Typhlitis Caused by Intestinal Serpulina-Like Bacteria in Domestic Guinea Pigs (Cavia porcellus)

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Received 19 May 1997/Returned for modification 15 July 1997/Accepted 14 November 1997

Between January 1992 and December 1996, Serpulina-like bacteria were demonstrated in intestinal tract lesions from 37 of 88 guinea pigs submitted to the University of Ghent, Belgium, for necropsy because of disease and death from different unknown causes. All infected animals had a history of sudden death with minimal introductory clinical signs. Occasionally, they produced yellow, slimy feces or showed nervous signs, but the condition always had a fatal outcome within 24 h. When larger colonies of guinea pigs were involved, the disease spread very rapidly unless treatment with ronidazole was initiated. Lesions consisted of a catarhal or hemorrhagic inflammation of the colon and cecum (typhlitis). Electron microscopy demonstrated the presence of large numbers of Serpulina-like organisms adhering to the cecal mucosa of these animals. Attempts to isolate the agents failed. The organisms did not stain by an immunofluorescence technique for the detection of Serpulina hyodysenteriae. The present data provide evidence that intestinal Serpulina-like organisms can be important as a cause of disease in guinea pigs.

Serpulina-like organisms have been observed in the intestinal tracts of numerous mammalian and bird species, including rodents, dogs, pigs, humans, and poultry (1–6, 8, 10–12, 24). Many of these spirochetes are normal inhabitants of the intestinal tract, but others have a pathogenic significance. The latter category of spirochetes usually causes cecal lesions, for example, dysentery in swine and spirochete typhlitis in chickens (1, 7, 21).

Intestinal spirochetosis has also been observed in guinea pigs. In 1977, McLeod et al. (14) described spiral to curved organisms along the brush border and in the crypts of the colon and cecum of a guinea pig suffering from Tyzzer’s disease. However, the investigators did not relate the presence of the spirochetes to clinical disease. Zwicker et al. (26) reported the presence of spirochetes in the cecum and colon of a guinea pig with acute diarrhea. Histologically, large numbers of spirochetes were present in the walls of both the cecum and the colon, and they were associated with severe necrosis and inflammation. Although the bacterium was not observed in this animal, Zwicker et al. (26) ascribed the clinical signs and lesions to Bacillus piliformis. Starting in 1992, we often found lesions of hemorrhagic typhlitis-colitis associated with sudden deaths in guinea pigs. Cytologic, histologic, and electron microscopic examinations demonstrated large numbers of spirochetes in these lesions. Other agents that might explain the acute deaths were not found. Outbreaks of this condition could be controlled by the administration of ronidazole. These findings may indicate an etiologic relationship between intestinal spirochetes and clinical disease.

The present article gives an overview of the clinical history and necropsy findings for 37 guinea pigs suffering from intestinal spirochetosis submitted to our institute between January 1992 and December 1996.

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tetraside in distilled water. For scanning electron microscopy, samples were dehydrated in alcohol and acetone for subsequent drying to the critical point in liquid carbon dioxide, glued with carbon cement onto aluminum stubs, sputtered with colloidal gold, and examined with a Philips 501 scanning electron microscope. Samples for transmission electron microscopy (TEM) were block stained with 2% (wt/vol) uranyl acetate in distilled water and were dehydrated in etha-

Field observations. When spirochete infections were diagnosed in larger guinea pig colonies, a 7-day treatment with ronidazole was given at a concent-

\begin{table}
\centering
\begin{tabular}{|l|c|c|c|c|c|}
\hline
Clinical history & Macroscopic lesions & Additional signs & Total \\
& Cecum and/or colon & Duodenum & Internal organs & Vitamin C deficiency & Flagellar parasites \\
\hline
Sudden death without signs & 12 & 0 & 11 & 3 & 4 & 14 \\
Diarrhea and sudden death & 17 & 4 & 16 & 1 & 5 & 17 \\
Nervous signs and sudden death & 4 & 1 & 2 & 2 & 3 & 4 \\
Skin disease & 0 & 0 & 0 & 0 & 0 & 1 \\
Anamnesis missing & 1 & 0 & 1 & 0 & 0 & 1 \\
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\end{tabular}
\caption{Clinical history and necropsy observations for 37 guinea pigs naturally infected with Serpulina-like spirochetes}
\end{table}

