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Review Article

Bacterial Diseases Causing Diarrhea in Foals: Epidemiology, Disease Conditions and Diagnosis

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Abstract

A review articles was conducted to determine the most common causes of bacterial diarrhea in foals in Minnesota. Up to 80% of Foals infected by bacterial diarrhea in first months of their births and causing deaths.so that our articles summarize the epidemiology of bacterial agents in diarrheic foals along with their diseases condition and diagnostic methods. we detected that most common isolated bacterial diseases is Sallmonellosis, clostradium perferings type A, C, E. coli and Lawsonia intracellularis that difficult to be isolated in normal bacterial growth media need to be tissue culture like virus and most highly sensitive test for diagnosis bacterial diarrhea was PCR.

Keywords: Diarrhea; Foal; Horse; Epidemiology; Clinical Signs; Diagnosis

Introduction

Diarrhea is one of the most common problems of foals, with up to 80% experiencing one or more episodes of diarrhea during the first 6 months of their life [36].

The range of infectious agents that cause diarrhea in foals is poorly described, at least when compared to other domesticated species. Recently, several new agents have been incriminated as causes of diarrhea in newborn and suckling foals. In addition, there are new diagnostic tests available that may aid in differentiating new and established causes of diarrhea. Many of these tests involve primary bacterial culture with subsequent molecular analysis. The approach to diagnosis requires knowledge of likely pathogens, what tests are available, and how to interpret data from the lab. In addition, the types of samples to be requested should be guided by the animal's age, the number of foals affected, and physical examination findings [42].

Isolation of an organism from the feces of foals with diarrhea does not directly indicate that the diarrhea is caused by that agent. *Clostridium perfringens* (biotype A), *Rhodococcus equi, Bacteroides fragilis*, and rotavirus are examples of potential enteric pathogens that may be recovered from feces in the absence of disease. Unfortunately, our knowledge of normal flora or changes that occur in the flora in response to disease is lacking. For example, does the fact that *Aeromonas hydrophila* is more frequently recovered from the feces of foals with diarrhea than from those of healthy animals [14]. It is not clear if this bacterium is responsible for the diarrhea or does it one reflect a change in normal flora and bacterial shedding in response to changes induced by different pathogens. Controlled inoculation studies are required to verify a more definitive role for some of these potential pathogens.

Here, we summarize the epidemiology of bacterial agents in diarrheic foals along with their disease conditions and diagnostic methods.

Salmonellosis

Salmonellosis is caused by a variety of *Salmonella* spp., all of which are gram-negative, motile, non lactose fermenting rods belonging to the family Enterobacteriaceae [27]. All *Salmonella* spp. are classified as S. enterica or S. bongori. S. enteric isolates, can be further separated into 6 subgroups or subspecies (I to VI), including subspecies enterica, which accounts for most cases of human and domestic animal disease. The Kauffman-White scheme divides *Salmonella* subspecies into several serogroups (designated by the letters A through I) based on their heat-stable somatic O antigens. The most important serogroups in veterinary medicine are A, B, C, D, and E [21]. Based on antigenic properties (somatic, capsular, and flagellar antigens), subspecies can be divided into a number of serovars or serotypes [27]. Within *Salmonella* enterica, over 2,000 different serotypes of veterinary and medical importance affect the gastrointestinal tract [27]. The most frequently isolated serotypes in horses include *S. Typhimurium*, *S. Anatum*, *S. Newport*, *S. Krefeld* and *S. Agona* [22]. The only host-adapted serovar reported for horses is *S. Abortusequi*, causing abortions between 5 and 10 months of gestation [34].

Epidemiology

Several non-host adapted *Salmonella* serotypes have been reported to infect horses of all ages and breeds; however, young horses are more susceptible [21]. Three types of *Salmonella*-infected horses have been described: carriers without fecal shedding, carriers with fecal shedding but without diarrhea, and shedders with diarrhea [10]. The source of infection for these horses can be the environment, shedding from infected horses, or infected animals of other species. In the environment, *Salmonella* organisms have been able to survive in soil, feces, water, waste, rodents, birds, and insects for long periods of time (up to 2 years), becoming an important source of infection [44].

Several studies have described the prevalence of *Salmonella* shedding in diverse populations of horses such as breeding farms and slaughter houses [12,43], where large numbers of congregated horses are vulnerable to infection and shedding. Results of several studies show a higher prevalence of *Salmonella* shedding in equine hospitals (1.4 to > 20%) compared to other operations (0.8 to 3%) where horses are housed in their natural environment and where transportation, health problems, and other factors are not common [8].

Variable prevalence patterns of *Salmonella* shedding have been observed, depending on the geographic region, season of the year, and the diagnostic method used to identify the organisms [17,50]. Prevalence of *Salmonella* shedding in horses admitted to veterinary teaching hospitals as determined by fecal culture has been reported to range from 1.4 to > 20% [30,47] and 0.8 to 3% on farms, in stables and other types of operations [12,32]. Higher prevalence has been reported in studies where PCR was used as the diagnostic technique due to the higher sensitivity of this test [18,26]. It is difficult to compare results from different studies due to different types of samples tested (feces, lymph nodes, rectal biopsies), number of samples tested per horse (1 or more), diagnostic technique (bacterial culture, PCR) and the different populations of horses tested (general hospital population, horses with clinical signs of salmonellosis,and animals from necropsy).

