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Review

A review on major bacterial causes of calf diarrhea and its diagnostic method

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Calf diarrheic diseases result from complex interactions of the environment. Infectious agents and the calf itself are the major constraints for raising replacement stock. Calf diarrhea is a multi factorial disease entity that can have serious financial and animal welfare implications in both dairy and beef sucker herds and is one of the most common diseases reported in calves up to 3 months old. Among the bacterial causes of diarrhea in neonatal food animals, *Escherichia coli* and *Salmonella* species are the most common and economically important ones. *Clostridium perfringens* and *Campylobacter* species have also been identified as causes of enteric diseases in calf diarrhea other, Non-infectious factors, such as insufficient uptake of colostrum, poor sanitation, stress, overcrowding in the calf pens and cold weather, could cause neonatal calf diarrhea. The most prominent virulence factors identified in bacterial diarrhea are expression of fimbrial (pili) antigens that enables the bacteria to adhere and to colonize the luminal surface of the small bowel and elaboration of one or more enterotoxins that influence intestinal secretion of fluids. Various laboratory methods have been applied for the detection of infectious agents of calf diarrhea in fecal sample such as, bacterial culture, electron microscopy, molecular based techniques (PCR, DNA microarray) and serological techniques (enzyme-linked immunosorbent assay, latex agglutination test). Accurate and rapid early confirmation of the etiology in the disease outbreak as well as improving the various management factors are advised, for effective control and prevention of enteric disease in newborn calves. Treatment with rehydration solutions and provision of dry and warm conditions are vital in the treatment of calf diarrhoea.

Key Words: Bacteria, calf diarrhoea, *Escherichia coli, Salmonella* species, risk factor.

INTRODUCTION

The future of any dairy production depends, among other things, on the successful raising of calves and heifers for replacement. Under modern dairy production in the developed world, the average length of time a cow stays in a milking herd is about four years and, therefore, 25% of the milking herd must be replaced each year (Bath et al., 2012).

Generally, calf diarrhea result from complex interaction of the environment, infectious agents and the calf itself are the major constraints for raising replacement stock. The impacts of calf diseases could be direct and indirect through increased treatment expenses, decreased lifetime productivity and survivorship (Waltner-Toews et al., 1986a; Randhawa et al., 2012).

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Calf diarrhea is a multifactorial disease entity that can have serious financial and animal welfare implications in both dairy and beef sucker herds. It has been estimated that 75% of early calf mortality in dairy herds is caused by acute diarrhea in the pre-weaning period and also, a commonly reported disease in young animal and still a major cause of productivity and economic loss to cattle producers and also a cause of high morbidity and mortality in the cattle industry worldwide (Uhde et al., 2008; Bartels et al., 2010). Diarrhea is one of the most common diseases reported in calves up to three months old (Svensson et al., 2003). However, calf diarrhea was perceived as a minor problem by dairy producers, while the beef producers did not consider it a problem at all (Roderick and Hovi, 1999).

Various infectious agents such as viruses, bacteria, and protozoa are involved in calf diarrhea (Smith, 2009). Among these agents that have been implicated in calf diarrhea, bovine corona virus (BCoV), bovine rotavirus (BRV) group A, and bovine viral diarrhea virus (BVDV) act as viral agents, *Salmonella* species, *E. coli* K99+ and *Clostridium* species act as bacterial agents and *Cryptosporidium* spp. as a protozoan agent (Bhat et al., 2012; Bhat et al., 2013; Singla et al., 2013). *Salmonella* species and *E. coli* K99+ are known as the most common pathogens identified in scouring calves less than two months of age (Acha et al., 2004).

According to Cho (2012), 80% of diarrheic calves tested were positive for at least one of the target enteric pathogens, suggesting that the infectious factor is still a major cause of calf diarrhea. More than 50% of the diarrheic calves tested were concurrently infected with more than one pathogen. Co-infection with two pathogens were the most common finding (31%) with up to six pathogens detected in 1% of the fecal samples from diarrheic calves. The majority of diarrheic cases were identified among 0 to 4 week old calves and concentrated among calves at 0 to 2 weeks of age. High frequency of co-infection by multiple pathogens in young animals emphasizes that interventions for calf diarrhea should be focused on husbandry and management strategies, including assurance of colostrum intake, hygiene, reduction of population density, or modified components of the sand hills calving system (Larson and Tyler, 2005). Many of these enteropathogens cause severe intestinal lesions, alterations in enzyme activity, and alterations in nutrient transport mechanisms, or a combination of these effects. Infectious diarrhea of neonatal animals is one of the most common and economically devastating conditions encountered in the animal agriculture industry (Wudu, 2008).

Non-infectious factors, such as insufficient uptake of colostrum, poor sanitation, stress and cold weather could cause neonatal calf diarrhea. Due to its poor immune capability, a newborn calf is vulnerable to infection. The main risk factors increasing the exposure to infection and further lowering the defense mechanism within the calf in early life are: poor hygiene and overcrowding in the calving facility, high relative humidity, low temperature of the incoming air, contamination of the incoming air inadequate ventilation, close proximity to adult cows, mixing of different age groups and poor stockman ship or motivation of the herdsperson responsible for the calves (Lance et al., 1992).

