Isolation of \textit{Yersinia pseudotuberculosis} from Bovine Calves with Enteric Disorders

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(Accepted for publication, October 6, 1983)

Yersiniosis due to \textit{Yersinia pseudotuberculosis} has been reported from all continents with most cases occurring in the northern hemisphere, especially Europe. \textit{Y. pseudotuberculosis} has been isolated from a wide range of animals and birds and most outbreaks occur in laboratory animals (8). Isolation of \textit{Y. enterocolitica} from man, cat, and pig in India was reported for the first time in 1980 (9) but \textit{Y. pseudotuberculosis} as a bovine pathogen has never been ascertained with definite proof. The authors believe that this communication records the first authentic isolation of \textit{Y. pseudotuberculosis} from young bovine calves suffering from enteric disorders in India.

During the winter months (January-February), six buffalo calves 6–9 months age belonging to an organized National Dairy Research Institute, Karnal, India, succumbed within one week of illness in spite of routine antibiotic (streptomycin) and supportive therapy. These calves had presented symptoms of intermittent diarrhea, listlessness, depression followed by nervous excitement and accelerated respiration, pulse and temperature. Immediate postmortem examination of the dead calves was conducted and after gross lesions were recorded, intestinal and abomasal contents along with other internal organs were appropriately collected and transported to the laboratory for bacteriological examination. Portions of intestine and other organs were also processed for histopathological examination.

The material was streaked directly on blood agar (10% sheep blood), modified MacConkey's agar (MMA), and Salmonella-Shigella-desoxycholate (SSD) agar plates (5, 11). No enrichment medium was used for processing these samples. The streaked plates were incubated at 37°C for 24 hr and examined after further incubation at room temperature (23.0±1.0°C) for 24 hr. The suspected and purified colonies were characterized culturally and biochemically (4). Antibiotic sensitivity was determined by the single disc diffusion method on nutrient agar (2). The biochemically characterized \textit{Yersinia} isolate was confirmed and serotyped by Dr. S. Toma, Central Public Health Laboratory, Ontario, Canada, whereas \textit{Escheri-
Fig. 1. Gross changes in the intestines, still connected with the fore stomach, showing extensive serosal thickening and glossy appearance. Arrow indicates congestion of the mucosa.

Fig. 2. Microscopic changes in the mucosa of the small intestine showing necrosis of the tip of the villi and surface epithelium, infiltration of macrophages with few neutrophils and eosinophils and epithelial hyperplasia of the crypts of Liberkühn (H & E × 100).
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Autopsy consistently revealed pronounced serosal thickening of the walls of the lower fundus, and pylorus of the abomasum and intestines. The thickened serosa appeared glossy, soft, and translucent (Fig. 1) and its cut surfaces exuded a gelatinous substance. The mucosa of the abomasum and intestines was thickened, congested, and streaked with hemorrhages and the lumen of the intestines contained a whitish slimy exudate. Both lungs, liver, and heart were highly congested whereas the kidneys were pale and the pericardium was slightly thickened. Microscopically, the mucosa of the intestines revealed necrosis of the tip of the villi and surface epithelium and infiltration of macrophages with few neutrophils and eosinophils (Fig. 2). Dilatation, engorgement and at places extravasation of the blood vessels and a similar type of cell infiltration was marked in the lamina propria. Epithelial hyperplasia of the crypts of Lieberkühn was moderate. The submucosa showed moderate edema, degeneration and necrosis of the germinal center of the lymphatic nodules. The serosal connective tissues were markedly distended owing
to extensive edema (Fig. 3) and were infiltrated focally with inflammatory cells, viz. lymphocytes, macrophages, and neutrophils. In the kidneys, marked congestion with focal hemorrhages and degeneration of the tubular epithelium was seen. The hepatic cells were mildly degenerated and the central vein and sinusoids were acutely engorged. The lung parenchyma was highly congested and atelactic. In places, evidence of proliferative changes were seen in the mesothelium, and connective tissues of the pleura appeared as papillary projections with lymphocytic infiltration.

Bacteriological examination of the intestinal and abomasal contents yielded both minute nonlactose-fermenting and large lactose-fermenting colonies in an approximate ratio of 9:1. The nonlactose fermenting organisms were characterized as Y. pseudotuberculosis, serotype III whereas the lactose fermenters were E. coli, 01 and 0146. The Y. pseudotuberculosis strain isolated was negative for oxidase, indole, Voges-Proskauer test (37 or 25 C), Simmon’s citrate, malonate, hydrogen sulphide (TSI), lactose, sucrose, gas from glucose, raffinose, adonitol dulcitol, inositol, phenylalanine deaminase, arginine dihydrolase, and lysine decarboxylase. It was found positive for the methyl red test, nitrate reduction, catalase, mallow, and salicin (5 days). The strain was resistant to penicillin (5 units), tetracycline (10 μg), erythromycin (10 μg), and sulfadiazine (200 μg) whereas it was sensitive to chloramphenicol (30 μg), ampicillin (25 μg), kanamycin (30 μg), gentamycin (10 μg), polymyxin (250 units), cephaloridine (30 μg), nalidixic acid (30 μg), cloxacillin (10 μg), carbenicillin (50 μg), colistin (50 μg), furadantin (250 μg), and Septran (200 μg). Intermediate sensitivity was noted for streptomycin (25 μg) and neomycin (10 μg).

The etiology of enteric disorders, viz. diarrhea, in calves is a complex problem and in some cases association of different agents has been demonstrated. Though Y. pseudotuberculosis has been incriminated in bovine mastitis (7) and bovine abortion (6), it has neither been confirmed as an etiologic agent of calf intestinal disorders nor isolated from caecal contents and mesentric lymph node specimens from healthy cattle (12). Y. pseudotuberculosis serotype III, as reported here, has been recorded as the predominant serotype from intestines of healthy pigs in Japan (3, 10, 12). Y. pseudotuberculosis was considered as the primary etiologic agent of calf intestinal disorders, since direct plating of specimens on unenriched medium resulted in 90%. Y. pseudotuberculosis colonies whereas only 10% of the colonies were E. coli and the latter represented only a part of the intestinal flora of the calves under investigation. Unfortunately, serum samples from the calves were not available for serological study but the presence of prominent gross and histopathological lesions in the intestinal mucosa, not yet described in the literature, indicates the role of Y. pseudotuberculosis as an enteric pathogen. However, this can be confirmed after successful demonstration of an enterotoxin (?) or other virulence factor in a suitable model as done for other pathogenic enterobacteria. Surprisingly, the presence of Y. pseudotuberculosis and/or Y. enterocolitica could not be demonstrated in the feces of 226 healthy and diarrheic bovines (adult and young) examined subsequently on the same and nearby premises (1).
NOTES

The authors thank (i) Dr. S. Toma, Central Public Health Laboratory, Ontario, Canada, for confirmation and serotyping of *Y. pseudotuberculosis*; (ii) Dr. S.N. Saxena, National Salmonella and *Escherichia* Centre, Kasauli, India, for serotyping *E. coli*; and (iii) Dr. J.R. Sadana, Department of Veterinary Pathology, for his help in histopathological interpretation.

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(Received for publication, August 23, 1983)