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Diarrhea in Foals: Unravelling the Role of Bacterial Infections and Strategies for Diagnosis and Control

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Abstract

Diarrhea is a dangerous condition in foals that can result in dehydration and substantial economic losses. The most common bacterial causes of diarrhea in foals include *Salmonella* spp., *Clostridium perfringens* types A and C, *Clostridium difficile*, and *Escherichia coli*, in addition to less common bacteria as *Rhodococcus equi* and *Lawsonia intracellularis*. Diarrhea typically occurs within the first six months of a foal's life and can manifest as sporadic cases or outbreaks. While *C. perfringens* and *C. difficile* are part of the equine neonatal intestinal flora, they can lead to severe diarrhea. Accurate diagnosis of the causative pathogens is crucial for appropriate treatment selection and outbreak prevention. Management of diarrhea primarily involves fluid and electrolyte correction, accounting for factors such as age and physiological status. Symptomatic management may be sufficient, but specific treatments depending on the underlying cause may be necessary. Implementing strict hygienic measures is essential for disease prevention. Traditional diagnostic methods such as bacterial culture and microscopy have limitations, including labor-intensive procedures, low sensitivity, and the need for specialized laboratories and trained personnel. However, advancements in molecular diagnostics and commercially available kits have paved the way for antigen detection and molecular-based techniques, potentially replacing traditional methods. This review article aims to provide a comprehensive examination of bacterial diseases causing diarrhea in foals, focusing on their diagnosis and control. It also emphasizes the "One Health" concept, underscoring the interconnectedness of human, animal, and environmental health in addressing the challenges posed by bacterial diarrhea in foals.

Keywords: *Clostridium difficile*; *Clostridium perfringens*; *Escherichia coli*; One Health.

Introduction

Diarrhea is characterized by more frequent bowel movements and higher water content in the stool. It often occurs alongside enteritis [1]. Globally, foal diarrhea is a prevalent issue that leads to significant mortality in new-borns and substantial economic losses in horses and foals [2-4]. Within the first six months, 60% of foals are affected by diarrhea, with up to 20% suffering from infectious agent-related diarrhea [5]. The primary infectious causes in horses are bacterial, viral, and parasitic agents [6]. Diarrheic foals typically exhibit poor body condition and lack of appetite, with severe cases presenting symptoms like high fever, low white blood cell count, septic shock, rapid heart rate, and loss of appetite [7].

Studies have investigated the cause of diarrhea in neonatal foals, which is often attributed to a single pathogen but actually results from a combination of various enteric infections [8]. Bacterial causes are more common, while parasitic and viral causes are less [9]. *Salmonella* spp., *Clostridium* species, and *Escherichia coli* (*E. coli*) are the primary bacterial pathogens found in diarrheic foals [2, 4, and 10]. Gram-negative bacteria, particularly those in the Enterobacteriaceae family, are known to cause gastrointestinal disturbances and can thrive when the normal intestinal microflora balance is disrupted, leading to conditions like colitis in foals [11]. Diarrhea mechanisms include secretory, due to excessive electrolyte and water secretion into the gut, and mal-absorptive, due to the intestine's inability to absorb nutrients and water. Understanding these mechanisms is crucial for veterinarians before starting treatment [3].

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The microbes in gut play a vital role on disease progression and health maintenance into mammals, including horses. Horses depend on a hindgut fermenter that promote by the activities of bacteria, protozoa, fungi in the colon and cecum to produce energy and regulate metabolism [5]. The foal microbiota, which changes until two months of age, differs from that of older horses, indicating the need for controlled research on microbiota management as a preventive or therapeutic measure for foal diarrhea [12].

Diagnostic laboratory tests for blood and biochemical parameters serve as reliable indicators for diagnosing and treating equine diseases. Diarrhea can lead to severe dehydration, electrolyte loss, and acidosis, which are common causes of death [13, 14]. Hyponatremia is a frequent electrolyte imbalance in diarrheic foals and is often associated with the severity of diarrhea [15]. Bacterial culture, microscopy, and immunoassays are fundamental in diagnosing these infections [16].

Dehydration occurs rapidly in young foals (in a few hours, from six to eight hours) because of the small size of foals, shortness of their gastrointestinal tract, and not being able to reabsorb enough amount of liquid as horses.

To treat cases of diarrhea, administering fluid therapy and a targeted antibiotic is necessary [17]. Less than thirty-day-old foals must be inoculated by a broad-spectrum antibiotic to combat bacterial infections [2]. Nonetheless, the indiscriminate use of antibiotics is contributing to a global rise in antibiotic resistance [18]. The employment of probiotics, specifically those containing *Saccharomyces boulardii*, is common in the treatment of colitis in horses, as it can reduce both the intensity and the length of diarrheal episodes [19].

This review article aimed to spotlight on diagnosis and control of bacterial diseases causing diarrhea in foals, while highlighting the importance of the "One Health" concept. The review will discuss the most common bacterial agents associated with foal diarrhea, along with their diagnostic methods and control strategies to enhance the understanding of healthcare professionals, veterinarians, and researchers in effectively managing and preventing this perilous condition in foals. Furthermore, it will emphasize the interconnectedness of human, animal, and environmental health, underscoring the need for collaborative efforts and integrated approaches to address the health challenges posed by bacterial diarrhea in foals.

Diagnosis of Bacterial Diseases Causing Diarrhea in Foals

Diarrhea is the main common clinical complaint among foals. Affected foals are usually ranged from 3–12 months old, but those of 4–6 months old are of high risk of infection. Stress may be a predisposing factor. In spite of sudden death might be occurred in foals, fatalities are lower in well treated ones. Causes of foal diarrhea may be also secondary to non-infectious foal heat diarrhea; the common bacterial infections are *C. perfringens* and *C. difficle*, *Salmonella* spp., and *E. coli*. Although *E. coli* has a significant role as a mediator of systemic sepsis in newly born foals it is not the main reason diarrhea of foals. Nonconventional bacterial causes are *Enterobacter aerogens, Proteus mirabilis, E. coli, Citrobacter diversus, Salmonella enteric*a and *Proteus vulgaris* which represent the main causative agents of diarrhea in Arabian horses' foals. Gram-positive bacteria found in the diarrheal foals of Arabian horses were not given sufficient consideration [10]. Bacterial diseases that cause diarrhea in foals can be diagnosed as follows:

Case History

The information about the number of foals showing symptoms of diarrhea and their ages, vaccination, and deworming status are important data to diagnose the diseases causing diarrhea [20]. Failure of passive transfer of immunity might be studied [21]. The risk factors include; the following but not limited to; the large use of antibiotics and suboptimal deworming of mares [22].