Various laboratory methods have been applied for the detection of infectious agents in feces. Historically, virus isolation, electron microscopy, enzyme-linked immunosorbent assay, latex agglutination test, bacterial culture, direct microscopy of fecal smear and/or fecal flotation have been commonly used to test fecal samples for enteric pathogens (Fotedar, 200; Meir et al., 2010).

Acute infectious diarrhea encountered in a herd is often difficult to manage because of the large number of potential enteropathogens involved, differences in individual animal immunity within the herd, population dynamics, environmental stresses, nutritional status–and difficulty in establishing an etiologic diagnosis (Waltner-Toews et al., 1986b). The etiologic diagnosis is not determined for a large percentage of cases of neonatal diarrheas. However, from infectious bacterial enteropathogens, *E. coli, S. species* and *Clostridium* species are the important bacterial causes of calf diarrhea which can be diagnosed and confirmed using the available laboratory techniques. Accurate and rapid confirmation of the etiology early in the disease outbreak can aid in quick implementation of appropriate interventions or prevention measures in the herd to decrease economic losses (McGuirk, 2008; Meir et al., 2010). Therefore, the objectives of this review is to highlight the major bacterial causes of calf diarrhea and its diagnostic method in dairy calves, and assess the zoonotic importance of bacteria involved in calf diarrhea.

GENERAL DESCRIPTION OF BACTRIA INVOLVED IN CALF DIARRHEA

Farm animals are born into environments with many potential enteropathogens and are initially exposed to resident micro flora in the vagina of the dam and subsequently to microbes harbored by herd mates. Some microorganisms are potentially harmful, while others are necessary for normal development and function of the gastrointestinal tract. However, once exposed, the intestinal tract is susceptible to infection with potential enteropathogens, and in the absence of protective antibodies, various enteropathogens can become established and cause enteric disease.

Most cases of calf diarrhea are likely to be mixed infections, where more than one of the pathogenic agents is present. The major bacterial infectious agents that have been implicated in calf diarrhea are *S.* species, *E. coli* K99+, and *Clostridium* spp. mixed infections with rotavirus and cryptosporidium appear to be the most

common. Many cases of scour proceed very rapidly, causing severe dehydration and metabolic imbalance within a few hours of the onset of disease. *E. coli* (K99 and F41) can cause very severe scour and dehydration in calves of less than one week old. Diarrhea caused by *E. coli* can occur as early as 24 h after birth, but seldom occurs after three days of age unless it occurs as part of a mixed infection with rotavirus and cryptosporidium species. *E. coli* can also invade the bloodstream and cause colisepticaemia (Quinn et al., 1994).

Experimental work also suggests that infection with one agent makes the calf more vulnerable to other pathogens. Many surveys indicate that rotavirus is the most common single cause of calf diarrhea in all types of cattle herds, but particularly in dairy and single sucker herds. Cryptosporidium is the second most common pathogen causing calf diarrhea, and slightly more common in beef sucker units than in dairy herds. Coronavirus is the third most common diarrhea agent. Salmonella is isolated often from disease outbreaks, particularly *Salmonella Dublin* in calf rearing units where calves are bought in from multiple sources (Hall et al., 1988; Fagan et al., 1995).

The genus *Escherichia*

Escherichia coli commonly abbreviated as *E. coli*; is a gram negative rod-shaped motile or nonmotile, facultative anaerobic, non-spore forming member of the Enterobacteriaceae family found in the gastrointestinal tract of warm-blooded animals and humans (Frydendahl, 2002). *E. coli* was discovered by German pediatrician and bacteriologist Theodor Escherich in 1885 (Feng et al., 2002) and is now classified as part of the enterobacteriaceae family of gamma-proteobacteria. The harmless strains are part of the normal flora of the gut, and can benefit their hosts by producing vitamin K2 (Bentley and Meganathan, 1982) and by preventing the establishment of pathogenic bacteria within the intestine (Hudault et al., 2001; Reid et al., 2001). New strains of *E. coli* evolve through the natural biological process of mutation and through horizontal gene transfer (Lawrence and Ochman, 1998). *E. coli* is a facultative habitant of the gastrointestinal tract, and also found in the environment. However, the infection is present due to break of the protection barrier, extreme pathogenic bacteria type or immunosuppression. Clinical disease due to *E. coli* in calves may be present as enteric or septicemic illness, being one of the most important causes of neonatal mortality in dairy calves. (Lofstedt et al., 1999). Some strains develop traits can be harmful to a host animal. Diarrhea in calves is commonly caused by enterotoxigenic *E. coli* (ETEC) more recently; attaching and effacing *E. coli* (AEEC) and Shiga toxin-producing *E. coli* (STEC) have also been identified as causes of diarrhea and dysentery in calves (Mainil et al., 1993).

