Isolation of Non-O1 Vibrio cholerae Associated with Enteric Disease of Herbivores in Western Colorado

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Non-O1 Vibrio cholerae was isolated from a horse (Equus caballus), a lamb (genus Ovis), and two American buffalo (Bison bison) suffering from enteric disease in the western part of Colorado. In 1981, a foal died of apparent respiratory failure. Necropsy findings included heart failure and gastroenteritis. V. cholerae serovar 347 (Smith) was isolated from the colon of this animal. V. cholerae serovar 27 (Smith) was isolated in 1983 from the intestine of a feedlot lamb suffering from pneumonia and severe watery diarrhea. In 1984, an enteric disease occurred in a herd of American bison. The sick animals were depressed and separated from the herd, dying in about 3 days. Of approximately 100 adult bison, 7 died. Necropsy of one animal revealed that gross lesions were limited to the gastrointestinal tract. V. cholerae serovar 27 (Smith) was isolated from the abomasum, duodenum, and colon of this animal. A swab specimen from the intestine of another dead bison also yielded V. cholerae serovar 27 (Smith).

Non-O1 Vibrio cholerae is biochemically indistinguishable from O1 V. cholerae, the causative agent of epidemic cholera in humans, but does not agglutinate in O group 1 antisera. Some strains of non-O1 V. cholerae produce an enterotoxin identical to that of O1 V. cholerae (28). Those environmental and clinical isolates that do not produce cholera toxin can possess other virulence factors which make them pathogenic (3, 21). Human gastroenteritis caused by non-O1 V. cholerae is well documented and has been reported from widespread geographical areas (3, 9, 19, 20). Human infections are most often associated with seafood consumption, saltwater exposure, or foreign travel (7, 9, 20). In the United States, non-O1 V. cholerae has been found along the coasts of the Gulf of Mexico (20), the Chesapeake Bay (6, 11), and the Pacific Ocean (13, 14) and has caused some human infections in inland regions (9; S. M. Gelbart and M. Prabhudesai, Abstr. Annu. Meet. Am. Soc. Microbiol. 1985, C120, p. 230).

Although non-O1 V. cholerae was isolated from a variety of animals in India and Bangladesh (23, 24), no clinical symptoms of disease were reported in animals under natural conditions. We report here the isolation of non-O1 V. cholerae from herbivores with enteritis.

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CASE REPORTS

Horse. In July 1981, a foal (about 2.5 months old) died after being treated for 1 week for apparent viral pneumonia. The foal had respiratory difficulty and a temperature of 39.5°C and had lost 50 pounds in 3 days. No diarrhea was reported; however, lesions were found in the colon at necropsy. V. cholerae and Aeromonas hydrophila were isolated from these lesions.

Lamb. In August 1983, a lamb feedlot operator noticed sudden deaths of lambs approximately 6 months old. The cause of the outbreak was found to be Haemophilus somnus.

However, in one of the lambs necropsied (but not representative of the herd problem), severe fluid diarrhea and pneumonia were observed. Pasteurella haemolytica was isolated from the lungs of this lamb, and V. cholerae was isolated from the intestine. No similar cases were presented.

Bison. In September 1984, a bison raiser noticed an outbreak of disease in which 7 of approximately 100 adult bison died. The sick bison were depressed and separated from the herd, dying in about 3 days. Symptoms reported by the owner were diarrhea, vomiting, serous nasal discharge with green frothy ingesta running from the nose, lacrimation, and reddened conjunctiva. Specimens of the intestinal contents, abomasum, duodenum, and colon of the bison yielded V. cholerae upon culturing. All of the sick bison were from a herd purchased about a month earlier and pastured with his existing herd. The bison had been on a new pasture for about 1 week. Examination of the pasture revealed no toxic plants.

After completion of necropsy and microbiologic examination, the owner was advised to move the bison out of the new pasture and treat them with tetracycline, one of the antimicrobial agents that inhibits V. cholerae. The owner chose oxytetracycline because of the ease of administration and reported no further problems after initiating this therapeutic regimen.

