

Retrospective Study on the Cause of Bacterial Diarrhea in Horses in Minnesota

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Abstract: A retrospective study was conducted to determine the causes of bacterial diarrhea in horses Minnesota. The database of Veterinary Diagnostic Laboratory, University of Minnesota was searched over a 10 year period (2001-2010). A total of 1,260 fecal samples from diarrheic horses were received and tested. The most common pathogens found were *E. coli* (46%), *Salmonella* (27%), *Clostridium perferings* (19%) and *Lawsonia intracellularis* (8%).

Key words: *Salmonella*, *E. coli*, clostridia, lawsonia, diarrhea, horses

INTRODUCTION

Minnesota has the 9th largest horse population in the U.S, with over 150,000 horses. The \$39 billion contributed to U.S economy annually by horses, Minnesota is contributes almost \$1 billion.

Diarrhea in horses associated with high mortality (Baverud *et al.*, 2003). According to one estimate about 80% of foals have at least one episode of diarrhea during the first 6 months of their lives. Urquhart (1981) and in severe cases, death may occur even before the onset of diarrhea. There are a variety of known causes of diarrhea in horses. Diarrhea may be the result of noninfectious causes include carbohydrate/grain overload, right dorsal colitis, non-steroidal anti-inflammatory toxicity, sand irritation, feed changes in mature horses (Brewer and Koterba, 1988) and gastro duodenal ulceration in foals (Magdesian, 2005).

Many infectious agents have been implicated as causes of diarrhea in horses such as *Clostridium* sp., *Salmonella* sp., *Escherichia. coli* and rotavirus (Lester and Madigan, 2009).

Other infectious agents less commonly associated with acute diarrhea include *Cryptosporidium*, *Strongyloides*, *Coronavirus* (Guy *et al.*, 2000), *Aeromonas* (Browning *et al.*, 1991). *Rhodococcus* (Zink *et al.*, 1986), *Streptococcus* (Tzipori *et al.*, 1984) and *Bacteroides* species (Myers *et al.*, 1987). The identification of infectious organisms in horses with diarrhea have shown that majority of samples are positive

for one or more than one organism (Frederick *et al.*, 2009). Many of the clinical signs associated with acute diarrhea are indistinguishable. Affected horses typically show signs of depression reduced appetite, mild to moderate colic often before the onset of diarrhea. Diarrhea is often sudden in onset and commonly profuse, voluminous and possibly bloody with fever and brick red mucus membrane. Clinical progression leads to severe dehydration and profound electrolyte disturbances. There is systemic inflammation from absorption of endotoxin and other bacterial products across damaged mucosal lining of the large intestine leading to laminitis. Gastrointestinal protein loss leads to oedema along the ven trum and legs. Weight loss may be rapid and severe. (Brewer and Koterba, 1988).

Clostridial Colitis/colotyphlitis is associated with *Clostridium perfringens* or *Clostridium difficile* (Baverud *et al.*, 2003) in mature horses. *Clostridium difficile* is a Gram-positive, anaerobic, sporeforming bacillus. It is associated with a wide spectrum of diseases in humans as well as in several animal species including horses, dogs, ostriches, rabbits, cats and pigs (Baverud *et al.*, 1998). In particular, the toxigenic bacterium is an important cause of enterocolitis in horses often following antimicrobial treatment as well as in foals during outbreaks or in sporadic cases (Brewer and Koterba, 1988). The isolation rate of *Clostridium difficile* from healthy adults varied from 4.3-12.7 and 90% in adults while in foals it was 16.7-63% (Jones *et al.*, 1988) (Cl.diff in horses). The prevalence rate of 42% for

Clostridium difficile was recorded in Sweden in mature horses with isolation from healthy foals with prevalence rate of 29% (Baverud *et al.*, 2003). Since *Clostridium difficile* can be present in the normal intestinal flora of healthy adult horses, in addition to foals these animals could herefore play an important role potential reservoirs of toxigenic strains (Dwyer *et al.*, 1990).

