An Update on Equine Salmonellosis

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Abstract
Salmonella, a member of family Enterobacteriaceae, comprises a group of more than 2501 serovars. Salmonella genus comprises of 2 species namely S. enterica and S. bongori. Salmonellosis in horses is mainly exhibited as colitis and diarrhoea. Salmonella typhimurium, S. enteritidis, S. virchow etc. have been isolated from the horses suffering from gastrointestinal disorder. Besides these, horses are affected with an abortion causing host specific Salmonella serovar i.e. S. abortusequi which is primarily infecting members of equidae, however, occasionally it has been isolated from human and animal infections. Salmonellosis affects horses of all ages and is more drastic in foals as it can result in hemorrhagic diarrhea and septicemia however in adults main clinical signs include fever, depression and moderate to severe, watery diarrhea along with neutropenia. Infection is usually through horizontal transmission i.e. grazing on infected pastures or drinking contaminated water. Since treatment in salmonellosis is mainly symptomatic which includes maintaining the body fluid and relieving pain and antibiotics too have very limited role in clinical salmonellosis. Therefore, proper control of the infection depends mainly on control of the disease through improvement in general hygiene.

Keywords: Salmonella; Equine; Diarrhoea; Foals; Control; Antibiotics

Introduction
Salmonella, a member of family Enterobacteriaceae, comprises a group of more than 2501 serovars [1]. It is a gram-negative facultative anaerobic intracellular pathogen. As per the systematic classification, Salmonella genus comprises of 2 species namely S. enterica and S. bongori [2]. Salmonella enterica is further classified into 6 subspecies viz., S. enterica ssp enterica; ssp indica; ssp salamae; ssp arizonae; ssp diarizonae and ssp houtenae. Most of the serovars infecting warm-blooded animals belong to S. enterica ssp enterica including S. typhimurium, S. agona, S. abortusequi etc. S. typhimurium and S. agona are the most common serovars causing diarrhea in horses whereas S. abortusequi, is the most probable single pathogen responsible for infectious abortions in mares all over the world [3-5]. In horses main route of transmission is via faeco oral route. Salmonella infections affect horses of all ages and can range in severity from asymptomatic colonization to severe systemic illness. In adults mainly the gastro intestinal tract is affected but in foals (up to 4 months of age) is affected systemically causing septicemia. One abortifacient Salmonella viz., S. abortusequi causes abortion in mares and assumes great significance for the horse industry.

Source of infection

The potential source of infection in salmonellosis in horses include consumption of contaminated food or water, direct contact with shedders, contaminated environmental surfaces, equipment or handlers, ingestion of contaminated bird/vermin’s feces or dead insects or sometimes via aerosol exposure too. Since in salmonellosis animal become carrier and continues to shed the organism in its feces thereby contaminating its surroundings and thus act as potential source of transmission of the organism to other animals [6].

Salmonella organisms can persist in the environment for months to years depending on the serotype, moisture content and temperature conditions. Most of the Salmonella serovars after getting transmitted colonize and produce the disease but there are also a large number of Salmonella serovars which do colonize but do not normally produce clinical disease and are shed in the faeces [7].

Predisposing factor responsible for establishment of Salmonella

Predisposing factors that are responsible for the establishment of the Salmonella organism includes change in feed (either feed restriction or change in diet), seasonal variation, transportation, surgical intervention, exposure to any concurrent disease (colic or gastrointestinal disturbances), age of the animal, antibiotic administration etc.

It has been observed that changes in intestinal motility and volatile fatty acid production by normal flora may increase the ability of Salmonella to attach and proliferate. Horses those are put on antibiotics [8] or are on feed restriction or sudden feed change [9] are at a greater risk of getting infected. Exposure of transportation on an animal too has profound stress on the animal making it prone to Salmonella [10]. Major surgery, particularly abdominal surgery [11] and gastrointestinal disease [12] too make an animal susceptible for salmonellosis. In case of young animals, they are at a higher risk of getting infected mainly because of their naïve immune system and due to partially established microflora within the gastrointestinal tract and due to their habit of coprophagia [12].

Factors influencing infection

There are a variety of factors that will influence the development of clinical salmonellosis in horses. Some of these factors are related to the microorganism itself, such as the infective dose of bacteria and the virulence of the individual strain. Other factors that influence infectivity are related to the host and its individual susceptibility.