MATERIALS AND METHODS

Necropsy and histopathology. Necropsies of the foal, lamb, and one bison and histopathologic examination of tissues from the foal and bison were performed.

Microbiology. Samples from the colon of the foal, the intestine of the lamb, and the abomasum, duodenum, and colon of the necropsied bison were cultured on the following media: Trypticase (BBL Microbiology Systems, Cockeysville, Md.) soy agar with 5% sheep blood (BA) and Hektoen enteric agar (HE) (BBL). Selenite-F Enrichment (BBL) was inoculated with the same samples to test for Salmonella species. Swab specimens obtained from the intestines of two other dead bison and mailed to the Western Slope Animal Diagnostic Laboratory were inoculated directly onto thiosulfate-citrate-bile salts-sucrose (TCBS) agar. The cultures were incubated at 37°C for 18 to 24 h. HE plates were

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streaked with Selenite-F cultures, incubated for 24 h, and examined for lactose-nonfermenting colonies.

Eleven specimens of feces from the bison herd (after oxytetracycline treatment had been initiated) and two water samples from the pasture pond and ditch were incubated overnight at 37°C in alkaline peptone water (1% peptone, 1% NaCl [pH 8.4]). The alkaline peptone water enrichments were streaked onto TCBS agar, and the plates were incubated for up to 48 h and examined for growth typical of *V. cholerae*. Isolated colonies in the agar media were subcultured onto BA to establish stock cultures.

Bacteria were identified by methods outlined in the *Manual of Clinical Microbiology* (17), supplemented when necessary by reference to Bergey’s manuals (5, 15) and by use of the API 20E system (Analytab Products, Plainview, N.Y.). The fermentation reactions of bacteria were determined with the aid of the DMS Rapid CH system (Analytab Products). The directions of the manufacturer were followed when using the API 20E system and the DMS Rapid CH system. Isolates giving biochemical reactions typical of *V. cholerae* were tested for agglutination in O1 *V. cholerae* polyvalent antisera obtained from Harry L. Smith, Jr. (Vibrio Reference Laboratory, Jefferson Medical College, Philadelphia, Pa.).

Serotyping of the non-O1 *V. cholerae* strain isolated from the foal was performed by Harry L. Smith, Jr. Biochemical verification, measurements of cholera toxin production, and serotyping of the lamb and bison isolates with Smith antisera against non-O1 *V. cholerae* (25) were performed at the Enteric Bacteriology Section, Centers for Disease Control, Atlanta, Ga. Additional biochemical verification of *V. cholerae* isolates was done at the National Veterinary Services Laboratory, Ames, Iowa (for the lamb isolates) and at the Colorado Department of Health, Denver (for the bison isolates).

Samples from the intestine of the necropsied bison were examined for enteric parasites by direct microscopic examina- tion and for parasite ova and coccidial oocysts after concentration by zinc sulfate flotation. The direct microscopic examination of intestinal contents was used for the detection and presumptive identification of *Campylobacter* species. Wet-mount preparations were observed with the aid of dark-field microscopy for bacteria of characteristic size and morphology and exhibiting the corkscrew, darting type of motility of campylobacters (12, 15). No attempt was made to detect and culture enteric viruses and chlamydiae in specimens collected during necropsy of the animals.

**RESULTS**

**Histopathologic and microbiologic findings.** (i) Horse. The principal necropsy findings in the foal were chronic passive congestion resulting from heart failure due to a large patent ductus arteriosus. Thrombosis of the anterior mesenteric artery due to verminous arteritis caused poor circulation to the intestine. Additional findings were an inflamed and edematous gastrointestinal tract.

Microscopic lesions in the foal consisted of erosion and focal ulceration of the gastric mucosa, with chronic nonsuppurative inflammation and bacterial colonization. The intestine was hyperemic and edematous, and both mononuclear and polymorphonuclear leukocytes were found in increased numbers in the mucosa.

The BA plate inoculated with a sample from the colon of the foal had two types of hemolytic colonies in approximately equal numbers, and the confluent areas of hemolysis were greenish in color. Using the API 20E system, we identified these two colony types as *V. cholerae* and *A. hydrophila*. Only yellow-orange colonies were present on the HE plate; they were assumed at the time to be Escherichia coli.