Clinical Inspections

Fever, faecal consistency, and gastric distension can all be detected with clinical examinations. In addition to aiding in testing prioritization, this information may be utilized for prompt therapy [2].

Clinical Signs

Clinical signs vary where initial symptoms force the animal to stop movement and/or excessive motility, colic, and abdominal bloating. Faeces are considered the first coming which can depend on to judge the severity of diarrhea where it may vary from watery to pasty, with blood and/or casts. Foals dehydrate quickly and reach the very bad state of dehydration indicated by prolonged skin tent and sunken eyeballs. Also, salivation, anorexia weakness, and depression were seen. The body temperature has different degrees of fever or hypothermia [23]. Foals infected with enteritis commonly have signs of systemic inflammatory response syndrome (SIRS), including congested vital membranes, faint pulses, tachycardia, cool extremities, and abnormally rapid breathing of low temp [23]. When signs advance, recumbency, coma, and death may occur. Sick foals should be examined clinically in regular time (two hours at least). Palpate joints and the umbilical cord of foals have to be visually checked to monitor any localized bacterial infections [24].

Foals which were affected by *Clostridium* spp. dies rapidly before the symptoms of diarrhea appear. Feces are frequently bloody, and this may be

transient. Affected animals may show signs of hypovolemia and endotoxemia. *Clostridium difficile* and *C. perfringens* type A and C are the pathogens which mostly common, however, *C. perfringens* Type C is the most important pathogen of diarrhea in newly born foals [27, 25].

Laboratory Diagnosis

Laboratory diagnosis includes hematological and biochemical analyses, isolation and identification of the bacterial agents (culturing on specific media, microscopic examination, and biochemical tests) as well as molecular investigations [26, 28].

Hematological and Serum Biochemical Analyses

These analyses are highly effective in determining the origins of diarrhea and its treatment strategies. The detection of immature and toxic neutrophils, along with changes in neutropenia, are indicative of severe diarrhea [27]. Furthermore, an increase in packed cell volume (PCV) is associated with dehydration, signalling the need for immediate intervention. Diarrheic foals exhibit elevated levels of total white blood cells (WBC), platelets, red blood cells (RBCs), lymphocytes, and hematocrit [28]. Biochemical analysis of serum in these foals reveals reduced levels of albumin, calcium, urea, and sodium. Additionally, there is a noted decrease in overall serum proteins, globulins, γ-globulin, and β2-globulin, whereas creatinine levels, aspartate aminotransferase activity, and α2-globulin concentrations are found to be higher [10]. The effect of *Rhodococcus equi* (*R. equi)* on hemato-biochemical parameters are leukocytosis, neutrophilia, and microcytic anemia, and increased serum globulins [29].

Bacteriological Examination

In equine medicine, similar diagnostic methods for bacterial causes of diarrhea are employed as those used in human medicine. An optimal laboratory investigation involves analyzing freshly collected fecal samples submitted directly to the laboratory. In *C. difficile*, for instance, the recommended approach combines detection tests with culturing methods. However, culturing *C. difficile* is infrequently performed due to its limited clinical significance and the challenges associated with handling this obligate anaerobe. Consequently, researchers lack routine access to such isolates for molecular characterization and detection of antimicrobial susceptibility, toxin gene and virulence factors. Lack of some bacterial isolates hampers investigations into potential epidemiological changes or shifts in clinical presentation over time. Many diagnostic procedures rely on an enrichment step to enhance spore recovery. Additionally, an alcohol shock reduces the number of live bacterial cells, highlighting the need for diverse isolation methods based on sample types [30, 31].

One of obstacles facing basic laboratory examination is that *Clostridium* spp. incriminated with diarrhea die rapidly before the symptoms of diarrhea appear. Feces are frequently bloody, and this may be transient. Animal may show signs of hypovolemia and endotoxaemia. *Clostridium difficille* and *C. perfringens* type A and C are the pathogens which detected where the most pathogen of diarrhea is *C. perfringens* Type C in newly born foals [25].

Isolation and Identification of Clostridium difficile

In human and animals, *C. difficille* has associated with diarrhea and colitis as one of the outstanding characteristics of diarrhea and it happens afterwards the antibiotic therapy. It is a gram-positive anaerobic sporulating bacillus. The first factor stimulates the occurrence of the disease is the trouble of the gut flora. The normal intestinal flora act as a colonization barrier, it protects the gut from penetration of C. *difficile* in normal conditions. The colonization is penetrating when the flora is disturbed and the main important factor causes this disturbance is antibiotic therapy. The key virulence toxins of *C. difficille* are toxin A (TcdA) and toxin B (TcdB) [32]. *Clostridium difficile* could be cultivated on *C. difficile* agar with supplement. *Clostridium difficille* colonies are irregular, non-hemolytic, 2 to 6 mm in diameter, and creamy yellow to gray-white in colour. Colonies are coarsely mottled to mosaic.

Isolation and Identification of Clostridium perfringens

Clostridium perfringens is a gram-positive anaerobic rod and widely distributed in the environment. It is found in the decaying organic matter and soil. It is also one of the members of the normal intestinal flora in many animals. The bacteria are able to produce endospores which are highly resistant to different environmental conditions which mean they live in the environment for a long time. However, if the microbiota has been distressed and the organism produces spores, these spores have ability to convert into active vegetative cells which proliferate and immediately produce toxins [33]. *Clostridium perfringens* has five types from A to E producing four main exotoxins: α, β, ε, and i. The predominant gene in all strains is that encoding the toxin (CPA). The toxin encoding genes are used to differentiate *C. perfringens* from biotypes A to E [34]. The cpb2 toxin-process by *C. perfringens* has a significant role in the lethal spread of colitis in horses [35, 36]. Isolates with cpb2 -positive for equine disease can manufacture cpb2 toxin [37]. *Clostridium perfringens* enterotoxin (CPE) gene may be found in some of *C. perfringens* isolates. This happen due to an outcome of sporulating cells in an alkaline media. It helps on lysis of these cells which found to be hard to proteolytic enzymes. It will stick and enter the brush edge membrane causing the formation of toxin

in these cells which eventually leads to the death of cells [38]. Reports have found an association between cpb2 β2-positive *C. perfringens* toxin and cpe β2-positive *C. perfringens* enterotoxin with colitis and enteritis in equines [38]. NetF is a novel poison of *C. perfringens* type A and has a relation with lethal foal necrotizing enterocolitis [38]. There was an association between the presence of netF with separate type A strains of the inflammatory conditions known as necrotizing enterocolitis in foals. Both netF and enterotoxins have been reported to play a role in inducing enteritis in foals [39]. *C. perfringens* colonies are characterized by a large double zone of hemolysis on blood agar [40].