More virulent strains, such as *E. coli* O157: H7 cause serious illness or death in the elderly, the very young or the immunecompromised (Hudault et al., 2001). Identification of certain virulence factors has assisted in defining mechanisms by which various strains result to different diarrheal syndromes, commensal *E. coli* strains rarely contain virulence genes (Boerlin et al., 2005).

E. coli can be classified into six pathogroups based on their virulence scheme: ETEC; shiga toxin-producing *E. coli*; enteropathogenic *E. coli* (EPEC); enteroinvasive *E. coli* (EAEC); enteroaggresive *E. coli*; and enterohaemorrhagic *E. coli* (EHEC) (Kaper et al., 2004). ETEC, EPEC, and EHEC are the diarrheagenic types described as occurring in young farm animals. The importance of ETEC in the etiology of diarrhea among calves, lambs and pigs is well recognized and these organisms should not be confused with the rare EPEC and EHEC types that cause the less-common diarrheal syndromes. Among these pathogroups, the most common cause of neonatal diarrhea is ETEC stains that are producing the K99 (F5) adhesion antigen and also the heat-stable enterotoxin (Nataro and Kaper, 1998). Neonatal calves are most susceptible to ETEC infection during first 4 days after birth and develop “watery” diarrhea if infected (Foster and Smith, 2009). Following ingestion, ETEC infects the gut epithelium and multiplies in enterocytes in intestinal villi. The distal portion of small intestine is the most favorable environment of ETEC colonization due to the low pH (less than 6.5). The bacteria express the K99 antigen for the attachment (Francis et al., 1989). As colonized on the gut epithelium, heat stable toxin is induced by ETEC and causes the secretory diarrhea.

The genus *Salmonella*

Salmonella species are facultative anaerobic gram-negative rods within the family of enterobacteriaceae, they can survive and multiply in the environment as a result of fecal shedding. There are approximately 2,500 known serovars in the *Salmonella* genus (Davies, 2008). The most common serotype isolated is *Salmonella typhimurium*. *S. typhimurium* DT104 exhibits multiple resistances to the commonly used antibiotics (Jones et al*.,* 2002). *S. enterica* colonizes the gastrointestinal tract of a wide range of hosts (Wray, 2000). *S. enterica serovar typhimurium* and *S. dublin* are the most common etiology of salmonellosis in cattle (Sojka et al., 1977). *Salmonella* infection has a wide range of clinical manifestation from asymptomatic to clinical salmonellosis. Acute diarrheal disease is most common with *S. typhimurium* and systemic disease with *S. dublin* in cattle. Infected cattle can serve as source of zoonosis through food-borne or direct contact routes (Mead et al., 1999). *S. dublin*, host specific, and *S. typhimurium*, not host-specific, are quoted to be the most common serovar

in bovines (Venter et al., 1994), both affecting calves severely between six and twelve weeks of age and in the first three weeks of age, respectively (Bisping and Amtsberg, 1988). Due to management practices, the disease is more commonly found in dairy than in beef cattle (Venter et al., 1994). Source for infection are mainly latent carriers or contaminated environment, where *Salmonella can* persist for a long period. The infective dose, predisposing factors and the immunity status of the hosts determine the outcome of the infection (Venter et al., 1994). The peracute form is often fatal with signs of diarrhea and septicemia. The acute form goes along with fever, in appetence, diarrhea and polypnoea. In chronic cases of *salmonellosis*, calves are unthrifty, have long scruffy hair and are stunted (Venter et al., 1994).

The genus *Campylobacter*

The first description of a bacterium belonging to the genus *Campylobacter is* attributed to Theodore Escherich at the end of the 19th century. *Campylobacter has* long been recognized as a pathogen and commensal organism of animals and it is one of the most common causes of bacterial gastroenteritis in humans worldwide (Allos, 2001). It is adapted to the intestinal tract of warm-blooded animals and does not normally replicate outside this environmental niche and is widely distributed among animals. *Campylobacter jejuni* is the most common cause of bacterial gastrointestinal disease in many western industrialized countries (Tauxe, 1992). In contrast to most other animals, in which *Campylobacter* does not cause any symptoms, *Campylobacter* species, especially *C. jejuni*, may cause diarrhea in calves (Diker et al., 1990; Schulze, 1992). In addition to the potential risk of *Campylobacter* in contributing to enteritis in calves, calves might act as a reservoir for *Campylobacter* spp. and can be a source of human infection, either by direct contact or through fecal contamination of food and water.

Campylobacter species often inhabit the bovine intestinal tract, particularly of calves. The overall prevalence of *C. jejuni* in calves during the first 3 months of life on large calf farms was 39% in a Swiss (Busato et al., 1999). In a Danish study, (Nielsen, 2002) 20 out of 24 cattle herds were infected, and young animals had a higher prevalence than older animals. In 40% of infected herds, all *C. jejuni* isolates had the identical serotype and pulsed-field gel electrophoresis type. Prevalence of campylobacter infection in a multiple- herd study of adult beef cattle was 5% in California (Hoar et al., 2001).