RESULTS

For 51 of the 88 guinea pigs submitted for necropsy between January 1992 and December 1996, variable causes of disease but no spirochetes were detected. These animals are not fur-

Histologic changes in the cecum were characterized by numerous slender or filamentous helical gram-negative bacteria adhering to the apical surface of the cecal epithelium, causing a brush-border, ciliated appearance to these cells (Fig. 1A). Hyperemia and transmigration of neutrophils were observed in the mucosa. Occasionally, necrosis of the cecal crypt epithelium was noticed. The lamina propria contained a mild, diffuse infiltration of macrophages, plasma cells, neutrophils, and eo-

Scanning electron microscopy revealed spirochetes on the cecal epithelial surfaces of the three animals examined (Fig. 1B). The cecal segments were so heavily colonized by these spirochetes that the underlying mucosal surface was only scarcely discernible. TEM examination of the cecal segments (Fig. 1C and D) demonstrated many spirochetes intimately attached end on to the surfaces of the enterocytes and crypt epithelium. Four to seven periplasmic flagellae could sometimes be observed in the bacteria. Frequently, an electrondense invagination of the apical plasma membrane was observed when the spirochetes were attached to the plasma membrane. The number of microvilli was reduced.

Attempts to isolate the spirochetes remained unsuccessful. Indirect immunofluorescence experiments with \textit{S. hyodysenteriae} antibodies were negative.

Field observations. Practice information revealed that treatment with ronidazole at 100 mg/liter arrested the further spread of disease in guinea pig populations. Regimens with ronidazole at 50 mg/liter of drinking water either had no clinical effect or stopped the course of disease as long as the treatment lasted but with new cases occurring almost immedi-

Thirty-five guinea pigs infected with \textit{Serpulina}-like bacteria had died very suddenly, usually without introductory clinical signs. For 21 of these guinea pigs, the owners reported that the animals had briefly suffered from acute diarrhea, producing yellow, slimy feces, or had shown nervous signs such as convulsions, paralysis, and involuntary movements. In all animals, these clinical signs became fatal within a few hours. The signs occurred in guinea pigs of different ages, ranging from 1 week to 3 years, and were equally divided between animals of both sexes. When guinea pigs were kept in large colonies, the disease clearly spread rapidly, with numerous animals dying acutely in successive days. For two populations signs were emaciation and anorexia; in one of these two guinea pig pop-

The most typical macroscopic changes found in 34 of the infected animals consisted of dilatation of the cecum and colon and the accumulation of liquid contents with a greenish yellow appearance. Blood and mucus were found in the intestinal tract, especially in the cecum. In three animals, ne-

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FIG. 1. (A) Epithelium of the cecum of a guinea pig showing a compact layer of spirochetes (arrowheads) coating the surface of the enterocytes. HE staining was used. Magnification, ×40. (B) Scanning electron micrograph of the cecal mucosa of a guinea pig showing extensive colonization of the mucosal surface by spirochetes. Bar, 1 μm. (C and D) Transmission electron micrographs of thin sections of the cecal mucosa of another animal. Bars, 0.5 μm. Numerous spirochetes are attached end on to the luminal cells (C). At higher magnification (D), periplasmic flagellae (arrowhead) and intimate contact between the bacterial cells and the mucosal surface are seen.
DISCUSSION

Intestinal spirochetes have been found in many mammalian species. The pathogenicities of these agents are often not clear (7). The possible pathogenicity of spirochetes observed in the large intestines of guinea pigs therefore deserves careful consideration. To determine whether these organisms are pathogenic for guinea pigs, Koch's postulates must be fulfilled. Since no experimental infections were induced, the etiologic role of spirochetes in disease in guinea pigs remains uncertain. Nevertheless, several observations strongly suggest a pathogenic significance of intestinal spirochetes in guinea pigs. First, infected animals clearly showed macroscopic and microscopic lesions associated with the presence of the bacteria. TEM examination of cecal segments demonstrated many spirochetes intimately attached to the enterocytes. Furthermore, other agents that may cause similar clinical signs or lesions have not been found in these guinea pigs. Finally, the disease could be efficiently controlled with ronidazole, a therapeutic agent that is known to be active against several Serpulina-like spirochetes (7, 16).