Isolation and Identification of Salmonella

Salmonella is a gram-negative rod-shaped bacilli and a member of the Enterobacteriaceae family. It has 8 subspecies where the subspecies. It is responsible for 99% of mammalian diseases [41]. While numerous horses may harbor *Salmonella* within their bodies, they often do not excrete it and frequently remain asymptomatic. According to various studies conducted, less than 1% of horses in the country were found to be actively shedding salmonella on farms [41].

Incidence of *Salmonella* infection in neonatal foals is prevalent. *Salmonella* has virulence factors where these factors have the capability to stick to and conquer the intestinal mucosa producing entero- and cytotoxins and inducing local and systemic inflammatory reactions. *Salmonella* colonizes in the intestine triggering inflammation and huge mucosal damage of the colon and ileum [42]. There are a lot of methods used to improve diagnosis of *Salmonella* spp. in feces such as ELISA, and cultivation on a specific culture media. Molecular methods become more successful than conventional methods, especially in areas where conventional microbiological techniques are not practical. Furthermore, employing culture-enriched sequencing represents a valuable supplementary method for gaining deeper insights into the equine gastrointestinal microbiota [43]. The molecular methods, including conventional PCR and its modern versions, facilitate rapid detection of *Salmonella* spp. within a short timeframe, demonstrating their robustness and accuracy as detection technologies [44, 45]. The virulent gene (invA) is an appropriate PCR target gene for identification of *Salmonella* spp. and known as a global standard for the recognition of the genus of *Salmonella* [45]. For isolation of *Salmonella* spp., samples were injected into a specific broth Rappaport-Vassiliad (*Salmonella* Enrichment broth). The broth culture was plated in xylose-lysine-deoxycholate agar and then incubated. Suspected colonies of *Salmonella* spp. exhibit red colonies with a black center. These colonies are then inoculated in triple sugar iron and subsequently classified using biochemical tests [45].

Isolation and Identification of Escherichia coli

Escherichia coli (*E. coli*) is a gram-negative bacillus, rod-shaped, small in size, aerobic, motile, flagellated, non-spore-forming, oxidase negative, and toxin producer (endotoxin), and it is one of the family Enterobacteriaceae. Fecal samples are inoculated into nutrient broth (NB) as a primary culture of *E. coli*, followed by the culture into characteristic and differential media, eosin-methylene blue (EMB) agar and MacConkey agar. *E. coli* ferment lactose on MacConkey agar plate while on EMB agar it gives green metallic sheen colonies [46].

Foals are more susceptible to infection; especially neonates during first minutes to hours of life [47]. Neonates may suckle nearby objects, ground, or the mare's flank which may increase the chance to attract *E. coli* where it is widely spread in the environment. If the foal fails to nurse enough amount of the mare’s colostrum, it will develop bacteremia and then septicemia. Different strains of enterotoxigenic *E. coli* have been isolated from foals. *Escherichia coli* produce enterotoxins that affect epithelial cells to secrete electrolytes and water in excess, resulting in diarrhea [47].

The newly born foal has a single, passing inhabitant of cells in the intestine. These cells persist for the first 12-24 h of life and are specialized to engulf large colostrum proteins and transport them into the main circulation, in addition to engulf bacteria. If the foal fails to suckle enough mare's milk, it will attract bacteria and develop bacteremia and septicemia. When *E. coli* germs invade the intestinal mucosa of a foal, it can result in colibacillosis, which is an inflammation of the colon, enteritis, or both. In foals, colibacillosis is usually a secondary disease that usually occurs when the foal is being treated for other diseases, often with antibiotics [21].

Knottenbelt *et al.* [21] reported that there are three forms of *E. coli* diarrhea in foals: The first type is hypersecretory diarrhea caused by enterotoxins from *E. coli*. Enterotoxins cannot damage the intestinal mucosa but affect the secretion of electrolytes, water and bicarbonate into the lumen. The diarrhea is watery and profuse, but there is no leakage of protein. This phenomenon is not well known in foals. The second type is the most common, and this type is called "colibacillosis", where the bacteria invade the intestinal mucosa, leading to inflammation of the mucosa, which often leads to secondary septicemia. If the mucosal injury is very bad, blood and protein will leak into the intestinal lumen and can be seen in the feces. The third type is less common in foals. In this condition, *E. coli* bacteria damage the intestinal brush border, leading to poor absorption of fluids and nutrients. Protein levels in the stool are elevated.

Isolation and Identification of Rhodococcus equi

Rhodococcus equi is a facultative, gram-positive intracellular pathogen. It is the most common cause of pneumonia in foals [48]. *R. equi* is also a vital pathogen in newly born foals especially within the first 6 months of age. Animals infected with *R. equi* usually exhibit chronic and suppurative bronchopneumonia connected with the rise of the incidence of mortality, especially in foals not taken specific antibiotic treatment [22]. *Rhodococcus equi* is widely diffuse in horse farms and also widely spread all over the world. The pathogen is found on the surface layer of earth polluted with horse feces where in summer, dust particles covered with the bacteria are inspired by foals leading to pneumonia.

Infected foals suffer from mild fever, watery diarrhea, reduced appetite, dullness, and increased leukocyte count. In the 3rd week from the appearance of the disease, other symptoms may appear like swelling on fetlock joints and the hind hock joints, creamy and watery discharges from the eyes, conjunctivitis, and mild corneal opacity, and partial blindness. Bulging of eyeballs may also be noticed. Foals do not exhibit any symptoms of pain on palpation of the enlarged fetlock joints, but the walk is still abnormal, where these symptoms may be persistent in addition, appetite may still be normal. In 4th week of sickness, slight rise in rectal temperature was observed, however, the leukocyte count returned to normal value. After the first month from the occurrence of initial symptoms, watery diarrhea may appear again and the animal is completely off food [29].