Veterinary teaching hospitals are at high risk of nosocomial *Salmonella* infection in horses because of exposure of the hospital population to a common source of *Salmonella* organisms (e.g., contaminated feed) or by lateral transmission from infected patients. In some instances, these facilities have been forced to close temporarily (≥ 3 months) because of serious outbreaks of clinical disease. In 1981-1982, the University of California - Davis Veterinary Medicine Teaching Hospital (VMTH) experienced an outbreak of nosocomial salmonellosis due to S. Saint-Paul [47]. The outbreak resulted in severe disruption of hospital routine. In 1995, an outbreak of nosocomial salmonellosis due to *Salmonella* Infantis at the Colorado State University VMTH resulted in an estimated $500,000 in lost revenues and facility renovation [19]. The original source of the organism causing this outbreak was not determined. In 1996, an outbreak of equine salmonellosis occurred at Michigan State University VMTH [15,26], unique features of the outbreak included a high case fatality rate and zoonotic infection. Of the 18 horses associated with nosocomial infection, 8 (44%) died while hospitalized. In addition, the *Salmonella* Typhimurium isolate from a veterinary student had an antimicrobial resistance pattern identical to the outbreak strain. Pulse field gel electrophoresis patterns also suggested that the student was exposed to the outbreak strain.

Colic, long distance transportation [40], change in diet while hospitalized [41], with holding feed [23], use of common instruments such as nasogastric tubes or rectal thermometers [21], and antimicrobial therapy [22] have been identified as risk factors associated with isolation of *Salmonella* organisms from horses in

several US veterinary teaching hospitals. The common link among these factors is the alteration of gastrointestinal flora, volatile fatty acids and other substances normally produced in the gastrointestinal tract, which favors the multiplication of pathogenic organisms such as *Salmonella spp*. [13]. The season of the year has also been considered as a risk factor, due to the higher prevalence reported in summer months [12,17] or in fall [11]. Hot weather and high humidity associated with prolonged transportation can be important stress factors leading to a higher risk of *Salmonella* shedding [17,50].

Some of the horses infected with *Salmonella* organisms have been identified as subclinical or asymptomatic shedders. This group of animals may be chronically infected and not shed the organisms until exposed to stressful conditions [10]. They have been considered a potential source of contamination not only to the environment but also the other animals [11]. Prevalence of shedding among these horses also varies depending on the geographic region, season of the year, and the diagnostic method employed. Studies performed in a slaughter house, veterinary teaching hospitals, and breeding farms using fecal and tissue samples have reported prevalence between 1% and 70% in horses without clinical signs of salmonellosis. Studies based on fecal cultures reported prevalence between 0.8 and 5% [1,5,10,12], and studies using PCR identification reported 17 to 71.4% prevalence [27].

Disease conditions

Salmonella spp. is well-recognized pathogens causing neonatal septicaemia, with or without diarrhoea, and enterocolitis/diarrhoea in equids of all age groups. *Salmonella* has been significantly associated with fatal diarrhea in foals [26]. Virulence factors include the ability to adhere to and invade the intestinal mucosa, production of entero- and cytotoxins and stimulation of a severe local and systemic inflammatory response [24].

Most affected foals have moderate-to-severe clinical signs that include fever, diarrhea, dehydration, profound depression, and reduced appetite. Diarrhea can vary both in consistency and volume and may contain blood. Colic is common in the early stages of the disease. A complete blood count usually reveals neutropenia with a left shift and toxicity, which is replaced by a rebound neutrophilia as the disease becomes chronic. The fibrinogen usually is elevated.

Extraintestinal disease as a consequence of bacteremia is common in foals less than 2 months old. These extraintestinal diseases include bacterial uveitis, infectious synovitis, osteomyelitis, pneumonia and meningitis [17].