In none of the present 35 guinea pigs that died suddenly were agents such as Salmonella, Yersinia, and B. piliformis, which are mentioned in the literature as causes of sudden death in guinea pigs, found during necropsy, although we thoroughly checked for these organisms (15, 17, 25). Another important differential diagnosis is Clostridium difficile enterotoxemia. Diagnosis of this disease is rather difficult (18, 20, 23). At cytologic examinations, however, Clostridium-like bacteria were not detected. Moreover, there was never a history of antibiotic treatment before the clinical signs occurred (15, 17). Flagellar parasites, which were observed in 12 of the 37 animals (32%), are generally considered of low virulence for guinea pigs and are certainly not considered a cause of acute death (15, 17). This may indicate that Serpulina-like bacteria are an important cause of sudden death in guinea pigs living under domesticated circumstances, like the guinea pigs examined in our study.

Although most guinea pigs suffering from intestinal spirochetothis died very suddenly without premonitory signs of disease, it was striking that many of these animals were in bad general condition. Indeed, weight loss was noticed in 40% of the animals, 16% showed signs of vitamin C deficiency, and 32% had complementary infestation with flagellar parasites. This may indicate either that Serpulina-like bacteria affect the guinea pigs' general condition long before they induce acute death or that deaths associated with Serpulina-like bacteria are promoted by a general weakening of the animal through different causes such as vitamin C deficiency or infestations with flagellar parasites. In humans, it is well known that immunosuppression may result in chronic diarrhea associated with mass attachment of spirochetes end on to the surface epithelium of the lower intestine (7). Diarrhea associated with giardiasis and intestinal spirochetothis has been noticed in some human patients with AIDS (3, 19). In dogs, the role of Giardia in the pathogenesis of intestinal spirochetothis is unknown (3), but it cannot be ruled out that protozoa create an environment suitable for the development of the bacteria. Until now, all diagnoses of spirochetothis in guinea pigs have been made on the basis of morphological features of the bacteria in cytologic examinations. Therefore, it is possible that different species of spirochetes with similar morphologies occur in guinea pigs. Eventually, the virulence of these species for the host may also differ. A similar phenomenon has been observed in pigs, in which S. hyodysenteriae is highly pathogenic and Serpulina innocens does not have a pathogenic significance (5, 7, 8, 22).

Since our attempts to isolate spirochetes from guinea pigs have failed, their exact identity is still unknown. Further studies with different media and culture conditions will be necessary to try and isolate the spirochetes.

The lesions and clinical signs of guinea pig spirochetothis are to some extent similar to those of other mammalian spirochete diseases, such as S. hyodysenteriae infections in pigs. Different virulence mechanisms have been shown to contribute to the pathogenesis of the latter S. hyodysenteriae infections: motility, adherence, and production of a hemolysin and lipopolysaccharide. It is not known whether such virulence factors also occur in guinea pig spirochetes. Electron microscopic findings for affected guinea pigs, however, may suggest end-on adherence of the spirochetes to the cecal epithelium. Lesions on the internal organs of guinea pigs infected with spirochetes in the intestinal tract may indicate toxin activity.

Although Koch's postulates were not fulfilled, the present article provides evidence of a pathogenic role of Serpulina-like spirochetes in guinea pigs. When veterinarians or clinical laboratories are confronted with acute death in guinea pigs, they should take a spirochete infection into consideration. Since isolation of the responsible bacterium or bacteria has failed so far, the diagnosis must be made through cytologic and histologic examination of cecal lesions. Successful treatment with ronidazole may indicate, furthermore, that the diagnosis was correct.

ACKNOWLEDGMENTS

We are very grateful to the Flemish Institute for the Advancement of Scientific-Technological Research in the Industry (IWT, Brussels, Belgium) for its provision of a grant to Mia Vanrobaeys.

We thank J. Van den Bergh for preparation of the Warthin-Starry silver stains and C. Miry for performing the immunofluorescence tests.

G. Deboevere, P. De Laender, N. Dubois, M. Ledeq, C. Puttervils, A. Vandekerckhove, D. Vanrompay, and M. Vereecken are acknowledged for their skilled technical assistance.

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