(ii) Lamb. Necropsy of the lamb revealed that the ventral portions of the lungs were consolidated and covered with fibrin. Fluid and a mucopurulent exudate filled the lower airways. The intestinal wall was thin and moderately red- dened. The lumen was dilated and contained excess watery green feces containing shreds of mucosa.

BA plates streaked with intestinal contents from the lamb yielded mainly smooth, hemolytic, oxidase-positive colonies that imparted a greenish color to the hemolyzed areas of the medium. HE plates yielded predominantly yellow-orange colonies assumed to be *E. coli*. Further testing of subcultures of the hemolytic colonies showed them to be *V. cholerae*. No *Salmonella* species were isolated from Selenite-F Enrichment inoculated with specimens of the intestinal contents.

(iii) Bison. Gross lesions in the bison were limited to the gastrointestinal tract. The mucosa of the rumen was reddened, and the villi adhered to each other. The abomasal wall was thickened by edema, and the mucosa was dark red-purple. Shallow, coalescing erosions and ulcerations were seen in the small intestine and were most severe in the colon and cecum.

Histopathologic examination of tissues from the bison revealed that the most significant lesions were limited to the gastrointestinal tract and consisted of nonsuppurative inflammation. The abomasal mucosa was focally necrotic, and large numbers of bacteria were located deep within these necrotic foci. In some segments of the intestine changes were even more severe and the mucosa was nearly destroyed.

All BA plate cultures prepared from the specimens taken from the abomasum, duodenum, and colon of the bison yielded a predominance of large, smooth, hemolytic colonies that imparted a greenish color to the hemolyzed areas of the medium. HE plates inoculated at the same time as BA plates yielded only yellow-orange colonies. No *Salmonella* species were isolated after incubation of specimens of intestinal contents in Selenite-F Enrichment. Smooth, yellow colonies appeared on one of the plates of TCBS agar streaked with "mailed-in" swabs of intestinal contents from other dead bison. The hemolytic colonies on BA and the smooth, yellow colonies on TCBS agar were oxidase positive and identified as *V. cholerae*.

No enteric parasites or *Campylobacter* species were observed microscopically in specimens from the bison intestine.

No colonies of *V. cholerae* appeared on the TCBS agar plates streaked with alkaline peptone water enrichment cultures prepared from the 11 specimens of feces collected from the bison herd after the initiation of oxytetracycline therapy. Similarly, alkaline peptone water enrichment cultures of the two water samples taken from the water sources serving the bison herd did not yield growth typical of *V. cholerae* when plated on TCBS agar.

**Characteristics of *V. cholerae* isolates.** Excellent identification of *V. cholerae* was obtained with the API 20E system. All the animal isolates failed to agglutinate in antiserum against *V. cholerae* O group 1. The non-O1 *V. cholerae* strain from the horse was Smith serovar 347; the strains from the lamb and bison were Smith serovar 27. Cholera toxin production by non-O1 *V. cholerae* strains from the lamb and bison was not demonstrated by either the Y-1 adrenal cell
assay or the enzyme-linked immunosorbent assay; the V. cholerae strain from the horse was not tested for toxin production. The characteristics of the non-O1 V. cholerae isolates are shown in Table 1.

**DISCUSSION**

The non-O1 V. cholerae strain isolated from the foal may have contributed to the gastroenteritis, but the A. hydrophila strain also could have been involved. Perhaps both of these organisms were opportunistic invaders that multiplied because of impaired intestinal circulation due to heart failure and verminous arteritis.

The pathogenic role of V. cholerae in the intestinal infection in the lamb cannot be determined from the case presented in this report; however, the lamb did have a voluminous “rice-water” stool, a condition reported for some non-O1 V. cholerae infections in humans (1, 8, 19).