Salmonellosis, a (Brewer and Koterba, 1988) serious infectious disease of equids is associated with a greater death rate than other bacteria. It is caused by different serovars of *Salmonella enterica* subspecies enteric. The disease often precipitates as fatal septicemia and severe diarrhea in foals and colitis/typhilitis in equids of all ages (Smith *et al.*, 1978; Powell *et al.*, 1988; Traub-Dargatz *et al.*, 1990). The overall prevalence of fecal *Salmonella* was 13% with different salmonella serotypes. It is also suggested that a history of exposure to antimicrobial drugs and abdominal surgery were associated with *Salmonella* shedding in adult horses with gastrointestinal tract disease. Foals with gastrointestinal tract disease are more likely to shed *Salmonella* organisms than adult horses with gastrointestinal tract disease (Ward *et al.*, 2005). Horses recovering from acute Salmonellosis may act as a source of environmental contamination with *Salmonella* sp. for a variable period of time (Palmer and Benson, 1985). Case fatality rate of 38% was reported in salmonellosis (Hartmann *et al.*, 1996).

Proliferative enteropathy is caused by the obligate intracellular organism *Lawsonia intracellularis* in domestic and wild animal species (Lawson and Gehart, 2000). *Lawsonia intracellularis* is an obligate intracellular bacterium that causes proliferative enteropathy in a variety of domestic and wild animals (Lawson and Gehart, 2000).

Horses are the second most commonly affected domestic animal after pigs (Lavoie and Drolet, 2007). The disease has been reported from North America, Europe and Australia (Lavoie and Drolet, 2007). Horses that shed the organism appear healthy, indicating subclinical infection of *L. intracellularis* is detection of fecal shedding of *L. intracellularis* indicates the presence of the agent in the equine population (Lawson and Gehart, 2000). The transmission of infection in foals may occur through the ingestion of feed or water contaminated with *L. intracellularis* infected feces from feral or domestic animals. High prevalence of *L. intracellularis* fecal samples was observed in opossums (20%), coyote (50%) (Pusterla *et al.*, 2008), red foxes (7%) and wolves (9%) in Solavak (Tomanova *et al.*, 2003).

Escherichia coli are the most common isolate from foals suffering from Neonatal sepsis. Reports of sepsis in foals indicate the reemergence of gram-positive bacteria

such as *Enterobacter* sp. and *Enterococcus* sp. as the major causes of systemic sepsis coupled with resistance to multiple antimicrobials (Tanowitz and Chan, 2000) in Pennsylvania (Marsh and Palmer, 2001). This retrospective study was conducted to determine the prevalence of bacterial pathogens in for horses diarrhea diagnosis over a 10 year period.

MATERIALS AND METHODS

Animals: Fecal samples from horses submitted to the Veterinary Diagnostic Laboratory at University of Minnesota over a 10 year period (200-2010) were included in this study. During this period, a total of 709 samples were received from diarrheic horses.

Methodology: All fecal samples were processed with bacteriological culture for *Salmonella*, *Clostridia*, *E. coli* and *Lawsonia*. Different media were used for culture of different bacteria. Sheep blood agar was used for culture of *E. coli* at 37°C for 24 h, Macconkey's agar for *Salmonella* at 37°C for 24 h and anaerobic blood agar plates for *Clostridia* at 37°C for 24 h. Brilliant green agar and xylose Lysine desoxycholate agar media were used as selective media for *Salmonella* at 37°C for 24 h and latter poly agglutination test was performed for confirmation. For *E. coli* enrichment broth was used as selective media at 42°C for 24 h. *Salmonella* isolation by the following fecal samples were extended in 100 mL buffered peptone water and incubated at 37°C for 18 h. After pre-enrichment, fecal samples were selectively enriched in Tetrathionate Broth (TTB) and in Rappaport Vassiliadis medium at 42°C for 24 h. Thereafter, a loop of inoculum from the enrichment broths was streaked onto Brilliant Green Agar (BGA) and Hektoen Enteric Agar (HEA) plates and incubated at 37°C for 24 h (Cohen *et al.*, 1994). Three to five presumptive *Salmonella* colonies (transparent colonies with reddish periphery on BGA and smooth, bluish-transparent colonies with or without black center on HEA) were picked up and characterized through conducting tests 17 for motility and sugar fermentation test. Isolates were serotyped using standard tube agglutination test with factor-specific and group (Edwards and Ewing, 1972). Fecal samples for isolation *Escherichia coli* inoculated into Columbia 5% sheep blood agar and MacConkey agar plates and were incubated at 37°C overnight. Colonies with phenotypic characteristics of *E. coli* and that were lactose fermenting on MacConkey agar were selected for identification with the API 20E system (Cruickshank *et al.*, 1975). The fecal samples were cultured on blood agar plates (containing 5% sheep blood) and on *C. difficile* selective agar and