Diagnosis

Several diagnostic techniques have been used to detect *Salmonella* organisms. Polymerase chain reaction (PCR) and aerobic bacterial fecal culture are the most frequently used. Diagnosis of salmonellosis requires bacteriologic isolation of the organisms from appropriate clinical specimens such as feces, blood, or tissues. Serial fecal cultures are indicated to detect fecal *Salmonella* shedding [14]. Laboratory identification of the genus *Salmonella* is achieved by biochemical tests and the serotype is confirmed by serologic testing. Specimens are plated on several non-selective and selective agar media (blood, MacConkey, eosin-methylene blue, bismuth sulfite, *Salmonella*-Shigella, and brilliant green agars) as well as into enrichment broth such as selenite or tetrathionate. After 18 to 24 hours incubation in enrichment broth at 37º C, the isolate is subsequently subcultured the various agars and incubated for 18 to 24 hours at 37º C. Non-lactose fermenting, H S producing colonies are then selected, isolated for purity, inoculated onto urea agar slants, and incubated at 37° C for 18 to 24 hours to determine urease activity. Urease-negative organisms are identified using a commercial system (API 20E, Biomeriex Vitek, Inc). Biochemical identification of *Salmonella* has been simplified by systems that permit the rapid testing of 10 to 20 different biochemical parameters simultaneously. Following biochemical identification, the presumptive identification of *Salmonella* can be confirmed by antigenic analysis of O and H antigens using polyvalent and specific antisera. Approximately 95% of all clinical isolates can be identified with the available group A-E typing antisera. In addition, *Salmonella* isolates are tested for antimicrobial sensitivity by using the minimum inhibitory concentration method and commercially prepared plates (Radiometer America, Westlake, OH). *Salmonella* isolates are then sent to a central or reference laboratory, such as the National Veterinary Services Laboratories, Ames, Iowa for confirmation and serotyping [13]. Polymerase chain reaction (PCR) is a more sensitive diagnostic test that has been used for the detection of *Salmonella* either alone or in conjunction with bacterial culture. The advantages of using PCR in fecal samples are that it is relatively rapid molecular identification test, highly sensitive and requires the submission of fewer samples compared to bacteriologic cultures [18]. Bacteriologic cultures required at least 3 to 5 serial fecal samples to accurately assess an animal's shedding status [24,26]. The disadvantages of PCR are that it cannot discriminate between organisms that are alive or dead, or between pathogenic infections and tran-

sient bacteria, the organism is not available for serogrouping, serotyping and antimicrobial sensitivity testing (important information to determine if the infection is nosocomial in origin), and its lower specificity compared to bacterial culture [18,26]. PCR has also been used for environmental samples showing a higher sensitivity than bacterial culture [18]. It has been used for environmental monitoring in hospitals where extreme sensitivity is needed, in attempts to identify locations that harbor *Salmonella* organisms [18,26]. Since PCR uses specific target genes located in specific parts of the bacterial genome, one of the potential disadvantages that this diagnostic test can have is that bacteria can lose that specific area of the genome, resulting in false negative results [23,26].

Enzyme-linked immunoabsorbent assay (ELISA) is another test used for the detection of *Salmonella* antigens. Several ELISA tests are available in commercial kits; they are based on the O antigen (serotype-specific) and have been used for screening milk from bulk tanks in commercial dairy farms [26]. ELISA has also been used for detection of antibodies against *Salmonella* organisms in blood serum samples in humans, pigs, cattle and poultry [3]. Some limitations of this assay include cross-reactivity between serotypes [25] and difficulties in detecting specific serotypes, especially those that are poorly invasive [3]. ELISA has not been used on feces due to cross-reactivity with other enteric organisms [10,15].

Pulse field gel electrophoresis has been used to characterize biotypes of *Salmonella* enterica isolates, especially in outbreak investigations. This procedure provides information about the bacterial genotype by separating variably sized fragments of chromosomal DNA after digestion with one or more restriction endonucleases [10].

Intestinal Clostridiosis

The common causative agents of this condition are *Clostridium perfringens* biotypes A and C and *Clostridium difficile.* These gram-positive organisms can be found in the intestinal tracts of domestic animals and are widely distributed throughout the environment including soil. They produce potent exotoxins that are responsible for a variety of intestinal diseases in domestic animals. Enteric disease induced by Clostridium species are recognized more commonly during the early neonatal period and there are reports of biotypes A, B, C, D, and E being associated with enteric disease of foals; most studies suggest that biotypes A and then C are the most important (7).

Clostridium perfringens

Categorization into *types A-E* is based upon the expression of one or more major lethal toxins and the combination of toxins to certain defined subsets (53). *Types A* and *C* are the best described pathogens in foals [24]. Alphatoxin is produced by all types of *Cl. perfringens*, but is not thought to be a significant enteric virulence factor [13]. Beta-toxin is produced by *type C* while β2-toxin is produced by *type A*. Beta and β2-toxin have similar biological activities causing haemorrhage and necrosis of the intestinal wall, both toxins have been incriminated in equine necrotizing enterocolitis [24,25]. To date, β2-toxin has not been reported as a pathogenic factor in foal diarrhoea. Enterotoxin is produced by 2-6% of all isolates and types, but most commonly by *type A*; the role of enterotoxin as a virulence factor remains controversial.

Epidemiology

Cl. perfringens was cultured from 64% of neonates (age 8-12 h) and from more than 90% of foals (age 3 days) in moderate to large numbers in a study of 128 healthy mares and foals.

[26]. Eighty-five percent of the isolates were *type A* while the β2-toxin gene and the enterotoxin gene were identified in 12% and 2.1% of the isolates, respectively. In this study, *Type C* was identified in one foal requiring intensive treatment for enterocolitis.