Non-O1 V. cholerae serovar 27 most likely contributed to the outbreak of the gastrointestinal disease in the bison because (i) it was the predominant organism isolated from all the cultured tissues; (ii) it was isolated from two dead bison; (iii) no other enteropathogenic bacteria were isolated from the intestinal contents; (iv) some tissue damage of the intestinal wall observed in the dead bison appeared to be similar to that described in infant rabbits infected experimentally with cholera toxin-negative strains of non-O1 V. cholerae (18); (v) no enteric parasites were observed in specimens of intestinal contents or in the histopathologic preparations of tissues from the dead bison; (vi) antibiotic treatment to control V. cholerae appeared to resolve the disease outbreak in the herd; and (vii) non-O1 V. cholerae is an enteric pathogen and the herd of bison had an enteric disease.

Malignant catarrhal fever and bovine virus diarrhea occur in bison, but gross and microscopic examination of appropriate tissues revealed no lesions characteristic of these diseases. Although other viruses and chlamydiae cannot be entirely eliminated as potential agents in this outbreak of acute diarrheal disease in the bison, we are not aware of reports of these organisms causing enteritis in adult animals. *Campylobacter* species are common inhabitants of the intestinal tracts of animals, and some species have been associated with intestinal diseases of cattle (2, 10). Direct dark-field microscopic examination of specimens from the intestines of the bison did not reveal bacteria with the characteristic morphology and motility of campylobacters. Although this procedure may not be as sensitive as selective culture methods for the detection of Campylobacter species, it was an indication that this pathogen, if present, was not present in large numbers in the gastrointestinal tract of the dead bison examined.

The source(s) of the non-O1 V. cholerae in these cases of gastroenteritis in herbivores in western Colorado remains unknown. Perhaps V. cholerae is more widespread in inland areas in this country than previously thought. Non-O1 and O1 V. cholerae strains are usually found in brackish water along coastal areas (6, 11), although they have been isolated in inland areas. In several surveys conducted in Kent, England, V. cholerae was found to occur sporadically in small numbers in fresh water but regularly in large numbers in brackish water during the summer months (16, 26). Brackish water can be found in nonmountainous areas of western Colorado, especially in mid to late summer, when all of these outbreaks occurred. The surface water in these areas has a relatively high concentration of salts, particularly in areas where the water is stagnant and evaporating.

Our inability to detect V. cholerae in the water samples taken from the pasture where the bison herd grazed may have been due to a number of factors, including the limited volume of water tested, the incubation temperature (37°C) of the enrichment broth, competing microflora, or nonculturable but viable cells or all of these. V. cholerae, *E. coli*, *Salmonella enteritidis*, and *Shigella* species may exist in aquatic environments in a viable but nonculturable state (4, 22, 27).

To our knowledge, this is the first report of V. cholerae associated with enteric disease in herbivores under natural conditions. The strains isolated from the bison and lamb did not produce cholera toxin; however, it is not unusual for these types of non-O1 V. cholerae to still be pathogenic (18, 21). The finding of V. cholerae in these herbivores points to another possible vector, in addition to aquatic birds (16, 26), for this organism in the environment in inland areas of the United States. However, we cannot rule out the possibility that V. cholerae may be indigenous to certain waters,