Diagnosis of *R. equi* is very difficult, but becomes easier if pneumonia is present. Virulent *R. equi* strains are the only bacteria capable of expressing a 15-17 kDa virulence-associated protein (VapA) and have numerous 85-90 kb virulence-associated plasmids containing the equine pathogenic vapA gene [49]. VapA-positive strains are distributed among ranches with endemic diseases if compared with that periodic infection [50]. Alveolar macrophages engulf both virulent and avirulent strains of *R. equi* through phagocytosis. Nevertheless, it is only the VapA-positive bacteria that possess the ability to inhibit the fusion of phagosomes with lysosomes. This interruption of phagocyte cells ultimately contributes to the development of pneumonia [51]. Several studies have been found on virulent *R. equi* strains isolated from horses in different geographical areas of the world. To date, 12 plasmid types have been reported in VapA-positive strains of horses [52]. Each specific plasmid type is characteristic for special topographical area, which may add in the epidemiological studies.

Rhodococcus equi was isolated from the lung tissue after euthanasia [48], cultured on NANAT medium, Nutrient Agar (NA) and Sheep Blood Agar (SBA) and then incubated at 37°C up to 72 h. The plates were then examined for typical colonies after 48 hours and after 72 hours as 1-2 mm pink, irregular, confluent, mucinous colonies.

In terms of macroscopic findings [29], multiple large abscesses containing white, cheesy material were also found in both lungs. A single large abscess containing cheesy material was found, with adhesions to the small and large intestine. The small intestine and colon were moderately congested, while the cecum was severely congested. On other side, histopathological findings showed necrosis which is diffused in the alveolar parenchyma and bronchi. There was necrosis of the bronchial epithelium and bronchioles filled with inflammatory exudate mixed with erythrocytes. There were focal areas of massive necrosis where there were intact and dead neutrophils marked by fibrous tissue proliferation. Diffuse infiltration of macrophages was found in the lung tissue. The lamina propria and the submucosa of the intestine, colon and cecum were necrotic. The blood vessels of the intestine were sparsely congested, and there was widespread infiltration of lymphocytes, neutrophils and macrophages along with plasma cells in the lamina propria and the formation of lymphocytic aggregates in the submucosal areas and goblet cell hyperplasia.

Isolation and Identification of Lawsonia intracellularis

Lawsonia intracellularis is a gram-negative bacterium. It is an obligatory intracellular bacterium which is affecting mostly foals aged from 4 months to 7 months old causing proliferative enteropathy [53]. *Lawsonia intracellularis* invades rapidly dividing epithelial cells, allowing the bacteria to grow continuously and move the infected cells to colonize large areas of the intestinal epithelium, resulting in expansion and elongation of the epithelial cells. The small intestine mucosa becomes thick, irregular, and corrugated. The normal villus structure is substituted by strongly branching glandular epithelium with a poorly advanced microvillus brush border [53]. Animals infected with equine proliferative enteropathy (EPE) show lethargic, peripheral edema, stiffness, diarrhea, swelling of the forelimb distal part, and also swelling in the submandibular area, and loss a lot of weight. Necropsies exhibit the main lesions. There are many abnormalities in hematological and biochemical values such as anemia, leukocytosis, hypoproteinemia, fibrinogenemia, and electrolyte and acid-base abnormalities. On ultrasound examination, thickening of the wall of the small intestine and sometimes distension of the small intestine with fluid or gas can be seen [54].

Diagnosis of the disease is difficult and relies mostly on postmortem examination with Warthin-

Steiner silver stain, where curved bacilli can be seen at the apex of the cytoplasm. Immunohistochemistry using monoclonal antibodies against *L. intracellularis* has been interpreted as more sensitive than other staining methods [55]. *Lawsonia intracellularis* DNA sequences can be detected in frozen, formalin-fixed, or paraffin-embedded intestine specimens by PCR analysis [56]. Molecular and immunohistochemical techniques have been effectively used to differentiate *L. intracellularis* DNA from clinical cases of equine proliferative enteropathy [54].

Serology was investigated using immunoperoxidase monolayer assay (IPMA) to detect the presence of antibodies to *L. intracellularis* and proved to be reliable pointer for active or new infections [54].

Other diagnostic methods for *L. intracellularis* as a culture of the organism are impractical because the growth of the organism needed cell cultures and lowering oxygen atmosphere. PCR is considered a sensitive and selective method, detecting as less as 10^3 bacteria/g in stools; however it was difficult to find a detectable and constant titer of bacteria in animals [57]. In contrast, immunoperoxidase (IPX) staining showed remarkable sensitivity in detecting *L. intracellularis* in stool, and the lower sensitivity of PCR may be due to the presence of fecal inhibitors [55]. Serology may play a beneficial role in detection of IgG produced by *L. intracellularis*. Meanwhile, the serum samples collected before an outbreak, failed to detect antibodies against *L. intracellularis* [55].

Pathological findings (macroscopic/Post-mortem): The small intestinal serosa was hypercongested (Fig. 1a). The bowel wall thickening was associated with marked wrinkling and thickening of the mucosal folds and a reduced intestinal lumen (Fig. 1b). The mucosa was strongly undulated, characterizing the thickening of the intestinal folds (Fig. 1C) [58]**.**

Microscopic findings: Hyperplasia of immature intestinal cells and absence of goblet cells are noted.

Immature epithelial cell proliferation is most commonly characterized by the presence of organisms in the lumen of the small intestine (the terminal portion of the small intestine), large intestine, or both. Affected tissue sections are characterized by a reddish, thick, “garden hose”-like mucosa. The thickening of the intestine prevents normal gut function. In recent studies using fecal PCR in healthy foals with diarrhea, it was not detected in foals younger than four months of age [59]**.**

Histological sections of the small and large intestine showed enterocyte hyperplasia of the crypts. Crypts were rarely dilated and their lumen was filled with cell debris and neutrophils (crypt abscess). In addition, crypts were present in some areas of the submucosa. Immunohistochemical staining showed antigen labelling at the cytoplasmic apex of enterocytes and in macrophages in the lamina propria of the large intestine, duodenum and ileum, and at the cytoplasmic apex of enterocytes [29]. Abdominal ultrasound is very useful in the diagnosis of *L. intracellularis*. The hyperplasia often results in thickening of the small intestine and/or colon.

Nonconventional Bacterial Cause of Diarrhea in Foals

Other nonconventional bacteria that cause diarrhea in foals such as *Enterobacter aerogens, Proteus mirabilis, Proteus vulgaris, Enterobacter agglomerans,* and *Citrobacter* spp. were also identified [10].