A significant association was found between *Cl. perfringens* and diarrhea in foals less than age 6 months but the overall mortality was low [6,7]. Foals less than 1 week of age were more likely to die. No association could be established between *Cl. perfringens* isolation and antimicrobial treatment and, despite characterization of 17 different genotypes, no pathogenic sub-population could be identified [18]. Detected enterotoxin in 8/28 diarrhoeic foals (28.6%) and in 0/4 (0%) control foals while [8] failed to identify enterotoxic isolates more commonly in diarrheic foals than in healthy controls and enterotoxin gene was not identified in any the fatal cases [9]. Suggested that either a predisposing factor is necessary For development of disease or a molecular marker for pathogenicity remains unidentified.

Disease conditions

Initial reports on *Clostridium*-induced diarrhoea focused on the rapid progression of the disease and high fatality rate, the most prominent clinical signs being sudden death, colic, bloody

diarrhoea and clinical and haematological signs of systemic sepsis [19,24]. This clinical syndrome appears to be most prominent in neonatal foals less than 10 days of age [24,44]. Less severe clinical presentations include mild diarrhea often with spontaneous recovery [3]. Foals affected with *Cl. perfringens type C* were more likely to die or be subjected to euthanasia than foals affected with *type A* [24].

Diagnosis

This is difficult to achieve unless the presence of *Clostridium* and its toxins are correlated with expected pathological lesions, commonly established only with *post mortem* examination (Jones 2000). Quantitative culture of less than 100 CFU/ml has been suggested to be within the normal range [25], but heavy growth can be found in 42% of apparently healthy foals 3 days in age [16]. Recovery of the organism can be greatly improved with specific culture methods [11]. Cytology of fecal samples might be a useful rapid screening test in severely affected animals [22] but a negative stain does not rule out clostridial disease. A commercially available ELISA for detection of enterotoxin (Immunocard Toxin A Test) 2 has been used in the clinical setting to diagnose enterocolitis associated with enterotoxigenic *Cl. perfringens* [18,23]. A reverse passive latex agglutination test designed for the detection of enterotoxin yielded a high number of false positive results [19]. As enterotoxigenic strains alone do not account for the overall association of *Cl. perfringens* with diarrhea and are rarely found in fatal cases [17,28], some authors suggest isolation of the organism and PCR multiplex assay for determination of the toxin genotype to obtain a diagnosis [25]. However the organism selected for genotyping might not be representative for the entire fecal sample.

Clostridium difficile

Despite several publications in recent years about the role of *Clostridium difficile* in equine diarrhea [19,23,35], many aspects of this pathogen are yet to be clearly elucidated. *Clostridium difficile* produces several 5hydrolytic enzymes and at least 5 toxic factors, *toxins A* and *B* being the best described. When competing microflora suddenly become diminished, *Cl. difficile* can replicate rapidly releasing toxins into the intestinal lumen. Release of toxins results in disruption of the enterocyte cytoskeleton and tight junctions, severe inflammation of the *lamina propria* and micro-ulceration of the colonic mucosa [17,28].

Epidemiology

The organism is frequently isolated from the faeces of healthy, untreated puppies and human neonates [15,38]. and has also been found in almost one third of healthy, asymptomatic foals less than 14 days old [6]. The organism and its toxins were also identified in non diarrhoeic foals age 1-3 months treated with antimicrobial drugs [5,6]. Few healthy mature horses carry the organism [6,18]. In contrast to man, where antimicrobial therapy is a main risk factor [28], disease can develop in foals without previous antimicrobial therapy [34].

Disease conditions

Early reports in equine neonate's age 1-3 days concentrated on cases of largely fatal haemorrhagic necrotizing enterocolitis. The main clinical signs being colic, systemic sepsis and rapid deterioration. Foals surviving long enough often developed bloody diarrhea [42]. Other reports described clinical presentations ranging from mild to severe diarrhoea [38] to asymptomatic carriage and shedding of the organism and toxins in foals treated with antimicrobial drugs [5]. Most foals are less than age 2 weeks but cases have been reported in foals and horses of all age groups [19,45].

Diagnosis

Cl. difficile-induced diarrhoea currently requires detection of toxigenic strains or toxin in the faeces of the affected individual, as 25% of *Cl. difficile* isolates lack the genes to produce toxins.

Techniques used to identify toxigenic strains include culture in combination with cell cytotoxicity, cytotoxin assay (Cytoxi Test) or PCR, to identify toxin or toxin-encoding genes and detection of toxin directly in the faeces by the use of enzyme immune assay (EIA) [13]. Recovery of the organism from faecal samples is greatly improved when samples are stored anaerobically until processed and when selective culture media are used, typically incorporating cycloserine and cefoxitin. Survival of the organism decreased dramatically to a median survival length of 1 day when samples were stored aerobically. Toxins proved to be more stable and could still be detected by ELISA after 60 days of aerobic storage at 4°C [20]. Several EIA are marketed for the detection of either *toxin A* (Immunocard Toxin A Test2; Culturette Brand Toxin CD Enzyme Immuno Assay3; TOX-A TEST4) or *toxins A* and *B* (TOX A/B TEST) most of them performing with high sensitivity and specificity when compared to tissue culture. The only organisms that cross reacted with the TOX A/B TEST was a toxigenic strain of *Cl. sordellii* that produces 2 immunologically related toxins [13].

