Gastrointestinal infection with *Brachyspira spp* in a filly with chronic diarrhea: a case report

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Abstract

An eleven month old filly was referred to the Research and Teaching Hospital of the Faculty of Veterinary Medicine at the University of Tehran. The animal presented a three-week history of diarrhea and previous treatments had failed to effect a recovery. Based on the history, clinical findings and results of laboratory investigations, it was assumed that a gastrointestinal disorder was affecting the filly. After the exclusion of other possible etiologies, *Brachyspira spp*. became the most likely causative agent. Despite supportive therapy in hospital, the clinical condition of the filly deteriorated over the course of eleven days and the animal died of inanition and consequent multiple organ dysfunctions. Subsequent pathological investigation confirmed a *Brachyspira spp*. infection of the gastrointestinal tract. This report is believed to be the first equine case of *Brachyspira*-associated gastroenteritis.

Introduction

Chronic diarrhea is a common presentation for a number of colonic disorders in horses. Those that are commonly associated with chronic diarrhea with a duration of more than two weeks in horses include: (I) cyathostomiasis; (II) chronic idiopathic colitis; (III) salmonellosis; (IV) alimentary lymphosarcoma; (V) granulomatous colitis; (VI) eosinophilic colitis; (VII) ingestion of sand; (VIII) chronic liver disease; (IX) peritonitis; (X) lymphangiectasia; and (XI) as a consequence of acute diarrhea. However, even with concerted effort a definitive antemortem diagnosis is achieved in fewer than 30% of cases (Radostits *et al.,* 2007). The less common differential diagnoses for chronic diarrhea in an adult horse or foal include: (I) NSAID drugs toxicity; (II) squamous cell carcinoma; (III) amyloidosis; (IV) abdominal abscess; (V) clostridiosis; (VI) antibiotic-associated diarrhea; (VII) cryptosporidiosis; (VIII) rotavirus infection; (IX) *Rhodococcus equi;* (X) *Lawsonia intracellulare*; (XI) gastric ulcer; and (XII) *Strongyloides westeri* (Kahn, 2005; Hines, 2004; McConnico, 2004). Recently, *Brachyspira spp.* has been implicated as a cause of chronic diarrhea in horses in both Australia and Japan (Radostits *et al.*, 2007).

The genus *Brachyspira (*formerly *Serpulina*) currently consists of seven species of anaerobic intestinal spirochetes that colonize the large intestine of animals and birds (Hampson, 2003). Classic Brachyspirosis predominates in swine and poultry populations (Radostits *et al.,* 2007; Stephens *et al.*, 2001; Jamshidi *et al.*, 2007). However, there have also been reports of bovine, cervine (Shibahara *et al.*, 2000), canine (Fellstrom *et al.*, 2001) and human (Tasu *et al.*, 2003) enteric Brachyspirosis. The existence of equine intestinal spirochetes was initially reported in 1964 (Davies, 1964). At that time, they were considered to be a part of the normal intestinal flora (Davies, 1985). Invasive intestinal spirochetosis due to *Brachyspira spp.* was originally described in horses from Japan (Shibahara *et al*., 2002) and to date there have been only two other reports of *Brachyspira*-associated diarrhea in a horse, worldwide (Shibhara *et al.*, 2002; Lester, 2005; Hampson *et al.,* 2006). In addition, the presence of *Brachyspira pilosicoli*, the etiological agent for swine colonic spirochetosis, has been demonstrated in diarrheic horses (Hampson *et al*., 2006).

Case History

An eleven-month-old filly was referred to the Veterinary Research and Teaching Hospital of the Faculty of Veterinary Medicine at the University of Tehran with a three-week history of diarrhea. The history given by the owner was of initial dark and fetid feces, which progressed to a light and watery consistency after one week. The filly had been born in the wood factory of its owner and had remained in the same environment until presentation. The horse's feed consisted mostly of barley, alfalfa hay, wheat bran and wheat straw. After the

first occurrence of diarrhea, the feed was changed to wheat bran for one week and then to fresh alfalfa alone, based on the advice of a veterinarian.

Prior medications by the owner included the use of potentiated sulfonamides, penicillin/streptomycin and gentamicin administered intramuscularly in combination or alone, together with oral oxytetracycline. However, the antibiotics had failed to stop or control the diarrhea. Approximately five liters of fluid therapy with Ringer's solution had been given each day, along with doses of vitamin A and ketoprofen on three different occasions.