were incubated at 37°C under anaerobic conditions for isolation of *Clostridium perferingens* (Herholz *et al.*, 1999). Due to the inability to culture *L. intracellularis* from fecal material, documentation of infection or exposure to *L. intracellularis* in a susceptible animal relies on the detection of *L. intracellularis*-specific antibodies in peripheral blood or on the detection of *L. intracellularis* DNA in feces (Lawson and Gehart, 2000) in the present study detection of Lawsonia in fecal samples usually detected by PCR (Jones *et al.*, 2003).

RESULTS AND DISCUSSION

The result of bacteriological culture for *E. coli*, *Salmonella* sp., *Clostridium* sp. and *Lawsonia intracellularis* showed prevalence of 46, 27, 19 and 8%, respectively. The prevalence of *E. coli* was highest in 2006 (59%) while it was lowest in 2002 (11%). The prevalence of *Salmonella* sp. was highest in 2001 and 2003 (54%) while it was lowest in 2008 (2%). The prevalence of *Clostridia* sp. was highest in 2002 (39%) while it was lowest in 2001 and 2006 (15%). The prevalence of *Lawsonia intracellularis* was highest in 2004 (16%) while no sample was positive in year 2001 and 2002. The data up to March 2010 showed the highest prevalence of *Clostridia* sp. (43%) followed by *Lawsonia Intracellularis* (29%), *Salmonella* and *E. coli* (14%) (Table 1, Fig. 1).

This study determined the rate of prevalence of bacterial agents associated with diarrhea in horse in Minnesota. The bacteriologic isolates found in horses in the present study include *E. coli*, *Salmonella*, *Cl. peferengenes* and *Lawsonia intracellularis* and these organism were previously reported by Browning *et al.* (1991), Ewart *et al.* (2001), Jones *et al.* (1988) and Lavoie and Drolet (2007) in horses. During the present study the rate of prevalence of *E. coli* was highest than other pathogens (46%) which agree with other several studies, *E. coli* (Marsh and Palmer, 2001; Corley *et al.*, 2007; Henson and Barton, 2001; Wilson and Madigan, 1989).

This prevalence rate of *Escherichia coli* was higher than reported by Clark *et al.* (2008) in Canada (82/1026) 7.9%. The prevalence of *Salmonella* in the present study was 28% was higher rate than reported by Ward *et al.* (2004), *Salmonella* was isolated from 36 of 924 (3.9%) fecal samples and by Frederick *et al.* (2009), *Salmonella* sp. was 12%. Isolation of *Salmonella* from 6.8% fecal samples of equids confirmed endemic status of salmonella in india (Singh *et al.*, 2007).

In studies of horses admitted to VTHs in the United States, prevalence of *Salmonella* infection has been estimated to be 6% (California) Mainar-Jaime *et al.* (1998), 13% (Pennsylvania) Palmer and Benson (1985), 10-11% (Texas), Cohen *et al.* (1996, 1995), 7-9% (Colorado), Traub-Dargatz *et al.* (1990); Kim *et al.* (2001) and 5% (Michigan) Ewart *et al.* (2001). The present study showed that the prevalence rate of *Clostridium perferings* was 19% from fecal sample from horse with diarrhea in Minnesota in other hand in India *C. perferingens* was isolated from 5 of 21 (24%) fecal samples from 21 horses with intestinal diseases Herholz *et al.* (1999). A particularly high incidence of b2-toxigenic *C. perferingens* was found in specimens of intestinal ingesta and biopsy specimens of the intestinal wall from horses with typical or atypical typhlocolitis (75%) and a lower incidence was found in horses with other intestinal disorders (62%) in Switzerland (Jones *et al.*, 1988). About 2 of 17 (11%) foals were positive *C. perferingens* fecal culture in Georgia (Frederick *et al.*, 2009).