Pathogenesis and pathobiology of salmonellosis in horses

Intestinal colonization of Salmonella is central to entry into the animal system. Salmonella enter the body via oral route mainly because transmission is via faeco oral route. After the entry the bacteria overcomes the host defense mechanisms (gastric acidity, intestinal flora, peristalsis, intestinal mucus and lactoferrin), it passes through the stomach and enters the intestinal tract and interacts with the intestinal mucosa. Since the mucosal lining is lined by epithelial cells that are acting as barrier for the microbes so it poses difficulty for the microbes to pass through these epithelial cells. However, in the intestine, more that 50% specialised follicle associated epithelium (FAE) are antigen supporting M (Microfold) cells in addition to enterocytes that helps in the entry of Salmonella. After attachment of Salmonella to the enterocytes, there is a characteristic remodelling of the membrane leading to lamellipodial extension and the bacteria is then enveloped [13]. Also simultaneously the phagocytized and free Salmonella organisms travel via the lymphatics to regional lymph nodes where they persist in stimulating an inflammatory response [14].

Salmonella organisms have the ability to survive inside the macrophages as they inhibit the fusion of phagosome and lysosome [15] thus preventing bacteria being exposed to nicotinamide adenine dinucleotide phosphate oxidase (NADPH) and inducible nitric oxide synthase (iNOS) containing vesicles [16]. The ability to survive within macrophages is important for the infection and establishment of the organism in the animal’s body.
Although phagosomes acidify their contents to a pH of less than 5, *Salmonella* are able to slow the rate of acidification [17]. In the body the major macrophage killing mechanisms include NADPH oxidase, nitric oxide, and cationic antimicrobial peptides (defensins). Salmonella affects more to the animals suffering from chronic granulomatous disease as these animals do not exhibit respiratory burst involving NADPH oxidase mechanism thus providing ideal opportunity for survival of *Salmonella* in their body. The virulence in *Salmonella* has been attributed to the presence of pathogenicity islands that are clusters of genes on the chromosome with a related function that show different degrees of stability, and are suggested to have been acquired horizontally. They are frequently inserted at genes encoding tRNAs (transfer Ribonucleic acid), which themselves are often integration sites for bacteriophages which helps in microbial evolution. In *Salmonella* more than 10 *Salmonella* Pathogenicity Islands (SPI) have been found in *S. enterica* and all have genes which are associated with virulence functions [18-20].

**Mechanisms of diarrhea**

Salmonellosis in most animals is clinically exhibited via gastric disturbances accompanied by diarrhoea. Animal affected with *Salmonella* exhibit diarrhoea which is primarily in response of various exo/endotoxins, cytotoxins and enterotoxins produced by *Salmonella* organisms and secondly because of malabsorption of the nutrients due to inflammation in the intestinal tract [21]. Cytotoxins in the intestinal tract chelate essential cations and thereby reduce their absorptive capability leading to fluid loss from the body. Endotoxins exhibit profound inflammation and tissue damage via cytokines, enzymes and oxygen radical that are released by infiltrated neutrophils and macrophages. These inflammatory mediators lead to intestinal malabsorption resulting into diarrhea. Bacterial enterotoxins in general initiate diarrhea by binding to receptors that stimulate the cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP), which then activate enterocyte fluid hypersecretion [22] e.g. cholera toxin activates adenylate cyclase leading to an increased intracellular cAMP and activation of chloride pump that actively transport chloride ions inside the intestinal lumen and loss of sodium, potassium and water, creating alterations in osmotic and electrical gradients leading to accumulation of fluid in the intestinal lumen [23]. The virulence components of *Salmonella* i.e. adhesion molecules, toxins (cytotoxins, endotoxins, enterotoxins) and resistance to antibiotics etc. are encoded either on the microbial chromosome or on plasmids [24].

**Transmission**

The main route of transmission of *Salmonella* in horses is via faeco oral route. Horse suffering from diarrhea shed large number of *Salmonella* into the environment which acts as the main source of transmission. As per one study [25], an equine alimentary tract contains 120 to 150 liters of ingesta. On an average it excretes 14 liters of feces per day and a horse with clinical salmonellosis sheds 104 - 105 organisms per gram of feces [26], therefore, large volumes of diarrhea can become significant source of environmental contamination. Clinically normal horses too can shed *Salmonella* organisms transiently. Horses are not considered to be carriers of these bacteria as there are no known strains that are host adapted to the horse.

**Clinical signs**

Salmonellosis is one of the most commonly diagnosed infectious causes of diarrhea in adult horses mainly manifested either with subclinical carrier to acute, severe diarrhea or sometimes even death. Four clinical syndromes of equine salmonellosis have been described: 1) asymptomatic infection, 2) mild infection (fever, anorexia, and depression), 3) severe acute diarrhea, and 4) bacteremia [27]. In the subclinical form animal may or may not be an active shedder but transmit the bacteria either directly or indirectly via environmental contamination, water or feed sources. In the mild clinical course which may last for 4 - 5 days is usually self-limiting and there are signs of depression, fever and anorexia. Animal may have neutropenia and the feces of the animal are soft but not exactly watery. In the acute form there is sudden onset of severe depression, anorexia and there is profound neutropenia, frequent abdominal pains, systemic signs of toxemia including fever, mucous membrane injection with prolonged capillary refill time (CRT), anorexia, and depression. Signs of circulatory shock may be present especially if infection has advanced to bacteremia, including tachycardia, cool extremities, poor pulse quality and weakness. In this stage the feces are fluid filled and are foul smelling.