Escherichia coli

Escherichia coli (E. coli) is one of many species of bacteria living in the lower intestines of mammals, known as gut flora. When

located in the large intestine, it assists with waste processing, vitamin K production, and food absorption. Discovered in 1885 by the odor *Escherich*, a German pediatrician and bacteriologist, *E. coli* are unable to sporulate. Thus, treatments which kill all active bacteria, such as pasteurization or simple boiling, are effective for their eradication, without requiring the more rigorous sterilization. As a result of their adaptation to mammalian intestines, *E. coli* grow best *in vivo* or at the higher temperatures characteristic of such an environment, rather than the cooler temperatures found in soil and other environments [4].

The enteric *E. coli* (EC) are divided on the basis of virulence properties into enterotoxigenic (ETEC - causative agent of diarrhea in humans, pigs, sheep, goats, cattle, dogs, and horses), enteropathogenic (EPEC - causative agent of diarrhea in humans, rabbits, dogs, cats and horses); enteroinvasive (EIEC - found only in humans) verotoxigenic (VTEC - found in pigs, cattle, dogs and cats); enterohaemorrhagic (EHEC - found in humans, cattle, and goats, attacking porcine strains that colonize the gut in a manner similar to human EPEC strains); and enteroaggregative *E. coli* (EAggEC - found only in humans) [49].

Epidemiology

Enterotoxigenic *E. coli* (ETEC) are important causative agents of diarrhea1 illness among neonatal farm animals. Various ETEC strains from calves and pigs have been extensively studied in order to define their virulence attributes and pathogenic mechanisms. In contrast, little is known about possible virulence attributes and the enteropathogenic potential of strains of *E. coli* recovered from foals. While some strains of *E. coli* that possess ETEC-associated virulence attributes have been isolated from the feces of diarrheic foals [24,27,48,49]. their importance in foal diarrhea1 illness has not been investigated. Although K88-positive ETEC have been recovered from diarrheic foals [17] and K88-positive pig strains have been shown to adhere to equine (BBM) brush border membrane [12] no evidence exists that K88-positive ETEC are causative agents of foal diarrhea.

Disease conditions

Escherichia coli are the most common cause of systemic sepsis in newborn foals but it is an uncommon primary enteric pathogen. Reports suggest that *E. coli* can mediate diarrhea in foals less than one month old. The diarrhea is profuse and watery but non fetid [49].Pathogenic strains of *E. coli* (0111a, K792) cause classical ultrastructural changes to the intestinal microvilli of ileal explants harvested from month-old foals [4].Recovery of *E. coli* from feces is very common but these isolates typically lack the appropriate virulence factors required to create intestinal disease.

E. coli possessing KS8 fimbriae and belonging to serotype 0149 is a common cause of diarrheal illness among pigs during both the neonatal and post weaning periods [23]. ETEC strains that express K88 fimbriae show a high degree of host specificity, which is conferred primarily via the fimbriae [33,43]. The KS8 fimbriae enable the bacteria to bind to specific intestinal epithelial cell receptors and thereby colonize the intestinal epithelium. After binding, ETEC secrete enterotoxins which also bind to specific receptors, and ultimately cause the epithelial cells to become hypersecretory [46]. Strains lacking fimbriae and enterotoxins do not cause this secretory diarrhea, and hosts lacking corresponding epithelial cell receptors for ETEC fimbriae and enterotoxins are unlikely candidates to experience ETEC induced diarrhea.

Diagnosis

Diagnosis is achieved by culture from feces and then detecting virulence factors with PCR [30]. Isolation of *E. coli* in specific microbiological cultural media with characterstic traits is important [19]. *E. coli* resistance genes from horses are similar to those found in other animals and humans, specifically by sequencing the TEMβ-lactamase PCR products to identify genes responsible for resistance to: ampicillin, chloramphenicol, trimethoprim and tetracycline which have great economic effect on veterinary antibiotic treatment [2].

Lawsonia intracellularis

Lawsonia intracellularis is an obligate intracellular small, Gram negative, curved-shaped rod [5,11,37]. The name L. intracellularis was formally given to the organism in 1995 in honor of the Scottish scientist G. H. K. Lawson as the primary discoverer of the bacterium [4]. L. intracellularis is a member of the delta division of Proteobacteria [35] and is taxonomically distinct from other intracellular pathogens [2]. DNA sequences of the 16 S ribosomal RNA gene from L. intracellularis were found to be closely related to Bilophila wadsworthia [10] and the sulfate-reducing proteobacterium, Desulfovibrio desulfuricans [35], with 92 and 91% homology, respectively. L. intracellularis is classified as a Gram-negative, microaerophilic, obligate intracellular, non-flagellated, non-spore-forming, curved or S-shaped bacillus [7], but recently, a long, single, polar flagellum has been observed by electron microscopy in multiple pure culture

isolates of L. intracellularis [9]. The bacterium measures 1.25-1.75-mm long and 0.25-0.43-mm wide comprising a trilaminar outer envelope separated from the cytoplasm membrane by an electron-lucent zone; neither fimbriae nor spores have been observed [29].

