Porcine proliferative enteropathy: Etiology and diagnosis

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Summary— *The intracellular organism of swine proliferative enteropathy has been identified as a new genus and species, unrelated to any of the* Campylobacter *species. A specific DNA probe has been developed that identifies this organism in diseased tissue. Primers prepared from this specific probe provide the basis for a sensitive assay to detect the intracellular organism shed in the feces of live swine with proliferative enteropathy.*

Porcine proliferative enteropathy or enteritis is a transmissible disease of weaned pigs of all ages. Recognized variously as intestinal adenomatosis, necrotic enteritis, regional ileitis, and proliferative hemorrhagic enteropathy, the disease presents either as an acute hemorrhagic syndrome that results in death, or as a nonhemorrhagic syndrome that causes chronic weight loss. In all manifestations, intestinal cryptal epithelial cells proliferate, thickening the walls of the ileum, jejunum, or large intestine. Small, curved, intracellular organisms are present in the apical cytoplasm of proliferating cells (Fig 1). The identity and origin of these intracellular organisms and their relationship to the pathogenesis of the disease are only now being resolved.

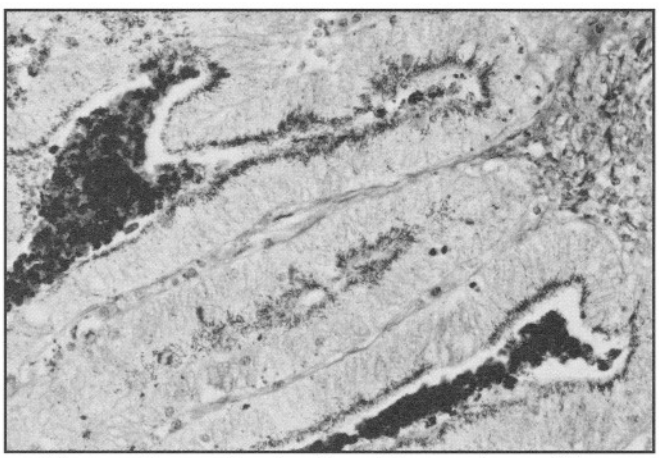

Fig 1.— Ileum of a pig affected by porcine proliferative enteropathy. Dark line along the apical cytoplasm of crypt cells are many small, curved intracellular rod bacteria.

Previous studies, using immunohistochemical techniques and antigenic analyses, have suggested that the intracellular organisms observed in all forms of the disease were similar, if not identical, to each other.[5,6] These organisms morphologically resemble the *Campylobacter* species. Several species of *Campylobacter* with a similar curved morphology and size are cultivated from the porcine lesions of proliferative enteropathy, including *C. coli*, *C. hyointestinalis*, and *C. mucosalis*. So far, however, transmission and immunohistological studies have shown that none of these culturable organisms is the intracellular organism of proliferative enteropathy.[6] Attempts to culture the intracellular organism on cell-free artificial media have failed, but the organism has been cultivated intracellularly in a rat enterocyte cell culture.[4]

Development of DNA probes specific for the intracellular organism

The consistent presence of the intracellular organisms in proliferating pig intestinal epithelial cells suggests that the organism is involved in the disease process. Because clinical signs are unreliable to diagnose the proliferative enteropathies, a definitive diagnosis can only be made at necropsy or slaughter. An antemortem diagnostic test would enable surveillance and monitoring procedures. Recently, specific DNA probes to detect the intracellular organism have been generated and characterized.[2] For this, intracellular organisms were purified directly from the lesions of a pig with proliferative enteropathy, without culturing. Probes were produced that hybridized with porcine mucosa obtained from pigs with proliferative enteropathy, but not with normal mucosa. The probes hybridized equally with mucosa obtained from each of the various clinical forms of proliferative enteropathy. The probes failed to hybridize with any of the commonly isolated porcine *Campylobacter* species, including *C. hyointestinalis*, *C. mucosalis*, and *C. coli*. Conversely, species-specific DNA probes for *C. mucosalis* and *C. hyointestinalis* do not react

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with the intracellular organism. Therefore, the intracellular organism is an as yet unidentified species.

Assay for detection of the intracellular organism in feces

A sensitive assay based on amplification of a portion of the DNA targeted by this intracellular organism-specific probe was developed to detect the intracellular organism in the feces of live pigs. Primers prepared from these probes were used in the polymerase chain reaction to amplify DNA of the organism in samples of infected pig feces to numbers that could be detected using a diagnostic test.[3] Following extraction of DNA from fecal samples, as few as 10^3 organisms per gram of feces were detected. No amplification product was produced from amplifications performed on DNA extracted from the feces of healthy pigs. This test is currently being evaluated at the University of Minnesota in field outbreaks and epidemiological surveys.

Molecular identification of the intracellular organism

Finally, the identity of the intracellular organism has been determined using a novel genetic technique. A portion of the genome of the intracellular organism was isolated, without culture, and amplified so that it could be sequenced and compared to similarly obtained sequences from other organisms. This technique is called 16S ribosomal DNA sequence phylogeny and was successfully used to definitively identify the intracellular organism of proliferative enteropathy.[1] Again, intracellular organisms were released and purified from the proliferative cells without using culture techniques. After DNA purification, highly conserved primers and the polymerase chain reaction technique were used to amplify the 16S ribosomal DNA. The products were then sequenced and the sequences were compared to those of the *Campylobacter* species and other groups of organisms. The sequences obtained did not align with any *Campylobacter* species, but did align with a nonrelated organism, *Desulfovibrio desulfuricans* (91% similarity). Therefore, phylogenetic analysis shows that these intracellular organisms are related to the strictly anaerobic sulfate-reducing bacteria, rather than to any *Campylobacter* species.

Conclusions

- DNA probes specific for the intracellular organism provide a simple method to diagnose and identify the organism. The observation that the probes hybridize to the intracellular organism from all forms of the disease confirms that the organisms seen in various clinical and pathological manifestations of proliferative enteropathy are similar or identical. These intracellular organism-specific probes may be used to detect and identify the intracellular organism from tissues of pigs with proliferative enteropathy.
- The lack of antemortem diagnostic techniques has limited investigations of the epidemiology, prevalence, and economic impact of proliferative enteropathy. Using primers to amplify specific DNA in feces is a sensitive and specific technique to identify the disease without necropsy. The use of the assay will facilitate disease monitoring in the field and experimental studies of the pathogenesis and epidemiology of proliferative enteropathy.
- Finally, the 16S ribosomal-DNA-sequence results show that the intracellular organism of porcine proliferative enteropathy is not related to *Campylobacter* species and that it differs from other described genera as well. It is most closely related to a group of anaerobic sulfate-reducing bacteria, specifically to *Desulfovibrio desulfuricans*. The 16S ribosomal-DNA-sequence data, coupled with the unique curved bacillary shape, habitat, and growth characteristics will eventually warrant this organism's classification as a new genus and new species. The name "*Ileobacter intracellularis*" has been proposed for this organism. Until validly published, it will be referred to in the literature by the vernacular name, ileal symbiont intracellularis (IS-intracellularis).

Identification of the intracellular organism of proliferative enteropathy as a new genus and species, unrelated to the *Campylobacter* species, will affect the direction of research on the pathogenesis of the disease. Now that the identity is known, researchers can develop cultivation procedures for this newly identified organism.

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