Molecular Diagnosis

Polymerase chain reaction (PCR)

PCR is a highly sensitive and specific diagnostic technique compared to conventional bacterial culture methods. Bacteria isolates is confirmed by PCR after purification of deoxyribonucleic acid (DNA). Detection and amplification of DNA were used in *C. difficile* and *C. perfringens*. Also, *Salmonella*; targeting PCR expansion of invA gene is used for its direct identification from fecal samples [60]**,** *E. coli* (16s rRNA gene) [61] and *R. equi* (gene coding the 16S subunit of rRNA and vapA gene of the virulence plasmid) [62].

Genotyping of *C. perfringens*, and *C. difficile* isolates Using PCR: where fecal samples were exposed to detection of different toxins encoding genes such as α, β, β2 toxin consensus, enterotoxin, ε toxin and NetF genes [26].

Abdominal Ultrasonography

Ultrasonography is a rapid and non-invasive method that helps in the assessment of the various organs, wall thickness, intestinal fillings, and grade of visceral swelling. It can also be used to detect the sites for aggregation of abdominal fluid [63].

Control of Bacterial Diseases Causing Diarrhea in Foals

Treatment

Treatment of various cases of diarrhea was similar. Supportive care is playing an important role and is considered a large part of a successful outcome. It is essential to treat affected foals quickly before lesions become advanced. The treatment depends on four main steps (Table 1): fluid therapy, oral and/or intravenous nutrition, intestinal protectants and adsorbents and fundamentally broad-spectrum antimicrobials for the primary disease and bacteraemia [64].

Fluid Therapy

While foals with mild cases of diarrhea may be kept on oral or suckling fluids, badly affected foals should get supportive treatment to solve hypovolemia. Colloids, crystalloids, or combinations of both of them were given. Lactated Ringer solution can replace the crystalloid fluids for foals [47]. Hypertonic solutions are usually not given to the foals because they cannot tolerate a great amount of sodium. Synthetic colloids should be given as 3 ml/kg to 5 ml/kg boluses if needed [47]. Disturbance in the electrolytes should be corrected, this is very important for helping foals cure. This is also helping in balancing the acid-base condition of foals. To correct the decrease in the blood volume (hypovolemia) in neonatal foals, this is performed by giving intravenously (IV) fluid boluses (10 to 20 mL/kg) slowly (Over 20 to 30 min). Re-evaluation of the hydration should be done through laboratory data, symptoms, and urine production after giving each bolus. To keep the fluid rates for the young foals less than 60 days old, they need to be given nearly 80 to 120 ml/ kg/ day. This rate is lowered to 60 ml/kg/ day for foals older than 60 days [47]. Hyponatremia can be partially corrected by raising the plasma sodium concentration from 2 mEq/L to 4 mEq/L. Hypokalemia can be treated by giving fluids containing 10 to 40 mEq/L of potassium chloride, 20 mL/kg of physiological saline (0.9%) containing calcium, and 4 to 8 mEq/kg/min of dextrose [47]. The foals with very bad acidosis should be administered IV sodium bicarbonate therapy (1.3%) which improves symptoms of acidosis. Glucose is given at an initial dose of 4 mg/kg/min. This dose can be increased to 6 mg/kg/min and 8 mg/kg/min if necessary. Glucose should be given alone, apart from fluid therapy. This help in adjusting fluid and glucose [47].

Anti-inflammatory and anti-endotoxic treatment

Nonsteroidal anti-inflammatory drugs (NSAIDs) should be given with caution to foals less than one month of age because of their adverse effects on the gastrointestinal and renal systems [23]. The endotoxemia was treated by administering IV Polymyxin B at a dose of 6000 U/ kg. Foals with failure of passive transfer of immunity should be treated with the transfer of the plasma immunoglobulins [65].

Adsorptive Agents

Agents that play a role in adsorption such as kaolin/ pectin and bismuth subsalicylate should be given at a dose of 0.5 to 4 ml/kg, 1 to 4 times daily. Di-tri-octahedral smectites adsorbent can bind with the endotoxin, affects its absorption and neutralize the toxins of *C. difficile* and *C. perfringens* [23].

Nutritional Support

Foals without signs of colic are free to endure suckling, while in foals with abdominal disturbance it will be favourable to take GI rest for 12 – 24 h [25 23]. Fluid therapy should be given if a foal under 4 weeks of age is not nursing for more than 6 hours, and the foal should be reintroduced slowly and gradually. Foals could take 10% of body weight twice per day with 1 or 2 hrs intervals by a nasogastric tube.

Antibiotic Therapy

Generally, the antibiotic sensitivity test should be made before drug choice [10]. Metronidazole (15-25 mg/kg body weight every 8 h) is used to treat diarrhea caused by *C. difficile* bacteria. Vancomycin is used to treat severe cases of diarrhea associated with *C. difficile*. Bacitracin was used frequently in the past but is no longer used. The antibiotic used in the treatment of foals infected with *Salmonella* spp. is usually ampicillin or cephalosporin alone or with an aminoglycoside (amikacin or gentamicin) or fluoroquinolones. The treatment must persist after clinical recovery to prevent secondary infection [20]. Antimicrobials recommended for treating of *L. intracellularis* include oxytetracycline or chloramphenicol [53]. Treatment of *Rhodococcus colitis* is similar to that of *R. equi* lung infection using macrolide antimicrobials [48] (Table 1).

Antibiotic sensitivity test (Antibiograms)

The measurement of bacteria's susceptibility to antibiotics is known as the antibiotic sensitivity test. This test is conducted because bacteria can develop resistance to certain antibiotics. The results of the sensitivity test allow clinicians to change their choice of antibiotics from empirical therapy, where antibiotics are selected based on clinical suspicion and common bacteria causing the infection, to directed therapy [66].

Directed therapy involves selecting antibiotics based on knowledge of the specific bacteria and its sensitivities. The sensitivity test is usually performed in a clinical laboratory using culture methods, which expose bacteria to antibiotics. These methods often involve measuring the diameter of areas of no bacterial growth, called inhibition zones, around paper discs containing antibiotics on agar culture plates that have been evenly inoculated with bacteria [66].

The size of the inhibition zone can provide an estimate of the minimum inhibitory concentration, which is the lowest concentration of antibiotic that stops bacterial growth. Once isolated colonies of the pathogen are available, the bacterial sample is prepared and standardized prior to performing antibiotic susceptibility testing via disk diffusion. The antibiotic sensitivity test is widely used in clinical settings to determine antibiotic resistance profiles, guide treatment decisions, and predict therapeutic outcomes [67].

