The Prevalence of *Aeromonas* Species in Feces of Horses with Diarrhea

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Feces collected from 40 horses with diarrhea and 34 horses without diarrhea were examined to determine if an association existed between isolation of *Aeromonas* spp. and diarrhea. Samples were also examined for *Salmonella* spp., and identification of viruses and parasite ova. Neither *Salmonella* spp. nor *Aeromonas* spp. were isolated from the feces of 34 control horses. *Aeromonas* spp. were isolated from feces of 22 of 40 (55%) horses with diarrhea. *Salmonella* spp. were isolated from feces of 8 (20%) horses, and of these, 5 (12.5%) were also positive for *Aeromonas* spp. Twenty-nine isolates of *Aeromonas* spp. were recovered from the feces of 22 diarrheic horses. Of these isolates, more than 80% were susceptible on in vitro testing to amikacin, ceftiofur, chloramphenicol, and gentamicin. All isolates were susceptible to enrofloxacin. Diarrheic horses positive for *Aeromonas* were significantly ($P = .04$) older than diarrheic horses negative for *Aeromonas* spp. A significantly greater number of fecal samples were positive for *Aeromonas* spp. during March through August than samples examined in other months ($P = .014$). Results of this study indicate that *Aeromonas* spp. should be considered as a cause of diarrhea in horses.

Key words: Clostridia; Colitis; Enteritis; *Salmonella*.

Investigations into the etiology of diarrhea in horses are often restricted to confirmation or exclusion of salmonellosis.[1] Other bacteria recognized or suspected to cause diarrhea in horses or foals include *Clostridium* spp.,[2–4] *Campylobacter* spp.,[5,6] *Escherichia coli*,[7–9] *Streptococcus durans*,[8,10] *Bacteroides fragilis*,[11] *Actinobacillus equuli*,[5] *Mycobacterium paratuberculosis*,[12–14] *Aeromonas hydrophila*,[5,8,15] *Rhodococcus equi*,[16] and *Klebsiella pneumoniae*.[17]

Whether or not these bacteria are an important cause of diarrhea in the horse is unknown because of difficulties in isolation or because some may be part of the intestinal flora of normal horses. The prevalence of *Aeromonas* spp. in the gastrointestinal tract of horses, particularly in association with disease, is unknown. *Aeromonas* spp. may be a cause of diarrhea in foals, based on isolation of *A. hydrophila* from feces of two 3-day-old diarrheic foals.[8] Isolation of *A. hydrophila* from enteric lesions of horses lead to speculation that this organism was a potential enteric equine pathogen.[5] During a 2-year prospective study of diarrheic foals in Britain and Ireland, *A. hydrophila* was isolated from the feces of 9% (28/304) of the diarrheic foals.[15] *A. hydrophila* was associated with diarrhea and septic arthritis in a 4-day-old foal.[18]

Aeromonas spp. are gram-negative rods commonly found in water and soil. The genus *Aeromonas* is composed of at least 12 recognized species, each representing distinct DNA homology groups. In addition, there are several species of questionable validity and a number of unnamed DNA hybridization groups.[19,20] Members of this genus have long been recognized as pathogens of fish and reptiles.[21] *Aeromonas* spp. cause gastrointestinal and soft tissue infections in immunocompetent humans and disseminated infections in immunocompromised humans.[22,23] *Aeromonas* spp. are recovered from feces of humans with diarrhea more commonly than from feces of asymptomatic humans.[24,25] *Aeromonas* probably causes diarrhea in humans by adherence to mucosa and elaboration of toxins.[26,27] Known risk factors for disease in humans include swimming in or drinking untreated water and antimicrobial therapy with agents that are ineffective against aeromonads.[24]

From May 1993 through September 1995, 56% of 110 fecal samples from horses with diarrhea examined for enteric bacterial pathogens in our laboratory were positive for *Aeromonas* spp. Consequently, an investigation was undertaken to determine the prevalence of aeromonads in feces from healthy and diarrheic horses and the possible association between isolation of *Aeromonas* spp. and diarrhea in horses. Feces from horses with diarrhea were also examined for the presence of *Salmonella* spp., viruses, and parasite ova.

Materials and Methods

Horses

Feces were collected from 40 horses presented to our hospital from April 8, 1996, through April 17, 1997, for treatment of diarrhea. For most horses with diarrhea, feces were collected from an age- and sex-matched control horse examined at our hospital for reasons other than gastrointestinal disease. Feces from horses with diarrhea and from control horses were used for bacterial, parasite, and viral studies. Feces from control horses were not always available at the time of examination of diarrheic feces; in most instances, control feces were obtained within 1 month. For 6 horses, a matched control was not available. Information concerning signalment, duration of diarrhea, and drug treatment before and after onset of diarrhea was recorded for each clinical case.