Proliferative enteropathy (PE) has been described as an important enteric disease that has been recognized in pigs for over 70 years. Characteristic lesions of PE found in pigs were first described by [3]. It was not until the 1970s that intracellular bacteria were found within proliferating crypt cells in cases of PE in pigs [3,10]. A variety of Campylobacter species having morphologically similar features to L. intracellularis have been isolated from lesions of PE. Those include Campylobacter mucosalis [3,7], C. hyointestinalis [2,6], *C. jejuni* and *C. coli* [25]. Despite the routine recovery of these Campylobacter species in proliferative lesions, none of these organisms specifically cause PE or colonize intracellularly under experimental conditions (16; 18; 12; 1). It was not known until [16] inoculated rabbits with an extract containing intracellular bacteria from an intestinal lesion that did not contain Campylobacter that a new and novel intracellular bacterium was discovered. Convalescent serum containing antibodies from inoculated rabbits did not react to various isolates of Campylobacter but reacted to intracellular bacteria in formalin-fixed sections of PE-affected intestines [18]. Progress in cultivation of this organism ensued and Koch's postulates were fulfilled when pure cultures of the intracellular bacterium were shown to cause PE in pigs [17]. Initially, the bacteria were referred to as 'Campylobacter-like organisms or CLO' because of their similarities in morphology to Campylobacter species (28). Later, the intracellular bacteria were given the name Ileal Symbiont (IS) intracellularis and were identified as a distinct genus that differed from Campylobacter species [35].

Lawsonia intracellularis inhibits the proliferating crypt cells of the ileum mostly, but other cells of the small intestine can be inhibited also [11,15]. Due this inhibition the mucosal villi of especially the ileum consist of poorly differentiated crypt cells with limited brush border development and therefore decreased absorptive capabilities [11,13]. The bacterium needs host animals for replication and transmission occurs generally through fecal-oral route [15,22]. Infection with *Lawsonia intracellularis* can result in a disease with unique symptoms [14]. This infection is also known as proliferative enteritis, proliferative enteropathy, proliferative ileitis or intestinal adenomatosis [11,14].

Epidemiology

There is little known about the prevalence of *Lawsonia intracellularis* in horses. In pigs there is more result available. Several studies are performed in different countries [37]. Showed that 96% of US pig herds were seropositive for *Lawsonia intracellularis*. In Denmark the prevalence in pigs is 93, 7% [11]. Also in Canada there are high prevalence reported, 84,4% of the tested pigs tested positive for the bacterium [3,9]. Serologic studies shown that the prevalence of proliferative enteropathy (PE)-positive herds ranges from 60 to 90% in farms in Austria, Venezuela, Germany, Belgium and the Netherlands [40]. In the Netherlands *Lawsonia intracellularis* infection in horses was first reported by [16] in foals and in 2008 a study of the prevalence of Lawsonia intracellularis in horses was performed. This study showed that 23% of the tested foals after weaning had positive *Lawsonia intracellularis* antibodies titres and 20% were inconclusive [1]. All dams of foals that tested positive or inconclusive at pre-weaning screening were tested also after weaning of their foal. All these dams were tested positive for *Lawsonia intracellularis* [32]. In Europe natural outbreaks have only been reported in pigs, [21]. In dogs in the Czech republic the bacterium is also widespread, 74,7% of the tested dogs were positive [12].

[7]. showed that in the United States 45,5% of the foals on a endemic farm had a positive titer to *Lawsonia intracellularis*. Resident horses on farms with diagnosed cases of EPE had seroprevalence rates from 30% to 76% [5]. A prevalence study in Brazil showed a positive titer in 9,42% of the tested horses [40]. In the Czech republic there was a high prevalence found, 87,1% of the tested horses over one year of age were positive [1]. Another study by [3] showed that in May 100% of the mares on a endemic farm tested positive for antibodies against Lawsonia intracellularis. In the Netherlands 14% of the foals showed a positive *Lawsonia intracellularis* titer before weaning, after weaning it was 23% [1,13]. All mares of positive tested foals after weaning were positive for Lawsonia themselves [10]. The present results shown that 98,3% of the tested horses were tested positive for antibodies specific for *Lawsonia intracellularis*. To the authors' knowledge this is the first prevalence study in the Netherlands among adult horses, so no other data are available for comparison. Because of the high prevalence in horses without clinical signs, the conclusion is that a positive titre of antibodies against *Lawsonia intracellularis* is not a sensitive test for definitive diagnosis of equine proliferative enteropathy (EPE).

