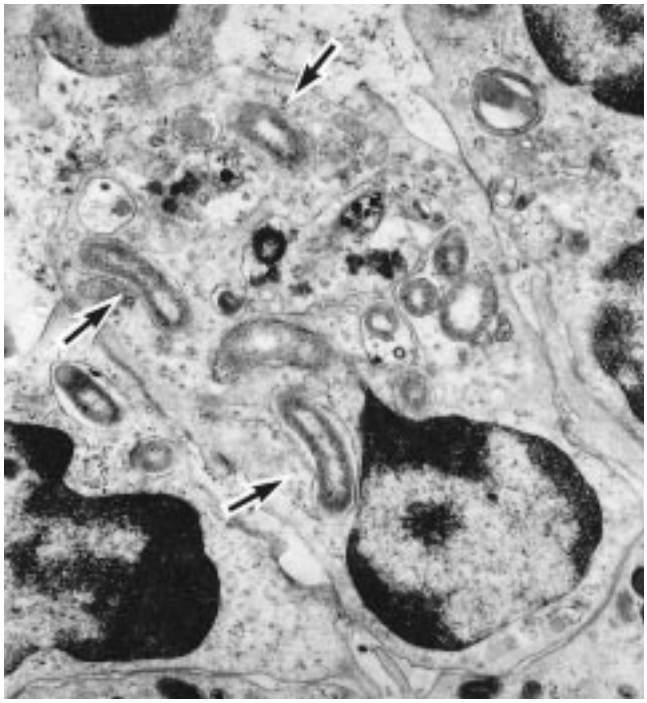

Figure 5. Phagocytosed bacteria (arrows) in the cytoplasm of a neutrophil in the lumen of a gastric gland. Uranyl acetate and lead citrate, x9800.

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.[4] All three are now known to be *Helicobacter* on the basis of 16S rRNA sequence analysis.[2] 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.[5] On the other hand, one report indicates the presence of serum antibodies in 6 of 22 calves, which reacted with epitopes from *H pylori*.[6] Moreover, spiral-shaped bacteria, including *H pylori*, may be found frequently in the bovine abomasum.[3] 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 canaliculus of parietal cells in gastric glands. *H bizzozeronii* (*H heilmannii*) also has been observed in parietal cells in a group of 45 rhesus monkeys infected with *H bizzozeronii*.[7] *H bizzozeronii* infection may be responsible for hypersecretion of acid.[8] Additionally, *H pylori* infection is related to increased gastric acid secretion in humans.[9] Moreover, inflammatory cytokines induced by *H pylori* infection may affect acid secretion in parietal cells.[10] This reindeer had histological evidence of inflammatory bowel disease; therefore, it would appear that clinical signs were caused by this disorder. *Helicobacter*-like organisms and rotavirus were present in the small intestinal lesions at the same time. Rotavirus infection can result in high mortality and this usually is attributed to mixed infection with such agents as coronavirus, adenovirus and toxigenic *Escherichia coli*.[11] The small intestinal lesions seemed to be caused mainly by rotavirus in the present case,[1] perhaps associated with *Helicobacter*-like organisms. Some *Helicobacter* species (for example, *H cinaedi* and *H fennelliae*) have been linked to proctitis and colitis in immunocompromised humans,[12] and furthermore, two cases of human enteritis associated with *H pullorum* have been reported.[13] Taken in the light of recent reports, our findings indicate that *Helicobacter* and rotavirus infection occurred in the gastrointestinal tract of a reindeer at the same time. The findings further suggest that *Helicobacter*-like organisms may play a role in the enteritis associated with rotaviral infection in reindeer.

The authors would like to thank Dr Y Ando for helpful advice and Mr T Fujisawa for his preparation of excellent photomicrographs.

References

1. Jones TC, Hunt RD, King NW. Disease caused by rotaviruses. In: Jones TC, Hunt RD, King NW, editors. *Veterinary Pathology*. 6th edn. Williams & Wilkins, Philadelphia, 1997:281-288.
2. Fox JG, Lee A. The role of *Helicobacter* species in newly recognized gastrointestinal tract diseases of animals. *Lab Anim Sci* 1997;47:222-255.
3. Braun U, Anliker H, Corboz L et al. The occurrence of spiral-shaped bacteria in the abomasum of cattle. *Schweiz Arch Tierheilkd* 1997;139:507-516.
4. Lockard VG, Boler RK. Ultrastructure of a spiraled microorganism in the gastric mucosa of dogs. *Am J Vet Res* 1970;31:1453-1462.
5. Jelinski MD, Ribble CS, Chirino-Trejo M et al. The relationship between the presence of *Helicobacter pylori*, *Clostridium perfringens* type A, *Campylobacter* spp, or fungi and fatal abomasal ulcers in unweaned beef calves. *Can Vet J* 1995;36:379-382.
6. Seidel KE, Kampschulte J, Lehn N et al. *Helicobacter pylori*: antibodies in sera of pigs and calves. *Berl Munch Tierarztl Wochenschr* 1996;109:434-439.
7. Sato T, Takeuchi A. Infection by spirilla in the stomach of the rhesus monkey. *Vet Pathol Suppl* 1982;7:17-25.
8. Dubois A, Tarnawski A, Newell DG et al. Gastric injury and invasion of parietal cells by spiral bacteria in rhesus monkeys. Are gastritis and hyperchlorhydria infectious diseases? *Gastroenterology* 1991;100:884-891.
9. el-Omar EM, Penman ID, Ardill JE et al. *Helicobacter pylori* infection and abnormalities of acid secretion in patients with duodenal ulcer disease. *Gastroenterology* 1995;109:681-691.
10. Tani N, Watanabe Y, Suzuki T et al. Effects of inflammatory cytokines induced by *Helicobacter pylori* infection on aminopyrine accumulation in parietal cells isolated from guinea pigs. *Dig Dis Sci* 1999;44:686-690.
11. Pearson GR, McNulty MS, Logan EF. Pathological changes in the small intestine of neonatal calves naturally infected with reo-like virus (rotavirus). *Vet Rec* 1978;102:454-458.
12. Totten PA, Fennell CL, Tenover FC et al. *Campylobacter cinaedi* (sp. nov.) and *Campylobacter fennelliae* (sp. nov.): two new *Campylobacter* species associated with enteric disease in homosexual men. *J Infect Dis* 1985;151:131-139.
13. Steinbrueckner B, Haerter G, Pelz K et al. Isolation of *Helicobacter pullorum* from patients with enteritis. *Scand J Infect Dis* 1997;29:315-318.

(Accepted for publication 11 September 2000)