Bacteriologic Studies

Approximately 5 g of feces was collected in sterile specimen cups at the time of admission from all diarrheic and control horses. Samples were processed for isolation of *Aeromonas* and *Salmonella* spp. using standard bacteriologic techniques. Specimens were cultured aerobically onto trypticase soy blood agar (Difco Laboratories, Detroit, MI) with 5% bovine blood, MacConkey agar (Becton Dickinson, Sparks, MD), Hektoen Enteric agar (Oxoid, Ogdensburg, NY), Salmonella–Shigella agar (Oxoid), and Cefusoldin–Irgansan–Novobiocin agar (Difco). All plate media, except for the blood agar, were incubated at 37°C under ambient conditions. Blood agar plates were incubated at 37°C with 10% CO_2. In addition, a 1% alkaline peptone broth was

inoculated for *Aeromonas* spp. enrichment, and tetrathionate broth (Difco) was inoculated for *Salmonella* spp. enrichment. These broths were incubated at 28°C and 37°C in air, respectively. Isolates suggestive of *Salmonella* and *Aeromonas* spp. and any atypical fecal isolate were examined using standard methods.[19,28] Definitive results of suspect *Salmonella* spp. were performed using an automated microidentification system (bioMerieux Vitek, Hazelwood, MO) and serologic methods. Oxidase-positive, gram-negative rods were screened for *Aeromonas* spp. using a 1% glucose broth containing a Durham tube for determination of gas production during glucose fermentation. Species were identified using conventional biochemical tests.[29]

Antimicrobial Susceptibilities

Disk diffusion assays on Mueller–Hinton agar (Difco) were employed to determine the antimicrobial susceptibility profile of each *Aeromonas* isolate. The methodology used to perform and interpret these tests was in accordance with the guidelines of the National Committee for Clinical Laboratory Standards (NCCLS).[30] After 16–18 hours of incubation, the plates were examined and zone margins were selected as the areas showing no visible growth. The sizes of the zones were interpreted using published standards and the isolates reported as either susceptible, intermediate, or resistant to the agents tested. Because interpretative criteria have not been established for *Aeromonas* spp., NCCLS standards for members of the family *Enterobacteriaceae* were used. The antimicrobials tested were amikacin, ampicillin, amoxicillin-clavulanic acid, ceftiofur, cefoxitin, cephalothin, chloramphenicol, enrofloxacin, erythromycin, gentamicin, trimethoprim-sulfamethoxazole, tetracycline, and ticarcillin.

Parasitology

Feces from all diarrheic horses and 8 control horses were examined for parasite ova by centrifugal fecal floatation using a sugar solution. Positive specimens were further examined using a modified Wisconsin sugar floatation method.[31,32] Numbers of ova, cysts, or oocysts were reported per 3 g of feces.

Viral Studies

Fresh fecal samples from all diarrheic horses were frozen at −80°C and later analyzed for presence of enteric viruses. Feces were examined for rotavirus using the Rotaclone Rotavirus Enzyme Immunoassay (EIA) test kit (Cambridge Biotech Corp, Worcester, MA) according to the manufacturer's instructions. Samples were examined for coronavirus by electron microscopy (Philips 301 transmission electron microscope, FEI Company, Hillsboro, OR) using negative staining with phosphotungstic acid at a magnification of 34,000×. A specimen was considered positive if 6 or more particles with peplomeric surface structures characteristic of a coronavirus were observed. Feces from 5 control horses were examined as negative controls for the Rotaclone Rotavirus EIA and electron microscopy.

Data Analysis

Data were analyzed using Statistical Analysis System (SAS release version 6.03) and EpiInflo (release version 6.04b) software. The relationship between age and isolation of *Aeromonas* spp. from horses with diarrhea was analyzed using the Wilcoxon rank-sum test.[33] The association of different variables such as antibiotic use, presence of diarrhea, and parasite isolation with isolation of *Aeromonas* spp. was assessed using the chi-square test for independence or Fisher's exact test. Probabilities lower than .05 were considered significant.

Results

The incidence of *Aeromonas* and *Salmonella* spp. from horses with diarrhea and from age- and-sex-matched horses

Table 1. Prevalence of *Aeromonas* and *Salmonella* species in horses with diarrhea and from age- and sex-matched control horses without diarrhea.