Lawsonia intracellularis needs host animals for replication and transmission occurs generally through faecal-oral route [22,31]. Fecal shedding within species has been identified as a possible source for infecting animals from the same species [21,37,39]. *Lawsonia intracellularis* can survive for 1 to 2 weeks in extracellulair conditions in feces, but the infectivity of the bacterium might be less [19]. The environmental contamination by feces must therefore be considered in cases with EPE.

Subclinically infected herd mates may also play a big role in transmission of the bacterium, since showing clinical signs, but they can shed the bacterium as well [19,20,35,40,41]. On farms with clinical cases of an infection with *Lawsonia intracellularis* other residential horses have been detected with positive antibody titers [11,21,28]. Transmission of antibodies can also occur by colostrum ingestion [18]. Showed that foals of a farm endemic for EPE (54% of the mares tested positive by IMPA at the time of foaling) had negative antibody titers prior to colostrum ingestion, after ingestion 54% of the foals were positive.

Many hosts have been recognized. These hosts shed the bacterium in their feces in the environment and spread there [32]. Infected feces may contaminate water or feed which is ingested by foals [19]. A correlation between pigs and foals infected with *Lawsonia intracellularis* has been proposed. However [10], found no correlation between the prevalence and the proximity of pig farms to the breeding farms and the use of pig manure on the fields. Also [20] found no other positive animals on a farm with a single foal positive for *Lawsonia intracellularis*. The farm had no history of any contacts with pig farms, so wildlife might have been the source of contamination. [7,22]. showed that rodents (especially mices) may act as a reservoir species and that they can participate in the spreading of the bacterium among domestig pigs and wild animals. Interspecies transfer of *Lawsonia intracellularis* must be considered as a real possibility for transmission of the bacterium [22,40]. Other possible mechanism of transmission of *Lawsonia intracellularis* as mechanical (eg. pitchforks, boots) vectors must be considered also [38,40].

Disease conditions

The disease is best described in pigs [21]. It is a major endemic disease with high economic importance in the swine industry [32,35]. Infection with *Lawsonia intracellularis* can be seen in several species, but is not believed to transmit directly between species [12]. The bacterium was found in wild boars and one fallow deer in a reserve in Czech republic [21]. It was also demonstrated in wolves, red foxes and red deer [18]. It also has been demonstrated: 74,7% of the tested dogs in Czech republic were positive for *Lawsonia intracellularis* [17,32]. Infection with *Lawsonia intracellularis* can also affect several other species such as, foxes, ferrets, rats, guinea pigs, rabbits, monkeys, ostriches, emus, sheep, deer and horses [20,28,44].

The disease in horses is called Equine proliferative enteropathy (EPE) and has been reported in various cases in the North and South of America, Canada, Australia and Europe [9,21,27,36]. In horses *Lawsonia intracellularis* most commonly affects foals [9,11,17,26]. Foals affected by *Lawsonia intracellularis* have symptoms such as diarrhea, profound dullness, fever, rapid weight loss, colic, rough hair coat and ventral edema [4,10,16,27]. The ventral edema is caused by severe hypoproteinemia [9,13,18,21]. Hypoproteinaemia can be caused by renal, hepatic and enteric factors [6]. Weanlings infected by Lawsonia intracellularis have hepatic and renal values within range, and the hypoproteinaemia is caused by protein-loosing enteropathy [4,9].

Diagnosis

Blood analysis often reveal leucocytosis or leucopaenia often with left shift, hypoproteinaemia, hypokalaemia, hyponatraemia, hypocalcaemia, hypomagnesaemia, hyperphosphataemia, anemia, azetomia, metabolic acidosis and dehydration [9,35]. Absorption test and urinalysis are usually within normal limits [9,33].

Due to the infection with *Lawsonia intracellularis* the mucosal crypt cell undergo excessive mitotic division and contribute to a hyperplastic thickening of the small intestinal loops [9,12,25,28]. This can be noticed on ultrasound of the abdomen (especially of foals with abdominal pain) can help assess bowel motility, detect distention of the small intestine, and measure bowel wall thickness. Abdominal ultrasonography of foals with clostridial enterocolitis may reveal gas and fluid-filled loops of large and small intestine. Segmental, marked thickening (6 to 12 mm; normal jejunum: 3 mm) of small intestine is often detected in foals infected with lawsonia intracellularis [9,12,25,28].

Diagnosis of an infection caused by *Lawsonia intracellularis* can be done by PCR on fecal or intestinal samples (30,39, 97, 98). Different serological methods, such as a immunoperoxidase monolayer assay (IPMA), indirect immunofluorescence antibody test (IFAT) and a blocking enzyme-linked immunosorbent assay (bELISA) are

available for detection of circulating antibodies against *Lawsonia intracellularis* [11,20,21,30,39,44] lawssonia is difficult to be cultured on bacteriological media, The preferred medium for growing Lawsonia susceptible tissue culture cells is Dulbecco's modified Eagle's medium (DMEM) with bovine serum at concentrations of 5-10% (43; 19).