The bacterial isolation in diarrhea depends mainly on the age of animal and the surrounding environment as well as weather, management which act as predisposing factor on animal for inducing diarrhea. But in the study interested mainly on the bacterial isolation in a horse fecal sample admitted to veterinary diagnostic lab not concerned with animal age and other factors. So the presence of *E. coli* in large percent does not indicate occurrence of diarrhea because it most common in the feces of foal (Corley *et al.*, 2007) also adult horse shedding salmonella in the feces for long time and become

Table 1: Bacteriological culture results for different isolates in horse in Minnesota (2001-March, 2010)

Years	No. of samples examined	No of positive isolates			
		<i>E. coli</i>	<i>Salmonella</i>	<i>Clostridium perferingens</i>	<i>Lawsonia intracellularis</i>
2001	13	4 (31)	7 (54)	2 (15)	0 (0)
2002	28	3 (11)	14 (50)	11 (39)	0 (0)
2003	161	58 (36)	87 (54)	14 (9)	2 (1)
2004	80	40 (50)	14 (18)	13 (16)	13 (16)
2005	100	50 (50)	13 (13)	27 (27)	10 (10)
2006	80	47 (59)	15 (19)	12 (15)	6 (7)
2007	114	66 (57)	18 (16)	18 (16)	12 (11)
2008	48	25 (53)	2 (4)	17 (35)	4 (8)
2009	78	34 (44)	21 (27)	15 (19)	8 (10)
2010	7	1 (14)	1 (14)	3 (43)	2 (29)
Total	709	328 (46)	192 (27)	132 (19)	57 (8)

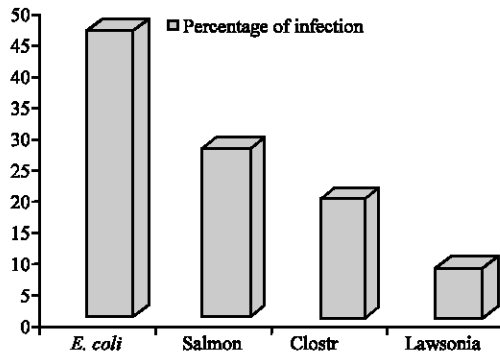


Fig. 1: Percentage of the bacterial diseases causing diarrhea in horse (2001-2010) and indicate the highest percentage with *E. coli* followed by salmonella, *Clostridium perferingens* and *Lawsonia intracellularis*

sthece of infection (Guy *et al.*, 2000). Also the bacterial isolation of clostridium does not give any indication about its infection because its spore found in horse feces and no identification to *Clostridium* sp. especially *Clostridium perferingens* and *Clostridium difficile* so this isolation should followed by further test to identify the toxin and species of clostridium because several study showed the virulence and pathogenic effect of *Clostridium* sp. in horse. The seroprevalance of *Lawsonia intracellularis* in horse feces indicate presence of infection and need more attention because most study in *Lawsonia* mainly during infection not with a servay with other bacteria. *Lawsonia intracellularis* most commonly infect foal and its shedding in feces very little unless accompanied with diseases (Pusterla *et al.*, 2008). The review gives a clear picture of bacterial diarrhea in horses but the detection of 1 or multiple infectious agents in the feces of diarrheic horses does not necessarily indicate that these agents are the cause of disease. Further studying antimicrobial resistance associated with diarrhea in horse and identification of the pathogenic bacteria from non pathogenic, detection the period of shedding these micro organism in feces specially salmonella which cause sever economic losses in horse farm one also rapid detection for bacterial diseases in feces using PCR.

CONCLUSION

The present study showed high prevelance of bacterial agent in horse feces that indicate increase conncering in the management of horse environment, isolation these animals because it become source of infection to other surroundings due to shedding of these micro organism in feces provided and also need specific method of diagnosis for each bacteria, sensitivity test then treatment.

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