On clinical examination, the animal was in a poor condition with a significantly tucked up abdomen, which was consistent with a history of prolonged diarrhea and inappetance. The animal was conscious and alert. Vital signs revealed a body temperature of 39.1°C, pulse rate of 40 beats per minute and the visible mucous membranes appeared pink but dry. The significant findings on abdominal examination were splashing sounds in the areas of the colon and cecum, with these being most marked on ballottement. The normal ileocecal valve sounds could not be detected by auscultation. Nasogastric intubation revealed no reflux. The filly had a watery diarrhea with a green-tinted discoloration that contained undigested particles of alfalfa and some mucus particles.

A jugular venous blood sample was submitted for hematological and biochemical examination. The initial complete blood count (CBC) results are shown in the first column of Table 1. Repeated daily CBC results are listed sequentially in the subsequent columns of this table. Table 2 shows the results of the biochemical evaluation.

Fecal samples were collected for a direct smear, parasitological evaluation and bacterial culture on three different occasions but none of these revealed any specific pathogen.

The animal received a dose of Ceftiofur sodium (2.2 mg/kg) plus a single dose of injectable phosphorus (5ml). Based on the serum electrolyte profile, intravenous fluid therapy was commenced with normal saline, Ringer's lactate and 5% dextrose solutions at a level of approximately ten liters per day.

The day after the initiation of this therapy, the body temperature fell down to 37.3°C but the dehydration status of the foal had deteriorated clinically. The same day, the antibiotic was withdrawn from the treatment due to the compromising effect it could have had on the intestinal microflora. The goal of therapy at this point became focused on rehydration and the restoration of the intestinal microbial environment. The latter was hoped to be achieved by adding approximately five liters of diluted yoghurt as a probiotic compound to the feed, but due to a lack of resources, this treatment was only given once. Daily oral fluid therapy and venous rehydration continued to be given throughout.

Previous intramuscular injections by the owner had resulted in the formation of an abscess in the cervical region, which was detected as a warm swollen area on the neck. An incision and drainage was performed and the area was debrided and dressed. This was followed by the daily application of an antibiotic (Nitrofurazone), plus topical phenytoin ointment after sterilization with diluted iodine solution. A single injection of 6 milliliters of the therapeutic agent Theranekron (*Tarantula cubensis*/ spider venom) was administered intramuscularly, which is claimed to accelerate the wound healing process (Sardari *et al*., 2007).

In spite of the aggressive fluid therapy, the filly continued to show signs of advancing dehydration as a result of the severe fluid loss through the feces. This clinical deterioration was confirmed biochemically and accompanied by hypersegmentation (five to six segments) in the nuclei of many neutrophils.

The urinalysis results of a urine sample, collected by catheter on the eighth day of hospitalization, is stated in Table 3 and the serum biochemical profile for day nine is shown in Table 4.

On the ninth day of hospitalization, clinical examinations revealed that the heart rate was 93 beats per minute, the body temperature was 37.8°C, and that the mucus membranes were dry and severely congested. Following the administration of additional fluids, the heart rate came down to 72 beats per minute. Throughout all of the interventions applied during admission, the character of diarrhea remained unchanged. After eleven days of hospitalization, the animal died because of uncompensated dehydration, inanition and multiple organ dysfunctions. The results of the CBC from the final day are presented in the ninth column of Table 1.

Necropsy revealed a lean, dehydrated carcass. Examination of the intestines showed small dark patches over the glandular region of the stomach, which were slightly raised from the mucosal surface with an erosive appearance (Figure 1). The colonic and cecal mucosae were edematous, hyperemic and corrugated (Figure 2). Furthermore, there was an area of hemorrhage in the left apical lobe of the lung encased in fibrotic connective tissue, approximately with the diameter of ten centimeters.

Tissue samples were submitted for histological examination. The samples were first subjected to Hematoxylin and Eosin (HE) staining and then to a spirochete-specific stain (Warthin-Starry) with 1% silver nitrate (Shibahara *et al*., 2005). The results were as follows:

- Spleen: severe hemosiderosis, acute inflammation.
- Liver: acute hepatitis.
- Kidney: hyperemia, tubular necrosis.
- Brain: hyperemia, edema
- Intestines: severe hyperemia, moderate

Table 1: Results of serial hematological examination from presentation until death.