However, the current process requires transportation of patient samples to the clinical microbiology lab, and obtaining a pure culture of the pathogen which can take several days, leading to delays in diagnosis and treatment. These delays can lead to increased patient mortality, poor clinical outcomes, and broad-spectrum antibiotic use, contributing to antibiotic resistance. To address this challenge, it is important to develop technologies that can rapidly perform the antibiotic sensitivity test, enabling personalized therapies with narrow-spectrum antibiotics at the earliest stage of treatment [67].

Probiotics

Probiotics are defined as live, non-pathogenic, non-toxic microorganisms that have the potential to exert a beneficial effect on animal hosts at an appropriate dose. Probiotics are used in animal nutrition to enhance animal health, production and immune system [68]. Probiotics work by enhancing the epithelial barrier, adhering to pathogens, and enhancing the immune system by secreting bacitracin [69]. Probiotics are commonly used to treat colitis in horses. *Saccharomyces boulardii* decreased the severity and duration of diarrhea [19]. Probiotics should not be recommended for use in neonatal foals less than 24 hours of age due to the potential for reduced absorption of colostrum immunoglobulins. *Saccharomyces boulardii* and *S. cerevisiae* are commercially available yeasts in purified freeze-dried capsules or granules and can be found in commercial horse feeds and nutritional supplements for humans and horses. Horses with watery diarrhea are administered 20 g orally four times daily.

Some reports suggest that giving the probiotic Lactobacillus to foals has been effective in promoting their growth and may be effective in preventing diarrhea [70].

Prevention

Preventing infectious diarrhea in foals should depend on 3 main steps of prophylaxis: limit contact to microbes' pathogens (disinfection and isolation), raise immunity (vaccination), and the most the effective way is management practices [26, 71] where good management practices are the key to prevention. Prevention occurs according to Ramey, 2024 [72] as follow:

Prevent the foal from getting the infection

The mare should be cleaned twice, the first one before the baby comes and the second after foaling by washing the birth canal and her backside carefully to eliminate the manure, which has a lot of bacteria. Preventing infections in foals required a lot of washing the foal with water and soap. Clean carefully the mare’s udder to eliminate bacteria. It is almost effortless to clean the udder when it is full than if there are numerous cracks and fissures found [72].

Pay Close Consideration Regarding the Umbilical Cord

The umbilical rope must be permitted to dismiss spontaneously all alone. Once the umbilical cord is separated, it is a good idea to disinfect the cord remnant with an antiseptic solution. The better disinfectant to plunge is a half-percent solution of chlorhexidine, ordinary iodine, which has been generally utilized for quite a long time. However, it is excessively caustic material and hard to get. On the contrary, povidone-iodine is too effective. Chlorhexidine is usually obtained as a 2 or 4 percent solution; therefore, a 1/2 percent solution is readily available. On the first day, the umbilicus ought to be dipped regularly every six hours, multiple times (four times) in the first 24 hours [72].

Strengthen the Foal’s Immunity

The mare's first milk, colostrum, is a temporary immune defense shield for the newborn foal. It is essential to drink as much colostrum as soon as possible when you can. The foal just has a brief time (up to 24 hours) before its intestinal tract can ingest immunoglobulin from the mare’s colostrum. It is not useful to drain the mare's milk and feed the foal 4 to 8 ounces of colostrum from a container (bottle), even before the foal can stand. It is important that the foal does not extend its head to drink while the colostrum is being given to the foal at first. Doing that may permit the colostrum to go to the lungs and lead to pneumonia [72].

Ensure Vigor and Vitality

Foals should act healthy. They ought to be perched on their chests with the two feet forward in sternum position this is significant. The sternum position of the two feet supports opening the foal's chest, to allow the most amount of oxygenation. Within the first 5 min. from birth, the rate of the foal respiration should be greater than 60 breaths/ min. The foal baby should be standing during the interval from one to two hours after birth. The baby should suckle within 2 to 3 hours after birth [72].

Vaccination

Vaccinating animals is a crucial method to protect them from various infectious bacterial diseases. To safeguard a foal from such diseases, preventive measures should be taken even before its birth. It is advisable to move the mares to the farm where they will give birth about 4 to 6 weeks before their due date. This timeframe allows the mare sufficient time to develop an immune response against the pathogens present on that particular farm. The objective of vaccinating mares before parturition is to stimulate their immune response, leading to the production of antibodies that will eventually be concentrated in the colostrum. These antibodies are then passively transferred to the newborn foal. It is crucial for the foal to consume colostrum from

vaccinated mares within the first 12 to 24 hours of life, as this provides a high level of maternal antibodies that protect the foal from infectious diseases during the first months until it can develop its own antibodies. In most cases, maternal antibodies persist in the foal for 3 to 4 months, but in some cases, they may persist for up to 6 months or more. Other methods used to ensure passive transfer of antibodies to the foal include administration of frozen-thawed colostrum or a commercial colostrum substitute containing concentrated equine antibodies, as well as intravenous administration of plasma from highly immune donor equine. As a general guideline, foals from unvaccinated mares should not receive their first vaccination until they are 3 to 4 months of age, while foals from vaccinated mares should receive their first vaccination at approximately 6 months of age [22, 28].

Vaccination of mares against Clostridium spp.

The toxins produced by *Clostridium difficile* bacteria are proteins that can be converted to non-toxic toxins by treating them with formaldehyde. The term toxoid indicates to the toxin treated with formaldehyde. Donor horses are initially injected with toxoids, but once antibodies are produced, subsequent boosters may contain pure toxins. The horses' responses are monitored, and once their antibody levels are high enough, they are bled. The bled is done at intervals until the antibody levels fall when the animals are boosted again with the antigen. The plasma is separated from the horse's blood, which contains the globulin portion. Immunization of mares with the binding domains for Clostridium difficile toxins A and B elicits antibodies in the serum and milk that block toxin binding [73].

Vaccination of pregnant mare should be done with *C. perfringens* C and D toxoids before foaling. There is some evidence that immune protection of the newborn foal can be gained by vaccinating the mare at 6 weeks and again at 3 weeks before foaling with a *Clostridium* toxoid vaccine. This will stimulate the formation of antibodies to *Clostridium* toxin which will be transferred to the foal [74]. Foals younger than 6 hours are highly recommended to take *C. perfringens* types C and D antitoxin on farms with a history of *C. perfringens* diarrhea besides vaccination of pregnant mares with toxoid of *C. perfringens* C and D [74].