The genus *Clostridium* Clostridia species are gram-positive bacteria, obligate anaerobes capable of producing endospores. Individual cells are rod-shaped or spindle, and consist of around 100 species that include common free-living bacteria and most importantly pathogens (Lewis, 2011). Clostrodia species are responsible for a wide range of diseases in mammals and birds (Van Immerseel et al., 2004) and can be found in the intestinal tract of human, animals, insects and in soil. *Clostridium* organisms are normal flora of cattle and only become problematic with dietary stress, injury, changes in management, parasitism that results in production of potent toxins (Popoff and Bouvet, 2009). Clostridia species produce the highest number of toxins of any type of bacteria and are involved in severe diseases in animals. Most of the clostridial toxins are responsible for gangrenes and gastrointestinal diseases. Presence of the organism in the intestine is not sufficient to cause diseases. Clostriadial toxins are classified into 5 toxinotypes (A, B, C, D and E) according to the production of 4 major toxins, namely alpha (CPA), beta (CPB), epsilon (ETX) and iota (Rood, 1998). *C. perfringens* can produce up to 15 toxins including lethal toxins as perfringolysin O (PFO), enterotoxin (CPE) and beta2 toxin (CPB2) (Gkiourtzidis et al., 2001) summarized in Table 1.

Some type A strains produce an enterotoxin that causes diarrhoea in humans and most likely also in various domestic animals. *C. perfringens* type A strains have been associated with intestinal disorders in horses, piglets, dogs and calves. Thus, the detection of *C. perfringens* toxin types and subtypes is critical for a better understanding of the epidemiology of *C. perfringens* infections and may be helpful in the development of effective preventive measures (Baums et al., 2004).

Clostridium difficile has been implicated as an important etiological agent in antimicrobial associated diarrheal disease and pseudomembranous colitis (Bartlett et al., 1980). It is a recognized pathogen in neonatal pigs and may contribute to enteritis in calves while recent evidence suggests that the epidemiology of *C. difficile* associated disease (CDAD) is increasing in incidence and severity (Boerlin et al., 2005). These changes are due, at least in part, to the emergence of a more virulent *C. difficile* strain, designated NAP1 (based on its pulsed-field gel electrophoresis (PFGE) pattern, by restriction endonuclease analysis (REA) and toxinotype III by polymerase chain reaction (PCR) characterization of the pathogencity locus by PCR ribotyping.

PATHOGENESIS AND VIRULENCE FACTORS

Escherichia coli

Investigations into *E. coli* strains associated with individual cases or outbreaks of diarrhea among neonatal calves, lambs, pigs, and humans have helped to determine specific virulence factors that can be used to distinguish between pathogenic and commensal strains (Okerman, 1987). Virulence factors in *E. coli* include the ability to resist phagocytosis, utilization of highly efficient iron acquisition systems, resistance to killing by serum,

Table 1. Major lethal toxins of *C. perfringens* for type determination.

Type	Toxins			
	Alpha	Beta	Epsilon	Iota
A	++	-	-	-
B	+	++	+	-
C	+	++		-
D	+	-	++	-
E	+	-	-	++

Source: (Niilo, 1980). ++ = Produced as a predominant toxic fraction, + = Produced in smaller quantities, - = Not produced.

production of colicins and adhere, colonize, and invade the hosts' cells. Further to these are the secretion systems, production of cell surface molecules, transport and siderophore formation. Mortality survey confirmed scour as the main clinical sign (48%) in camel calves, which were born alive but died within the first month of life (Hamouda et al., 2010). The pathogenicity of STEC O157:H7 is associated with a number of virulence factors, including Shiga toxin 1 (encoded by the stx1 gene), Shiga toxin 2 (encoded by the stx2 gene), intimin (encoded by the eae A gene) and enterohaemolysin (encoded by the Ehly gene) (Kang et al., 2004).

Two of the more prominent virulence factors identified for ETEC strains are expression of fimbrial (pili) antigens that enables the bacteria to adhere to and to colonize the luminal surface of the small bowel and elaboration of one or more enterotoxins that influence intestinal secretion of fluids (Holland, 1990) through increased cellular concentrations of cyclic AMP (cAMP) or cGMP. Although, the association with serotypes or serogroups does not confer virulence, results of several studies have shown that ETEC strains are limited to a few serotypes or serogroups (Soderlind et al., 1988). The most common observed fimbriae on ETEC from calves with diarrhoea are F5, also named K99 and F41, but strains with F165 fimbriae have also been isolated (Contrepois et al., 1989). K99 antigen is a fimbrial adhesion distinct from the capsular polysaccharide K antigens (Orskov et al., 1975). Two biological classes of enterotoxins are produced by ETEC: heat labile (LT) and heat stable (STa and STb) (Scotland et al., 1985), and most bovine ETEC produce STa enterotoxin and K99 fimbriae (Kaeckenbeeck, 1981).