CBC \ Sample	1st 7/30/	2nd 8/1/	3rd 8/2/	4th 8/5/	5th 8/6/	6th 8/7/	7th 8/8/	8th 8/9/	9th 8/11/
PCV (%)	35(32-53)	42.8	42.3	48.5	52	58	53.5	56	55
Hemoglobin (g/dl)	12.5(11-19)	15.6	15.4	19	19.7	22.5	19.5	-	20.9
RBC (×10/µl)	14.5(6.7-12.9)	8.64	8.93	10	10	12	11	12	11
MCV (fl)	50(37-58.5)	51	49	49	48	49	49	-	48
MCH (pg)	16.9(12.3-19.7)	18	17.3	18	18.2	18	18.1	-	18.3
MCHC (g/dl)	34.3(31-38.6)	35.5	35.6	37.1	37.9	36.9	36.1	-	38.3
Total solids (g/dl)	7.1(5.8-7.7)	7.7	7.6	8.2	8.1	9	8.9	8.5	7.9
Fibrinogen (g/dl)	0.5(0.1-0.4)	0.4	0.4	0.5	0.6	0.4	0.4	0.6	0.3
WBC (/µl)	15000 (5400-14300)	14000	10500	10000	16100	14700	12100	12400	23300
Neutrophil %	80	83	72	88	83	80	81	90	85
Band cell %	-	-	4	3	2	1	1	1	2
Metamyelocyte %	-	-	2	1	-	1	1	-	-
Lymphocyte %	19	16	21	7	15	15	16	8	12
Monocyte %	1	1	1	1	-	3	1	1	1

Table 2: Serum biochemistry on first day of presentation.

Parameter	Result
Sodium (mEq/L)	130 (132-146)
Chlorine (mEq/L)	85 (99-109)
Potassium (mEq/L)	2.3 (2.4-4.7)
Glucose (mg/dl)	144 (89-112)
Albumin (g/dl)	4.1 (2.6-3.7)

Table 3: Urinalysis results from the eighth day of admission.

Parameter	Results
Color/ turbidity	Yellow/ semi turbid
Specific gravity	1.037
pH	6-7
Protein (mg/dl)	300-700
Glucose	+
RBC (n/ high power field)	15-20
Calcium (mg/dl)	25.48
Phosphorous (mg/dl)	5.41
Magnesium (mg/dl)	14.56
Sodium (mEq/l)	2
Potassium (mEq/l)	3
Creatinine (mg/dl)	386.5

Table 4: Serum biochemistry results from the ninth day of admission.

Parameter	Results
Calcium (mg/dl)	12.13 (11.2-13.6)
Phosphorous (mg/dl)	3.12 (3.1-5.6)
Magnesium (mg/dl)	2.64 (2.2-2.8)
Sodium (mEq/l)	127 (132-146)
Potassium (mEq/l)	1.3 (2.4-4.7)
Creatinine (mg/dl)	1.58 (0.9-2)
BUN (mg/dl)	47 (12-27)

multifocal to diffuse erosive enteritis/colitis, necrotic debris on the luminal surface, numerous *Brachyspira* organisms in the crypts.

• Stomach: severe hyperemia, mucus secreting cell hyperplasia, multifocal to diffuse erosive gastritis, increased number of lymphocytes and plasma cells in the lamina propria, numerous *Brachyspira* organisms in the necrotic debris.

Brachyspira was confirmed by Warthin-Starry stain in both the gastric and intestinal samples. (Figures 3, 4 and 5).