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**Salmonella causing abortion in equines**

*Salmonella Abortusequi* is probably the single pathogen responsible for abortion in equines, followed by *S. typhimurium* [5,28]. Equine salmonellosis leads to huge economic losses to equine breeders by means of abortion, breeding inconsistencies, foal mortality, still birth and high maintenance cost of infertile mares over long periods of time. Salmonellosis in equines prevails mostly as a latent infection and precipitates into active form as and when the infection overwhels the immunity of the susceptible host. Pregnant mares are more prone to infection in the latter half of gestation and develop acute placentitis leading to abortion with retention of placenta, which later develops into chronic metritis. The common route of infection in equines is ingestion of contaminated feed and water [6]. Infection is usually through horizontal transmission i.e. grazing on infected pastures or drinking contaminated water but vertical transmission has also been reported. Susceptible horses may pick up infection by grazing on the pastures or by drinking water contaminated through foetal fluid or membranes expelled at the time of abortion. Stallions may also play an important role in coital transmission.

Equines of all ages and both sexes are susceptible to *S. abortusequi* however; young, debilitated, stressed and aged equines are more frequent victims. Infected mares may give birth to either live or dead foetus at full term. Foals born alive from infected mares often die of salmonellosis with signs of pneumonia and enteritis [29] or may develop polyarthritis before death in subacute cases. In most cases infected mare’s show no premonitory signs except a few which become off feed for a day or two with slight pyrexia and sometimes diarrhoea. In stallions, *S. abortusequi* infection is associated with orchitis and arthritus whereas salmonellosis in males is usually mild, clinically or sub clinically but may result into mortality as in brood mares [30-32].

The common source of environmental contamination is excretion of organisms through expelled foetal fluids and membranes. Therefore, proper control of the infection depends mainly on control of the disease in breeding mares through improvement in general hygiene and immunoprophylaxis.

**Diagnosis**

The diagnosis of the disease is based on the clinical signs/severe neutropenia and isolation of *Salmonella* from feces, blood and tissues. Because *Salmonella* cannot be consistently cultured from feces, multiple (a minimum of three to five consecutive 10 gram fecal cultures) daily should be collected from each horse. Some of the authors have suggested to culture using rectal mucosal biopsies to increase the probability of isolating the organisms but the technique is not without risk to the horse.

**Treatment**

The treatment in case of salmonellosis is mainly supportive and includes administration of intravenous fluid (IV) (that is aimed at ameliorating the effects of endotoxemia) and electrolyte replacement and efforts to control the host’s response initiated by the systemic inflammatory response. Thus use of nonsteroidal anti-inflammatories, plasma, antiseria, polymyxin B and intestinal protectants are the mainstay of therapy. Antimicrobial treatment in adult horses is controversial and does not appear to alter the course of the colitis or decrease shedding of the salmonellae; however, it may reduce the likelihood of bacteremia [7]. Antibiotics should only be used in patients that are at a high risk of developing septicemia, such as in the case of neonates or immunocompromised patients. Selection of an antimicrobial should be based on the sensitivity of the organism isolated and the ideal antibiotic should be lipid soluble [7].

A polyionic isotonic fluid is used for volume replacement (40 - 80 L/day is required). Some of the workers have used probiotic therapy using *Lactobacillus* but did not achieve complete success in treating acute salmonellosis [33].

Most horses that recover from acute salmonellosis or subclinical infection will shed the organism transiently for several days to weeks. To confirm the end of the shedding period, five consecutive negative fecal cultures are recommended [34].

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Control

Feco-oral route that is considered the main route should be checked by following certain measures such as new arrivals and horses should be isolated from other horses, avoid combining mare/foal pairs with new arrivals.

Always provide clean, individual food and water for your horses. Avoid contact with manure when visiting equine facilities, and wear footwear that can be cleaned and disinfected. If we know about the infection in our farm then we should do environmental cleanup involving removal of all organic material (bedding, contaminated feed, manure), complete washing and disinfecing with either chlorine bleach or quaternary ammonium compounds and the areas should be encouraged to dry quickly by using fans and exposing to sunlight, where possible.

Conclusion

Thus, it could be concluded from the study that salmonellosis in horses is mainly caused by *Salmonella typhimurium, S. enteritidis, S. virchow, S. abortusequi* etc. It affects horses of all ages and the infection is transmitted mainly through infected pastures or contaminated water. Therefore to control the infection improvement in horses' general hygiene is of utmost importance.

Conflict of Interest

It is to certify that there is no conflict of interest.

Bibliography

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