Salmonella

In cattle, enteric salmonellosis is very common. Various stress factors influence the outcome of the infection (Fenwick and Collett, 2004). Initial infection may be followed by bacteremia and dissemination to several organs. In pregnant animals, abortion may occur. Resistant *Salmonella* infections of calves are very common, so disease caused by *Salmonella* is often very severe (Radostits et al., 2007). Animals that recover from *Salmonella* infections may become carriers for life, shedding organisms sporadically in their feaces (Radostits et al., 2007). The basic virulence mechanism of *Salmonella* includes the ability to invade the intestinal mucosa, to multiply in lymphoid tissues and to evade host defense systems, leading to systemic disease. For the pathogenesis of *Salmonella*, the organism should be capable of invading intestinal epithelial cells, surviving within macrophages and causing enteropathogenicity (Tsolis et al., 1999; Holt, 2000). *Salmonella* colonizes in M-cell, enterocytes and tonsilar tissue (Reis et al., 2003). In lymphoid tissue infection, *Salmonella* easily spreads throughout the whole body by invading mononuclear cell and phagocytes (Holt, 2000). *Salmonella* pathogenicity island 1 (SPI-1) and SPI-5 are known to be involved in the type III secretion system, and are mainly responsible for *Salmonella* induced diarrhea in calves (Tsolis et al., 1999). SPI-2 is involved in the second type III secretion system and is responsible for intracellular survival of the organism (Ochman et al., 1996).

Clostridium

Organisms are normal flora of cattle and only become problematic with dietary stress, injury, changes in management, and parasitism result in production of potent toxins. *Clostridium perfringens* are part of the normal intestinal flora. However, it causes enterotoxemia syndromes of cows and calves. The role of some of these toxins in the pathogenicity of disease has not been clarified yet. *C. perfringens* strains harboring cpb2 have frequently been associated with enterotoxaemia in sheep and goat (Uzal and Songer, 2008).

The virulence of *C. perfringens* is determined by its prolific toxin-producing ability, including enterotoxins. *C. perfringens* strains are divided into five toxin types (A, B, C, D and E) on the basis of the production of four major lethal toxins: α, β, ε and ı (Al-Khaldi et al., 2004). Type A strains produce alpha (α) toxin only; type B stains produce α, beta (β) and epsilon(ε) toxins; type C type strains produce α and β toxins; type D strains produce α

toxins and type E strains produce α and ı (iota) toxins. Among these types, type C has been frequently reported in conjunction with calf diarrhea (Rings, 2004) but not as frequently as some other enteric pathogens such as *E. coli* and *Salmonella*. The toxin is the main lethal toxin, and functions in cell lysis through hydrolysis of membrane phospholipids. The β toxin is highly trypsin-sensitive and induces mucosal necrosis. The toxin causes lethal enterotoxemia in domestic animal, and the ı toxin is responsible for dermo necrosis due to its high vascular permeability (Songer, 1997., Petit et al., 1999). Enterotoxin causes diarrhea and intestinal cramping due to its act on epithelial tight junction protein Beta-2 toxin, which is produced from all types of *C. perfringens*, has been recently reported and postulated to have a synergetic function with enterotoxin (Gurjar et al., 2008).

Most domestic animals are susceptible to all types of *C. perfringens* due to the ubiquitous nature of the bacterium in the environment. Newborn calves which have a low level of proteolytic enzymes (example, trypsin) in gastrointestinal track can be easily infected by *C. perfringens* type C as β toxin is recognized as the main virulence factor responsible for clinical signs seen in affected animals. Intestinal lesions in such affected animals are characterized by diffuse or multifocal hemorrhagic necrotizing enteritis and bloody fluid distension (Barker et al., 1993). The pathogenicity of *C. difficile* is due to its ability to produce an extracellular protein that has cytotoxic effects on cells in tissue culture (Donta et al., 1980), and can produce disease in experimental animals similar to that seen in humans.

Campylobacter

Campylobacter jejuni is one of the most important causes of food-borne illness in industrialized nations and diarrhea in children in developing countries (Lyerly et al., 1988). Despite its importance as a pathogen, its virulence mechanisms are just beginning to be understood. The ability of *C. jejuni* to enter non phagocytic cells is thought to be very important for its pathogenesis (Mcsweegan and Walker, 1986). Proteinaceous toxins are relevant in the context of enteropathogenicity which can be classified into two classes depending on their primary mode of action: enterotoxins and cytotoxins. Enterotoxins are secreted proteins with a capacity to bind to a cellular receptor, enter the cell, and elevate intracellular cyclicAMP (cAMP) levels. The prototypes of enterotoxin cytotonic are vibrio cholerae toxin (CT) and the closely related E. coli heat-labile toxin (LT) (Spangler, 1992). Cytotoxins are proteins that kill target cells cytotoxins which act as intracellular or form pores in the cells.

Cytotoxins with intracellular activity generally bind to the cells and are processed before they reach the cell cytoplasm. Different mechanisms of toxicity exist, of which two predominate: inhibition of cellular protein synthesis and inhibition of actin filament formation examples, of the first type of cytotoxin are shigella dysenteriae toxin and the related *E. coli* Shiga-like toxin (Stx) also known as verotoxin. The two toxins are closely related and contain two subunits, the A subunit with enzymatic activity and a pentamer of B subunits (Olsnes et al., 1981).