Vaccination of mares against Salmonella spp.

Salmonellosis is an important zoonotic disease [105 74], there are 2500 serovars or more of the genus *Salmonella* have been recognized which found its contribution to huge worldwide losses in both human and animals as a result of diarrhea [75]. Killed vaccines are strain specific and produce only short-term immunity and attenuated vaccines may become infectious in immunocompromised horses. A variety of vaccines including killed or live have been used to control *Salmonella* (Table 2) [76].

Foal Isolation

In the case of a neonatal foal that stops nursing, it begins to be depressed, or has bloody or profuse diarrhea, a veterinarian should examine it as soon as possible and foals should be isolated from healthy animals. In addition, the foal's buttock ought to be kept clean and a water repellent ointment used to protect from scalding. The detection process of a sick foal required that all animals in contact with the foal must remain in the same places due to the possibility they might be already infected or the possibility they become a source of infection to others. The foals may shed infectious agents in their feces after the appearance of clinical signs. Therefore handling them should be with much care. The isolation time of foal from others depends on the causative organism. Testing whether the foal infection status permits transfer to others should be done and this test can be done during isolation time [77].

Precautions of Biosecurity

Biosecurity precautions are essential; care must be taken during put on boots and overalls. Hands must be washed from time to time with a suitable disinfectant cleaner. Separate tools must be used to feed and stable care. Foot dips should be put in place near the foal's stable. The disinfectant depends on the causative organism [78].

Cleaning and Disinfection

Cleaning of foal stall must start with the entire removal of all fecal material and bedding. After doing that, in the case of the solid floor and walls, it must be scrubbed with water and detergent and finally rinsed. After an overall cleaning, the floor and walls ought to spray with appropriate disinfectants for the suspected microbe. Hypochlorite or bleach with a suggested dilution of usual house bleach (1:10) has a broad scope that can be used for the germicidal activity. In order to avoid the inactivation of bleach, organic matter should be used. Bleach today becomes the existing sporicidal disinfectant, and its utilization ought to be after removing organic matter. In the case of outbreaks of clostridia colitis, phenolic disinfectants may be used due to their wide germicidal effect in opposition to bacteria, viruses, and fungi. Phenolic compounds are active in the presence of organic matter but are not sporicidal. Today one of the more recent disinfectants is peroxygen compounds which are powerful against an expansive scope of organisms and organic matter cannot inactivate them and also effective against clostridia spores [6].

Conclusion

Enormous variety of enteric bacterial pathogens causing diarrhea in foals were discussed in this

review article. *Salmonella* spp., *Clostridium perfringens*, *Clostridium difficile* were the most predominant pathogens causing mortality in the diarrheic foals. Hematological and biochemical assays in foals represent good indicators when they are tested through laboratory assays for both the diagnosis and treatment of diarrhea. Detecting of various serotypes of *Salmonella* toxigenic, *Clostridium perfringens*, virulent *R. equi* (VapA-positive), and *Clostridium difficile* strains that may exist in feces of diarrheic foals shows that the health of human is at risk. In addition, it refers that the worker must be careful when dealing with foals that have fecal material infected with these organisms which were identified as human pathogens and can be easily transported to the human through fecal contamination of food and water. The management process and medical treatment of foals with diarrhea are based on the severity of diarrhea on foals and the associated clinical signs. Management of foals that have mild diarrhea is done through normal physical inspection and suitable hygienic measurements. Good utilization of bismuth subsalicylate, di-tri-octahedral smectite, kaolin/ pectin, sucralfate, and *S. boulardii* might be valuable in such cases. Foals that might have mild diarrhea and don't need prolonged treatment, diagnostic examining for infectious pathogens might be useful to control outbreaks of infectious diarrhea. For foals that have diarrhea, hypovolemia, and clinical signs of sepsis, continuous treatment, with nonstop checking and giving a lot of fluid therapy regularly is fundamental. This fluid therapy not only compensates for the fluids lost, but also overcomes imbalances in electrolytes such as chloride, sodium, and potassium. Also, glucose is recommended as IV within fluid solutions. If the foals face severe electrolyte disturbance and are not corrected, the rest of the organs may be affected adversely.

Recommendations

Increase awareness of the importance of the foal diarrhea problem to avoid and overcome its harmful effect on animal wealth. Giving foal high-quality colostrum which is essential to the health of foals because the colostrum includes antibodies that invade the bacteria and viruses within the first 30 days of a foal life. A veterinarian has to test the blood of a foal at 12-18 h after born to know if the quantity of the colostrum given to the foal is enough or not.

The mare should be vaccinated to produce antibodies against bacteria and viruses. These antibodies are considered the first line of defense for the foal to face harmful germs.

The mare's udder should be examined two times daily. If a mare has a full udder, it means the foal is not nursing adequately, and nutrition or fluids are not enough, or both and most probably the first signal of sickness. It must give attention to inspecting the foal, the inspection is done by taking temperature, pulse, respiration rate, and then noticing that if the tail of the foal is soiled by feces, it means the presence of diarrhea.

It is essential to inspect the foals that have diarrhea within less than one month carefully by a veterinarian because diarrhea threatens their life and maybe lead to death due to dehydration.

In the case of severe diarrhea, foals should be given intravenous (IV) fluid therapy. In addition, in order to soothe and coat the gastrointestinal tract, intestinal protectants are usually used.

Good management practices are the key to preventing foal diarrhea.

Owners must not give foals antibiotics when they detect that foal has diarrhea because the use of antibiotics randomly may complicate the treatment of foal by getting rid of "good" bacteria that exist in the foal's intestine.

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Declaration of Conflict of Interest

The authors declare that there is no conflict of interest.