Discussion

In 1964, the presence of spirochete organisms was confirmed in equine large intestinal contents (Davies, 1964). However, it was not until 2002 that the immunohistochemical and macroscopic examination of the cecal and colonic mucosae of a 24-month-old thoroughbred colt suffering from a mild diarrhea for seven months revealed the ability of the spirochete, *Brachyspira,* to act as an intestinal pathogen in horses. In the follow-up study, no other significant pathogen was detected in the large intestine of the horse (Shibahara *et al*., 2002). In the present case, both the gross and histopathological findings in the gastrointestinal tract at necropsy established that *Brachyspira* not only attacked the colonic mucosa but also invaded the small intestinal and gastric mucosae as well (figures 3, 4 and 5). In the cecal and colonic mucosae of the thoroughbred colt, invasion and cellular infiltration by *Brachyspira* was more extensive and the resultant lesions were more prominent than those of porcine, cattle and deer Brachyspirosis. It has been concluded that this might be partly attributable to differences between species in both the host and the organism itself (Shibahara *et al*., 2002). Shibahara also demonstrated that three different types of *Brachyspira* attacked the colonic mucosae in the colt and that one type was assumed to be *B. pilosicoli* or a closely related species. Following the previous report, isolation of *B. pilosicoli* from weanling horses with chronic diarrhea has been reported (Hampson *et al.*, 2006). In equids, the epidemiological and pathophysiological aspects of this infection are still unclear (Shibahara *et al*., 2005; Hampson *et al*., 2006), but in swine there are two distinct syndromes associated with *Brachyspira* infection: one is swine dysentery, which is caused by *B. hyodysentriae* and characterized by large bowel diarrhea with mucus and/or blood in the feces. The other is the porcine colonic spirochetosis association with *B. pilosicoli* infection in feeder swine. In the case

Figure 1: Gastric mucosa. Erosive lesions in the mucosa.

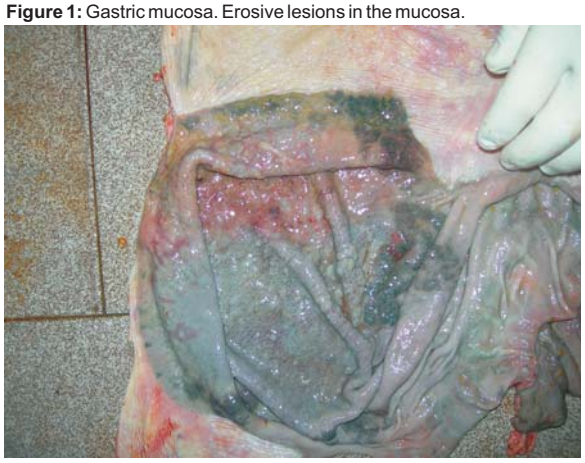
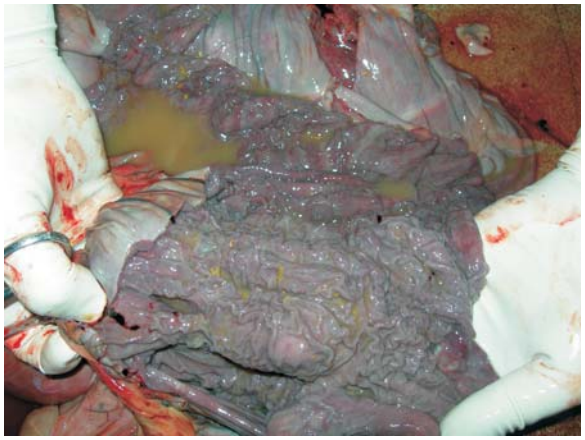


Figure 2: Cecal mucosa, corrugation, edema and hyperemia.



presented herein, the diarrhea was not bloody but there were mild intestinal erosions and enteritis with the infiltration of lymphocytes and macrophages and in later stages, there was goblet cell hyperplasia. It is thought that its pathogenesis is associated with malabsorption due to disruption of the mucosal surface and increased exfoliation of poorly differentiated cells at intercrypt extrusion zones that probably results in the enteric loss of plasma protein (Brown *et al.*, 2007; Radostits *et al.*, 2007).

The gross and microscopic pathologic findings of the case presented here shows similarities with those reported in porcine colonic spirochetosis and also those of previously reported equine cases, although no reports are currently available on the gastric involvement in *Brachyspira* infections either in swine or equine populations (Brown *et al*., 2007; Radostits *et al*., 2007; Shibahara, 2002). Although *Brachyspira* is known to colonize the large intestine (Hampson, 2003), previous reports have shown that the small intestine has been only mildly affected (Shibahara, 2002). In bovine spirochete-associated dysentery, the lesions are in the cecum and proximal colon (Shibahara, 2000).