Diagnosis

An etiologic diagnosis is useful in selecting specific diagnostic and preventative regimens for bacterial infections. Establishing an etiologic diagnosis for bacterial infections may be more important now that there is some indication that effective vaccines are being developed. Diagnosis of salmonellosis and higatoxigenic *E. coli,* can have public health implications. Once an agent has been identified, one of the major problems is in interpretation whether or not it is responsible for diarrhea in the individual or herd, because most agents can also be found in healthy calves. Identification of one agent in pathologic material does not preclude the possibility that other agents are also contributing to the condition (Naylor, 2002).

Culture techniques are designed to promote the growth and identify bacteria, while restricting the growth of the other bacteria in the sample. Often, these techniques are designed for specific specimens; for example, a sputum sample will be treated to identify organisms that cause pneumonia, while fecal specimens are cultured on selective media to identify organisms that cause diarrhea, while preventing growth of non-pathogenic bacteria. Specimens that are normally sterile, such as blood, urine or spinal fluid, are cultured under conditions designed to grow all possible organisms (Thomson and Bertram, 2001). Once a pathogenic organism has been isolated, it can be further characterized by its morphology, growth patterns such as aerobic or anaerobic growth, patterns of hemolysis and staining. Many scours, regardless of cause, show similar clinical picture.

However, the severity and character of the scour and the age of the affected calves can all help to make a professional judgment for the cause. Frequently, examination of faces samples from a group of calves can identify the organisms present in an outbreak. However, routine tests fail to identify any specific organism and further examinations are required to make an accurate diagnosis, for example, history of predisposing causes, isolation of *E. coli*, postmortem examination (Quinn et al., 2002) and the use of PCR to test for enterotoxigenic genes (Ahmed et al*.,* 2007).

Clinical finding

Disease produced by *E. coli* organism is associated with the feeding of inadequate levels of colostrum. Severe infection results in severe dullness, listlessness and

collapse in calves of less than one week old and is often fatal despite therapy. *E. coli* K99+ causes a watery diarrhea, dehydration, and weakness in 1- to 4-day-old newborn calves. The fimbrial adhesion F5 (K99) promotes the attachment of bacterial cells to glycoproteins on the surface of epithelial cells of the jejunum or ileum and bacterial enterotoxin also causes damage to the epithelial cells, resulting in fluid secretion and diarrhea (Acres, 1985).

Salmonellosis is a bacterial disease of humans and animals. The most common serovar infecting cattle is *S. dublin* and the second most common is *S. typhimurium* (Defra, 2005). The condition has different manifestations in infected animals. An acute generalized infection is seldom seen, but causes a severe condition that can be fatal without effective treatment in vulnerable individuals. An acute intestinal infection causes diarrhea, particularly in young animals. In adults, *S. typhimurium* infection is most commonly associated with diarrhea and dullness (Veling et al., 2002). Calves with *Salmonellosis* may become scepticaemic and die, or may suffer from necrosis of extremities as a sequel, especially the feet, tail and ear tips. A chronic intestinal infection can be asymptomatic clinically, and often leads to the presence of carrier animals that are not identified as *Salmonella* infected.

The diarrhea caused by *Salmonella* infection is characterized by watery and mucoid diarrhea with the presence of fibrin and blood. Even though *Salmonella* can cause diarrhea in both adult cattle and calves, infection is more common and often causes severe symptoms in 10 day to 3 month old calves (Fossler et al., 2005). *C. jejuni* is the most common cause of bacterial gastrointestinal disease in many western industrialized countries. Symptoms are often debilitating and typically include pyrexia, severe abdominal pain and diarrhea (Walker et al., 1986).

Clostridial diseases progress rapidly and sudden death is often the first and the only sign of disease. *C. perfringens* type C causes necrotic enteritis in newborn calves. Calves are suddenly depressed, weak, and may be distended or show abdominal pain. If diarrhea develops, it may have blood and tissue streaks. Affected calves may die before they develop diarrhea (Quinn et al., 2002).

Bacterial isolation

In feces samples, microscopy will show gram negative rods, with no particular cell arrangement. Then, either MacConkey agar or EMB agar (or both) are inoculated with the feces. A typical *E. coli* were identified by their characteristic colony morphology on MacConkey's agar, biochemical characteristics and by a slide agglutination test with rabbit antiserum to the atypical *E. coli*. On MacConkey agar, deep red colonies are produced as the organism is lactose positive, and fermentation of this sugar will cause the medium's pH to drop, leading to darkening of the medium. Growth on Levine EMB agar produces black colonies with greenish-black metallic sheen. The diagnosis of *E. coli* is also lysine positive, and grows on TSI slant with a (A/A/g+/H_2S-) profile. Also, the pattern of *E. coli* on, Indole, Methyl Red, Vagaues prouskare, and Citrat utilization is indole positive (red ring) and methyl red positive (bright red), but VP negative (no change-colorless) and citrate negative (no change-green color). Tests for toxin production can use mammalian cells in tissue culture, which are rapidly killed by shiga toxin. Although sensitive and very specific, this method is slow and expensive (Paton and Paton, 1998*).* Typically diagnosis has been done by culturing on sorbitol-MacConkey medium and then using typing antiserum. However, current latex assays and some typing antiserum have shown cross-reactions with non *E. coli* O157 colonies. Furthermore, not all *E. coli* O157 strains associated with HUS are non-sorbitol fermenters.

