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 perfringens (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 feaces 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/colytyphilitis 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 toxinogenic 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

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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 enterica. 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 broth 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 tubeagglutination 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 perfringens* (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 *Clostridium* sp. was highest in 2002 (59%) 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 *Clostridium* sp. (47%) 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*, *Clostridium perfringens* 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 VHIs in the United States, prevalence of Salmonella infection has been estimated to be 6% (California) Maimar-Jaime et al. (1998), 13% (Pennsylvania) Palmer and Banson (1985), 10-11% (Texas), Cohen et al. (1996, 1995), 7-9% (Colorado), Trab-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 perfringens was 19% from fecal sample from horse with diarrhea in minnesota in other hand in India *C. perfringens* was isolated from 5 of 21 (24%) fecal samples from 21 horses with intestinal diseases Herholz et al. (1999). A particularly high incidence of h2-toxigenic *C. perfringens* 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. perfringens* 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)

<table>
<thead>
<tr>
<th>Years</th>
<th>No. of samples examined</th>
<th>No. of positive isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td><em>E. coli</em></td>
</tr>
<tr>
<td>2001</td>
<td>13</td>
<td>4 (31)</td>
</tr>
<tr>
<td>2002</td>
<td>28</td>
<td>3 (11)</td>
</tr>
<tr>
<td>2003</td>
<td>161</td>
<td>58 (36)</td>
</tr>
<tr>
<td>2004</td>
<td>80</td>
<td>40 (50)</td>
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<tr>
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<td>100</td>
<td>50 (50)</td>
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<tr>
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<td>80</td>
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<tr>
<td>2007</td>
<td>114</td>
<td>66 (57)</td>
</tr>
<tr>
<td>2008</td>
<td>48</td>
<td>25 (53)</td>
</tr>
<tr>
<td>2009</td>
<td>78</td>
<td>34 (44)</td>
</tr>
<tr>
<td>2010</td>
<td>7</td>
<td>1 (14)</td>
</tr>
<tr>
<td>Total</td>
<td>709</td>
<td>328 (46)</td>
</tr>
</tbody>
</table>
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REFERENCES