Category	Horses with Diarrhea (%)	Control Horses
Total	40	34
Aeromonas only	17 (42)	0
Salmonella only	3 (8)	0
Both *Aeromonas* and *Salmonella*	5 (12)	0
Negative	15 (38)	34

without diarrhea is shown in Table 1. Neither *Salmonella* spp. nor *Aeromonas* spp. were isolated from the feces of 34 control horses. Of 40 horses presented for diarrhea, *Aeromonas* spp. were isolated from feces of 22 (55%, $P <$ .001). *Salmonella* spp. were isolated from feces of 8 (20%) horses ($P < .01$), and of these, 5 (12.5%) also were positive for *Aeromonas* spp. Fecal samples from 18 (45%) diarrheic horses were positive for intestinal parasites, 10 of which were also *Aeromonas*-positive. Seventeen (42.5%) diarrheic horses had low numbers (<100 ova/g) of strongyle-type ova and 4 (10%) were positive for *Parascaris* spp. ova. Feces from 3 (37%) of the 8 control horses tested for intestinal parasites were positive for strongyle-type ova. A fecal sample from 1 (2.5%) of the 40 horses with diarrhea was positive for rotavirus and feces from 4 (10%) of the diarrheic horses were positive for coronaviruslike virus particles. Two diarrheic horses with feces positive for coronavirus also had a positive fecal culture for *Aeromonas* spp. Feces from 1 of 5 control horses examined was positive for coronavirus by electron microscopy.

All 22 horses that were *Aeromonas*-positive had diarrhea; however, only 35% of the *Aeromonas*-negative horses had diarrhea ($P < .0001$). These findings were similar for *Salmonella*-positive and -negative horses. All of the horses that were *Salmonella*-positive had diarrhea, but 48% without *Salmonella* spp. also had diarrhea ($P = .0063$). No association was found between the presence of strongyles and diarrhea in the horses tested ($P = 1.0$).

Twenty-nine isolates of *Aeromonas* were recovered from feces of 22 diarrheic horses. Feces of 7 horses were positive for more than 1 species of *Aeromonas.* Of the 29 isolates recovered, 16 (55%) were identified as *Aeromonas caviae,* 9 (31%) as *A. hydrophila,* 3 (10%) as *A. veronii-sobria,* and 1 (3%) *Aeromonas* isolate that could not be identified to species.

Diarrheic horses from which *Aeromonas* spp. were isolated were significantly ($P = .04$) older than diarrheic horses from which *Aeromonas* spp. were not isolated (11 year median and 3 year median, respectively). No relationship was found between sex of horse and isolation of *Aeromonas* from the feces ($P > .05$). A significant association ($P = .014$) was found between season of year and the isolation of *Aeromonas* spp. Sixteen of 20 (80%) horses with diarrhea examined from March through August were positive for *Aeromonas* spp. In comparison, only 6 of 20 (30%) horses with diarrhea examined between September and February were positive for *Aeromonas* spp.

Agar disk diffusion antimicrobial susceptibilities were performed on 29 isolates of *Aeromonas*. None of the isolates were susceptible to ampicillin or erythromycin. Less than 25% were susceptible to amoxicillin-clavulanic acid, cephalothin, trimethoprim-sulfamethoxazole, or ticarcillin. Of the 29 isolates, 56% were susceptible to cefoxitin and 76% were susceptible to tetracycline. More than 80% of the strains were susceptible to amikacin, ceftiofur, chloramphenicol, and gentamicin. All isolates were susceptible to enrofloxacin. *A. caviae* isolates were more resistant to the selection of antimicrobials tested than were isolates of *A. hydrophila* and *A. veronii-sobria*. The 1 *Aeromonas* isolate that could not be identified to species was found to have the greatest degree of resistance; this isolate was susceptible only to amikacin, enrofloxacin, and gentamicin.

Clinical signs of respiratory disease were reported for 6 *Aeromonas*-positive horses at the onset of diarrhea. Gastrointestinal sand was thought to be the initiating cause of diarrhea in 2 *Aeromonas*-positive horses. Hospitalization, Cushing's disease, injury, septic peritonitis, or probable gastric ulceration occurred before the onset of diarrhea in 9 *Aeromonas*-positive horses. A possible initiating cause of diarrhea was not determined for 6 *Aeromonas*-positive horses (27%).