Diagnosis of salmonellosis involves isolation of *Salmonella* species in fecal cultures should be submitted for *Salmonella* culture in all cases of diarrhea or fever. Because *Salmonella* can be shed intermittently, repeated negative cultures must be obtained before ruling out salmonellosis. *S. typhimurium* recovered from calves and lambs were tested for their virulence using Congo red binding test, ability to produce hemolysin, adherence assay and cell invasion test and detection of inv A gene using PCR (Mohamed and Dapgh, 2007).

Accurate diagnosis of *Salmonella* require cultivation on specific media bacteriological testing of feces was undertaken for *Salmonella* using rapport and selenite brilliant green enrichment broths and sub-inoculation onto specific media such as; XLD, RVS, brilliant green agar, triple sugar iron agar, gram stain show medium sized gram negative rods and biochemical tests using API 20. Carriers of infections can be detected by culturing faces but because excretion is intermittent, repeated sampling and culture may be necessary. Serology may be useful but is best applied on a herd basis (Davies, 2008). No practical serological method exists for detecting individual carrier animals (Hansen et al*.,* 2006).

For isolation of *Campylobacter,* a small portion of fecal samples was suspended in 0.85% saline, filtered through 0.45 mm millipore filter papers. Filters were then cultured in Preston broth and incubated overnight at 37°C.

Cultures were then inoculated onto Preston agar plates and incubated for 48 h in an atmosphere of 5% oxygen, 10% CO2 and 85% nitrogen. Suspected colonies were identified based on their motility, hydrolysis of sodium hippurate and sensitivity to cefalotin and nalidixic acid (Achá et al., 2004). According to Klein et al. (2012), fecal samples were enriched in Bolton broth for 48 h at 42°C under microaerophilic conditions (10% CO2, 5% O2 and 85% N2). A loopful of this enrichment was streaked onto modified charcoal cefoperazon deoxycholate agar and a

second loopful onto CampyFoodAgar. Both plates were incubated at 42°C for 48 h under microaerophilic conditions. Additionally, fecal material without prior enrichment was directly streaked on modified charcoal cefoperazon deoxycholate agar and Campy Food Agar, and incubated at 42°C for 48 h. Morphological typical colonies were differentiated by aerobic incubation.

For clostridia, samples should be collected in cooked meat broth or thioglycollate broth media, and then aerobically incubated. Gram staining illustrated gram positive spore forming rods. Biochemical analysis is required to differentiate between different species, such as cultivation onto Egg Yolk agar for lecithinase and lipase activity, testing for hydrolysis of gelatin, digestion of casein, indole production and formation of acid from glucose-lactose-sucrose maltose fermentation test. Animal inoculation test by intramuscular injection in mice or guinea pig for toxin identification by neutralization test using polyvalent antitoxin, followed by specific monovalent antitoxin. *C. perfringens* is also identified by Nagler test and CAMP test. Florescent antibody technique for identification of *Clostridium novy* in acetone fixed liver impression smear (Quinn et al., 1994).

Molecular diagnosis

Diagnosis of *E. coli* infection currently relies on the phenotypic differentiation of pathogenic strains from nonpathogenic normal flora *E. coli* via bioassays or immunoassays for toxins and fimbriae. As with bacterial classification, identification of bacteria is increasingly using molecular methods. Diagnostics using such DNA-based tools, such as polymerase chain reaction, are increasingly popular due to their specificity and speed, compared to culture-based methods (Louie, 2000). These methods also allow the detection and identification of "viable but non culturable" cells that are metabolically active but non-dividing (Oliver, 2005). Detecting *E. coli* O157 in feces include ELISA tests, colony immunoblots, direct immunofluorescence microscopy of filters, as well as immunocapture techniques using magnetic beads (De Boer and Heuvelink, 2000). These assays are designed as screening feces to allow rapid testing for the presence of *E. coli* O157 without prior culturing of the feces specimen.

Polymerase chain reaction followed by a microarray hybridization step has been used for the detection and typing of *E. coli* virulence genes (Chizhikov et al., 2001). A serotype-specific DNA microarray for the identification of clinically encountered Shigella and pathogenic *E. coli* strains were recently described (Li et al., 2006).

Diagnostic microarrays based on the ArrayTube format were devised for virulence determinant detection as well as for protein-based serotyping of *E. coli* (Anjum et al., 2007). A novel ArrayTube assay, which incorporates oligonucleotide DNA probes representing 24 of the most epidemiologically relevant O antigens and 47 H antigens, has been described for fast DNA serotyping of *E. coli* (Ballmer et al., 2007). Serotyping is required and the use of mPCR is a useful accurate tool to detect toxic genes; shiga toxin and intamin that are responsible for signs of toxicity (Ahmed et al., 2007).