**TABLE 1.** Characteristics of non-O1 *V. cholerae* from horse, lamb, and bison

<table>
<thead>
<tr>
<th>Test or substrate</th>
<th>Reaction</th>
</tr>
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<tbody>
<tr>
<td>Indophenol oxidase</td>
<td>+</td>
</tr>
<tr>
<td>Arginine dihydrolase</td>
<td>-</td>
</tr>
<tr>
<td>Lysine decarboxylase</td>
<td>+</td>
</tr>
<tr>
<td>Ornithine decarboxylase</td>
<td>+</td>
</tr>
<tr>
<td>Indole</td>
<td>-</td>
</tr>
<tr>
<td>Urease</td>
<td>-</td>
</tr>
<tr>
<td>Simmons citrate</td>
<td>+</td>
</tr>
<tr>
<td>Kliger ion agar</td>
<td>Alk/A</td>
</tr>
<tr>
<td>H2S (in Kliger ion agar)</td>
<td></td>
</tr>
<tr>
<td>o-Nitrophenyl-β-d-galactopyranoside</td>
<td>+</td>
</tr>
<tr>
<td>Voges-Proskauer (37°C)</td>
<td>+</td>
</tr>
<tr>
<td>Motility</td>
<td>+</td>
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<tr>
<td>Gelatinase</td>
<td>+</td>
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<tr>
<td>Nitrate reductase</td>
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<tr>
<td>MacConkey agar growth</td>
<td>+</td>
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<tr>
<td>Hemolysis (sheep blood)</td>
<td></td>
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<tr>
<td>Growth in 0°C, 3%, or 5% NaCl</td>
<td>+</td>
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<tr>
<td>Growth in 7% NaCl</td>
<td>-</td>
</tr>
<tr>
<td>Growth at 25°C, 35°C, or 40°C</td>
<td>+</td>
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<tr>
<td>&quot;String test&quot; (17°C)</td>
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<tr>
<td>Gas produced from glucose</td>
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<table>
<thead>
<tr>
<th>Acid produced from glucose</th>
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<tbody>
<tr>
<td>Glucose</td>
<td>+</td>
</tr>
<tr>
<td>Lactose</td>
<td>+</td>
</tr>
<tr>
<td>Sucrose</td>
<td>+</td>
</tr>
<tr>
<td>Arabinose</td>
<td>-</td>
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<tr>
<td>Cellobiose</td>
<td>-</td>
</tr>
<tr>
<td>Inositol</td>
<td>-</td>
</tr>
<tr>
<td>Mannitol</td>
<td>+</td>
</tr>
<tr>
<td>Mannose</td>
<td>-</td>
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<tr>
<td>Gluconate</td>
<td>+</td>
</tr>
<tr>
<td>Glycerol</td>
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<td>+</td>
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<tr>
<td>Galactose</td>
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<tr>
<td>Maltose</td>
<td>+</td>
</tr>
<tr>
<td>Vibrio-statin O/129</td>
<td>-</td>
</tr>
<tr>
<td>Polyoxymylin B (50 U)</td>
<td>R</td>
</tr>
<tr>
<td>Cholera toxin</td>
<td>-</td>
</tr>
</tbody>
</table>

* +, Positive reaction; −, negative reaction; S, susceptible; R, resistant; Alk/A, alkaline slant, acid butt in 24 h.
* a, Lamb strain negative; horse and bison strains positive in 3 days.
* b, Lamb strain positive; horse and bison strains negative.
* c, The horse strain was not tested for cholera toxin.
especially brackish water (6, 26), and therefore that herbivores drinking the water may become transient or chronic carriers of the organism. Also, under the proper conditions, the organism may contribute to or possibly cause enteritis in herbivores.

It is important to point out that the V. cholerae in these cases was first detected by the hemolysis and greenish color on the BA plates. The HE plates had only yellow-orange colonies that were mistakenly assumed to be all lactose-fermenting coliforms (e.g., E. coli). However, V. cholerae does not grow slowly through sucrose fermentation. These colonies are easily mistaken for E. coli colonies. Microbiologists should be aware of this when cultivating for enteric pathogens. The hemolysis and greenish color have also been observed with A. hydrophila strains.

The isolation of V. cholerae is becoming more common in the United States because clinical microbiologists recognize its role in human disease. Normally, microbiologists in animal diagnostic laboratories do not attempt to culture V. cholerae from animals with gastroenteritis. We hope that this report will make veterinarians and microbiologists more aware of V. cholerae as another possible participant in enteric infections of herbivores.

ACKNOWLEDGMENTS

We thank Betty J. Ogg for her excellent secretarial assistance, Harry L. Smith, Jr., for serotyping the horse isolate and supplying the V. cholerae O group 1 antisera, and Archibald F. Alexander (Director, Colorado State University Animal Diagnostic Laboratories) for his review of the manuscript and helpful suggestions. We are grateful to Mr. R. R. Frentham, Foraging Section, Centers for Disease Control, for serotyping the lamb and bison isolates and testing them for the production of cholera toxin.

LITERATURE CITED