Corynebacterium (Rhodococcus) *equi*

Corynebacterium equi is a pleomorphic Gram-positive capsulated diptheroid microorganism. It is a saprophytic soil inhabitant and is an accidental or opportunistic pathogen [32]. Generally it causes a purulent bronchopneumonia in the young horse and causes abscessation of the lungs and other organs [10,22,37]. Two reports of enteritis caused by *C. equi* have been confirmed. In one, the only lesion was necrosis of Peyer's patches [37]. In the second, *C. equi* was cultured from a case of chronic diarrhea in foal and thickened small intestinal mucosa were positive for the organism [38].

Disease conditions

Approximately 50% of pneumonic foals presented for necropsy also have intestinal manifestations [49]. The intestinal form of *R. equi* infection is characterized by a multifocal ulcerative enterocolitis and typhlitis over Peyer's patches with granulomatous or suppurative inflammation of the mesenteric and/or colonic lymph nodes [49]. Occasionally, a single large abdominal abscess (usually in a mesenteric lymph node) often causing adhesion to the large or small bowel is the only finding [48]. Peritonitis may result and the organism may be isolated from the peritoneal fluid [2]. Clinical signs associated with the abdominal form of the disease may include fever, depression, anorexia, weight loss, colic, and diarrhea [2,50].

Marked gastrointestinal lymphatic obstruction associated with increased protein concentration in the peritoneal fluid and hypoproteinemia may lead to ascites giving foals a pot-bellied appearance. Such foals have a poor prognosis because of the extensive granulomatous inflammation of the colonic mucosa and submucosa, and mesenteric lymphodes.

Disease due to *R. equi* is rare in adult horses. In one adult horse, acquired combined immunodeficiency of unknown origin led to *R. equi* septicemia and lung abscessation [31]. Rarely, the organism has also been isolated from infertile mares and aborted fetuses (50; 29).

Diagnosis

A complete blood count (CBC), fibrinogen level, radiographs, and serology may help distinguish *R. equi* pneumonia from that caused by other pathogens. However, bacteriologic culture combined with cytological examination of tracheobronchial exudate are still the 'gold standards' used to arrive at a definitive diagnosis. Virtually all strains of *R. equi* produce membranolytic exoenzymes originally called'equi factors' [39]. These exoenzymes consist of a cholesterol oxidase and phospholipase C and AGID agar gel immune diffusion test detects mainly precipitating antibodies against these exoenzymes but the identity of other antigens sometimes detected is unknown. The vast majority of adult horses are negative for precipitating antibody on AGID; consequently, there are no false positives as a result of maternally-derived antibody [34]. In the same study, the earliest detection of such antibody in foals was at 26 days of age. After seroconversion, clearance of precipitating antibodies in sick foals receiving appropriate antimicrobial therapy has been reported to take 2-6 weeks [34]. Using a more sensitive synergestic hemolysis inhibition (SHI) assay, [42] demonstrated the widespread nature of subclinical infection in foals. As opposed to AGID, the SHI assay is quantitative but perhaps too sensitive for diagnostic purposes, detecting more subclinical infections than AGID [33]. The SHI assay is also more difficult to perform in the laboratory. Using ELISA, the mean values of anti-*R. equi* IgG antibodies in foals known or suspected to be ill with *R. equi* pneumonia were higher than in normal foals in a limited number of animal tested [46,48]. This ELISA was more sensitive than AGID [44]. The sensitivity and specificity of this ELISA in relation to bacterial culture of a tracheobronchial aspirate are however unknown. The use of polymerase chain reaction (PCR) based on the VapA gene sequence is a more rapid and sensitive mean of identifying *R. equi* in tracheo bronchial aspirate (TBA) samples than bacterial isolation especially if the foal is being treated with antimicrobial agents.

Other aerobic bacteria implicated in infectious diarrhea.

These bacteria include Aeromonas hydrophila, Yersinia enterocolitica, and Campylobacter species. Much of the data incriminating Aeromonas hydrophila as a potential pathogen of foals stems from work done in the UK in the early 1990 [14].

Conclusion

Salmonellosis is the most important bacterial infection in neonates, foal and adult horse causes several outbreaks in equine farms as well as zoonotic importance, *Clostradium perferingens t*ype A,C

is main causative agents in foals causing hemorrhagic enterocolitis and diarrhea detected infection by their Enterotoxine.K88-positive ETEC *enteritoxigenic E.coli* has been isolated from foal feces. *lawsonia intracellularis* recently recognized infection in horse breeding but mainly infect foals up to 6 months with proliferative enteropathy and symptoms of diarrhea. Diagnosis of these causative agents by first bacteriological isolation from fecal samples except Lawsonia.spp is difficult to be isolated in normal bacteriological media and need tissue culture media like viruses, the recent and sensitive method for detection most of bacterial diarrhea is PCR that what will use it for diagnosis the most common causative agent using multiplex PCR.

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