Only 2 *Aeromonas*-positive horses were presented with chronic diarrhea (more than 10 days duration). These horses had diarrhea for approximately 3 weeks at the time of euthanasia or death. *Aeromonas*-positive horses were often neutropenic ($<3 \times 10^3$ cells/μL) (8/17 horses, 47%), hypoproteinemic (serum total protein < 6 g/dL) (15/19 horses, 79%), and azotemic (>2.0 mg/dL serum creatinine or >20 mg/dL serum urea nitrogen) (11/16 horses, 69%). Ten of the 22 (45%) *Aeromonas*-positive horses died or were euthanized and 5 of the 12 (42%) *Aeromonas*-negative horses with diarrhea died or were euthanized (95% CI = 0.40 < odds ratio < 2.45; $P = .83$).

Antimicrobial drugs were administered to 9 of the 22 *Aeromonas*-positive (41%) horses shortly before the onset of diarrhea for treatment of respiratory disease (4 horses), injury (2 horses), a septic joint, laminitis, and prophylactically for castration. Of these 9 *Aeromonas*-positive horses, 4 had fecal isolates of *Aeromonas* spp. that were resistant to the antimicrobial administered (trimethoprim-sulfa products in 3 horses and oxytetracycline in another). One horse treated with trimethoprim-sulfa products had fecal cultures from which both *A. caviae* and *A. hydrophila* were recovered and both isolates were resistant to the antibiotic upon laboratory testing. The *Aeromonas*-positive horse given oxytetracycline was necropsied and intestinal contents yielded many colonies of *A. caviae* and *Salmonella* sp., serogroup B. Two phenotypic strains of *A. caviae* were recovered from the feces of this horse and both strains of *A. caviae* and the *Salmonella* isolate were resistant to tetracycline. Of the 18 *Aeromonas*-negative, diarrheic horses, 3 received antibiotics before the onset of diarrhea (17%); 2 received both procaine penicillin G and gentamicin, and the 3rd was given trimethoprim-sulfa. No significant difference ($P = .19$) was found in the previous use of antibiotics in *Aeromonas*-positive and *Aeromonas*-negative horses with diarrhea.

Discussion

This study supports the theory that *Aeromonas* spp. are a cause of diarrhea in horses because *Aeromonas* spp. were isolated only from diarrheic horses and not from age- and sex-matched controls. Furthermore, the isolation of *Aeromonas* spp. from 55% of horses with diarrhea was greater than isolation of *Salmonella* (20%). The high isolation rate of *Aeromonas* spp. possibly could be attributed to the intestinal environment of horses with diarrhea that may support proliferation of this organism. The *Aeromonas* isolation rate (55%) in this study is much higher than the 9% reported from diarrheic foals in Great Britain and Ireland.[15] The difference in isolation rate between these 2 groups of diarrheic horses may be related to the differences in age of horses sampled, environment, or specimen handling.

Aeromonas spp. have been implicated increasingly as the cause of acute gastroenteritis in humans.[27,34] In some populations, *Aeromonas*-induced diarrhea is thought to be the most common cause of bacterial gastroenteritis in humans; however, no well-documented outbreaks of *Aeromonas*-associated gastroenteritis are known.[27,35,36] In epidemiologic studies of human diarrhea, the rate of recovery of aeromonads from feces is usually much higher in patients with diarrhea than in those who are asymptomatic.[34,35,37,38] In our study *Aeromonas* spp. were not isolated from the feces of 34 control horses; however, in another study, *Aeromonas* spp. were isolated from the feces of 7 of 108 (6%) asymptomatic horses.[39] This difference in *Aeromonas* isolation rates from asymptomatic horses may be related to the seasonal temperature at the time of sample collection. Aeromonads are isolated most commonly from the feces of both symptomatic and asymptomatic humans during the warmest months of the year.[37,38] Because this study was conducted over 12 months, feces of control and diarrheic horses were examined during both warm and cold months. In the earlier survey,[39] feces were collected during May through November, which coincides with the highest occurrence of *Aeromonas* spp. in drinking water.[35,36,38] A correlation exists between isolation of *A. hydrophila* from animal feces and its presence in their drinking water. The prevalence of *Aeromonas* spp. in apparently healthy animals may reflect constant exposure to *Aeromonas*-contaminated water.[40] Furthermore, prevalence of *Aeromonas* spp. in feces of symptomatic and asymptomatic humans shows geographical variation.[36–38] The previous investigation was conducted in South Wales, this study was conducted in the southeastern United States.

Based on the data from this study, *Aeromonas* spp. may be associated with diarrhea in horses. Although a connection between the strain of *Aeromonas* cultured from feces and the drinking water source could not be made from the data collected during this study, drinking water should be examined as a potential source of *Aeromonas* spp. in diarrheic horses. Furthermore, challenge studies are also warranted to determine if previous exposure to antibiotics, with and without contaminated water, is a risk factor for gastrointestinal infection with *Aeromonas* spp. in the horse.

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