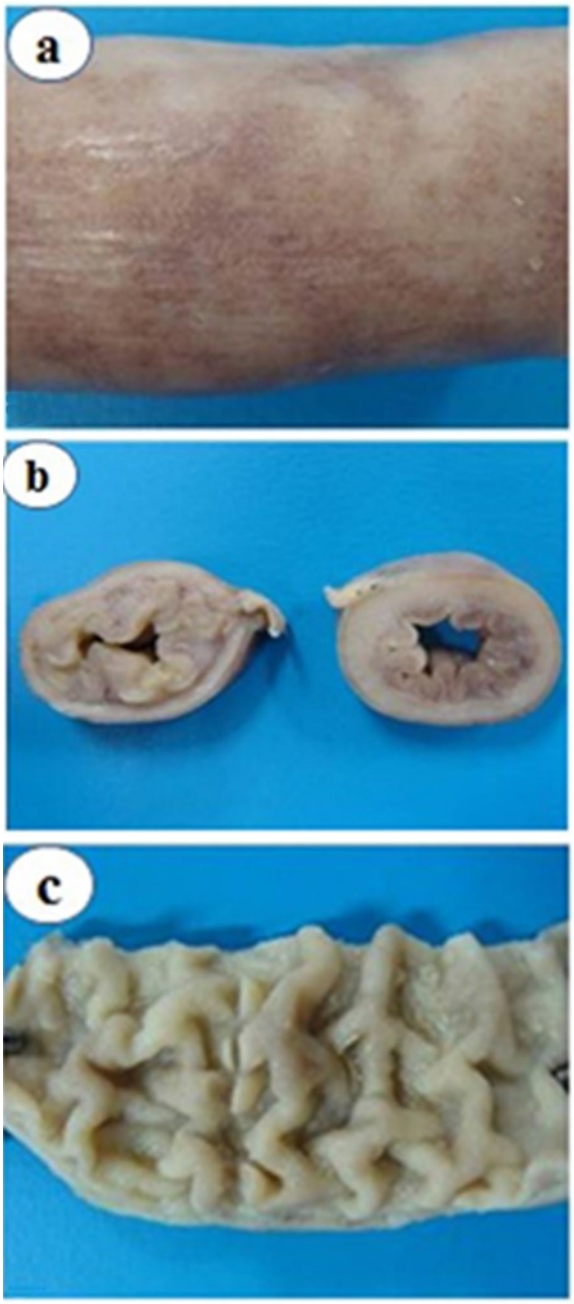

Fig. 1. Macroscopic findings of the intestine of a 7-month-old foal with *Lawsonia intracellularis* diagnosed with equine proliferative enteropathy (EPE) showing (a) serosal with multifocal to consolidative congestion, (b) cross-section of a portion of the small bowel segment with a thickened wall and a decrease in the intestinal lumen due to mucosal thickening, and (c) longitudinal section of a portion of the small bowel segment showing thickening of the intestinal folds [58].

TABLE 1. Treatments of foals with diarrhea [64]

Intravenous fluids	Gastrointestinal protectants/adsorbents	Antimicrobials	Other
Crystalloids	Di-tri-octahedral smectite	Penicillin	Lactaid
Hypertonic saline	Bismuth subsalicylate	Amikacin	Butorphanol
Colloids	Activated charcoal	Cefquinome	Flunixin*
Plasma	Kaolin	Ceftiofur	Ketoprofen*
Glucose	Sucralfate	Marbofloxacin	Glutamine
TPN	Omeprazole	Metronidazole	
Electrolytes	Ranitidine	Paramomycin	
Inotropes	Saccharomyces boulardii		

*Non-steroidal anti-inflammatory drugs should be avoided in foals

TABLE 2. Advantages and disadvantages of Live and Inactivated Vaccines [76].

Criteria	Live Vaccine	Inactivated Vaccine
Oral dosing	Good immunity	No or poor immunity
Duration of immunity	Long	Short
Requirement of adjuvant	No	Yes
Cross protection from related strains	Present	Rare
Safety on inoculation	Varies	Often safer
Potential contamination	Possible	Remote chance
Stability and maintenance	Poor and difficult	Good and easy
Secretary IgA and local mucosal immunity	Good	No
Reversion of vaccine strain to pathogenic	Possible	No
Persistence in the vaccine	Yes	No
Interference from normal flora of vaccine	Possible	No
Cost of the vaccine	Less	More

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الإسهال فى الأمهار: الكشف عن دور العدوى البكتيرية واستراتيجيات التشخيص والسيطرة

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الملخص

الإسهال مشكلة كبيرة في الأمهار حيث يسبب الجفاف وخسائر اقتصادية كبيرة. من أهم المسببات البكتيرية للاسهال الأكثر شيوعًا فى الأمهارهى *سالمونيلا* و*كلوستريديم بيرفرينجينز* نمط (أيه) و نمط (سي) *وكلوستريديم ديفيسيل وإشيريشيا كولاى* بالإضافة إلى البكتيريا الأقل شيوعًا مثل *رودوكوكس إكواى* و *لاوزونيا انتراسيليولاريز*. يحدث الإسهال عادة خلال الأشهر الستة الأولى من حياة المهر ويمكن أن يظهر في حالات متفرقة أو تفشيات. في حين أن *كلوستريديم بيرفرينجينز وكلوستريديم ديفيسيل* جزء من البكتيريا المعوية الطبيعية للأمهار إلا أنها يمكن أن تؤدي إلى إسهال شديد تحت ظروف معينه. التشخيص الدقيق لمسببات الأمراض المسببة أمر بالغ الأهمية لاختيار العلاج المناسب ومنع تفشي المرض. و يشمل التعامل مع مشكلة الإسهال علاج نقص السوائل و الأملاح في الجسم مع مراعاة عوامل مثل العمر والحالة الفسيولوجية. و علاج الأعراض قد تكون كافية للسيطرة على مشكلة الإسهال ولكن علاج مسببات الإسهال أيضا ضرورية. يعد تنفيذ تدابير صحية صارمة أمرًا ضروريًا للوقاية من المرض. إن طرق التشخيص التقليدية كزراعة البكتيريا و الفحص المجهرى لها قيود لأن إجراءاتها تتطلب عمالة مكثفة و ذات حساسية منخفضة و تحتاج إلى مختبرات متخصصة وأخصائيين مدربين. ومع ذلك، مهدت التطورات في التشخيص الجزيئي والمجموعات التشخيصية المتاحة تجاريًا الطريق لاكتشاف المستضدات والتقنيات القائمة على البيولوجيا الجيئية لتحل محل الطرق التقليدية. يهدف هذا البحث المرجعى إلى تقديم فحص شامل للأمراض البكتيرية المسببة للإسهال لدى الأمهار، مع التركيز على تشخيصها والسيطرة عليها. كما تؤكد على مفهوم "الصحة الواحدة"، مما يؤكد على الترابط بين صحة الإنسان والحيوان والبيئة في معالجة التحديات التي يفرضها الإسهال البكتيري لدى الأمهار.

الكلمات الدالة: الأمهار ، الاسهال ، الأمراض البكتيرية ، التشخيص ، السيطره ، الصحة الواحده.