Figure 3: Large clusters of intra-cryptal *Brachyspira* in the crypts of the small intestine, Warthin-Starry stain (x640).

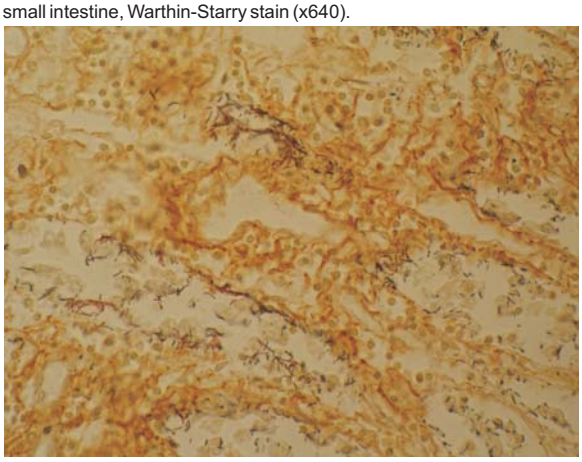


Figure 4: Large numbers of Brachyspira in the crypts of the colon following Warthin-Starry stain (x640).

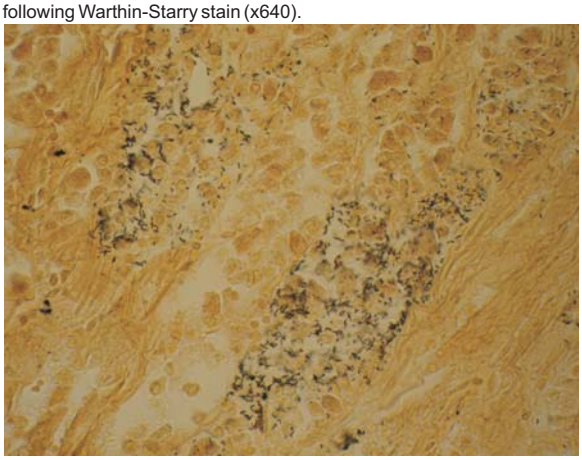
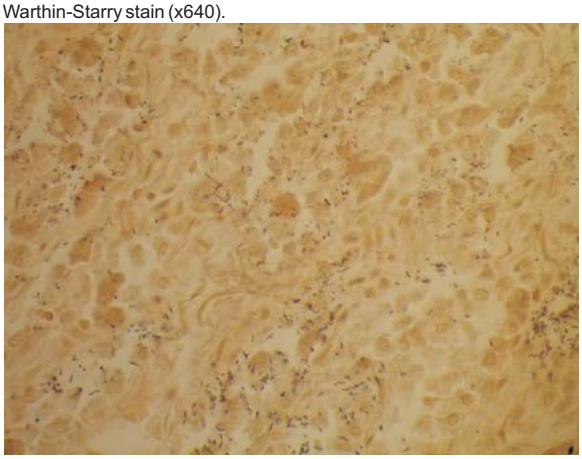


Figure 5: Extensive spirochete organisms in the gastric mucosa, Warthin-Starry stain (x640).



A case of chronic diarrhea with extensive fluid loss in a filly is presented in which the findings from necropsy and histopathology confirmed a *Brachyspira spp.* infection of

the alimentary tract with characteristic lesions. Alternative differential diagnoses for chronic diarrhea were excluded during admission by various methods including a full history of the presenting complaint (to exclude several important environmental causes), multiple fecal smears, aerobic and anaerobic cultures and floatation for parasites. It is important to note that spirochete isolation needs specific culture media and precise procedures, which were not available to the authors and the routine culture techniques, are insufficient for isolating these organisms adequately. It is therefore concluded that a form of gastrointestinal Brachyspirosis occurred in this filly and that the chronic diarrhea was probably related to this infection. Based on this case, the authors suggest that whenever a chronic diarrhea case is encountered, it would be wise to consider *Brachyspira* infection in the differential diagnosis list. For this, feces could be cultured for spirochetes or at list being investigated under dark field microscope. However, it is not yet possible to consider them as the causing agent. It is also recommended when Brachyspira infection is either suspected or assumed, it may be wise to examine the complete gastrointestinal tract by macroscopic and microscopic methods. This report is considered to be the first case of equine *Brachyspira spp.* gastroenteritis.

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