Various *Salmonella specific* primers and probes have been developed for molecular identification as well as detection in samples of these microorganisms. The use of pooled *Salmonella* enrichment broth cultures of bovine feces and PCR for the detection of the invA gene of *Salmonella* in feces appears to be an efficient method of *Salmonella* detection (Singer et al., 2006). *S. typhimurium* recovered from calves and lambs were tested for their virulence using Congo red binding test, ability to produce hemolysin, adherence assay and HEp2 cell invasion test and detection of inv Agene using PCR (Mohamed and Dapgh, 2007). Campylobacter PCR (Linton et al., 1997) and 16S rRNA gene sequencing on selected *Campylobacter* isolates were further identified and differentiated by matrix-assisted laser desorption/ ionization time-of-flight (MALDI-TOF) mass spectrometry analysis as described by Alispahic et al. (2010). For MALDI-TOF, colonies were grown on Columbia blood agar containing 5% sheep blood at 42°C for 48 h under microa erobic conditions. Single colonies were removed and analyzed. *C. perfringens* toxins were detected using quantitative culture followed by genotyping. Toxin detection can be performed by several techniques including an enzyme linked immunosorbent assay (ELISA) that detects CPA, CPB, ETX and *C. perfringens* (Uzal and Songer, 2008).

In vitro methods for detection of toxinogenic are based on detection of toxins or gene probes or Multiplex-PRC for detection of toxin genes. The use of gene probes and multiplex PCR assays for detection of toxigenic *C. perfringens* in affected animals has also been reported (Baums et al., 2004). Multiplex PCR assays have been highlighted as a rapid and accurate method for the detection and genotyping of *C. perfringens* in routine veterinary diagnostics, and provide a useful alternative to *in vivo* toxin neutralization tests for typing of *C. perfringens* isolates (Meer and Songer, 1997).

Serological diagnosis

Cho (2012), described that recently a commercial antigen-capturing ELISA kit in form of a dipstick (Bovine Enterichek, Biovet) was made available to bovine practitioners and producers for the rapid detection of *E. coli* K99+ in feces from diarrheic calves at acute stage of clinical disease with diagnostic sensitivity and specificity of 71.4 and 100% respectively in comparison to mrt PCR multiplex real-time polymerase chain reaction. Serology may be useful for the diagnosis salmonella but is best applied on a herd basis (Davies, 2008). No practical serological method exists for detecting individual carrier animals (Hansen et al., 2006). Serotyping used slide

agglutination test and antibiotic sensitivity test for detection of R factor plasmid (Quinn et al., 1994). Enterotoxins of *C. perfringens* have been detected by enzyme-linked immunosorbent assay (ELISA), immunoelectroforesis, latex agglutination and immunodiffusion (EL-idrissi and Ward, 1992).

Zoonotic importance

Increasingly, food animals and their products are being identified as important sources of infectious pathogens for humans. Many studies also showed that both healthy and diarrheic calves harbor STEC in their intestine (Roopnarine et al., 2007) and shed the bacteria for several months and in great quantities (Widirasih et al., 2004). In addition to economic losses, diarrhea in livestock is important because of the public health implications.

Numerous infectious agents causing diarrhea in animals are zoonotic and have been associated with food-borne diseases (Trevejo et al., 2005). Sporadic cases or large STEC outbreaks in humans are associated with the consumption of raw or undercooked meat of food animals and other foods contaminated by animal faces, and by contact with STEC-positive animals or with their environment (Paton and Paton, 1998).

Dairy and beef cattle are primary reservoirs of *E. coli* O157:H7 and they can carry it asymptomatically and shed it in their feces (Bach et al., 2002). Food products associated with *E. coli* outbreaks include raw ground beef and raw seed sprouts or spinach (Sabin, 2006) raw milk, unpasteurized juice, unpasteurized cheese and foods contaminated by infected food workers via fecal-oral route. *E. coli* and their subtypes (O26, O111, O118 and O157) are firmly associated with emergent food-borne diseases, especially Shiga toxin-producing *E. coli (STEC).*

In humans, EHEC a subset of STEC, is associated with severe systemic disease as haemorrhagic colitis (HC), haemolytic-uremic syndrome (HUS) and thrombotic thrombocytopenic purpura (TTP), especially in infants, young children and in the elderly (Nataro and Kaper, 1998).

Cattle have been identified as the main animal source of *S. typhimurium* infections in humans potentially, spread from cattle to humans can be via direct contact, contamination of the environment and contamination of meat and milk products. Milk has been reported as the source of human infection (Kavanagh, 2002). *C. perfringens* bacteria are the third-most-common cause of food-borne illness, with poorly prepared meat and poultry the main culprits in harboring the bacterium (Warrell et al., 2003).

The *C. perfringens* CPE mediating the disease is heat-labile (dies at 74°C) and can be detected in contaminated food, if not heated properly, and feces (Murray, 2009)

Control and prevention

Multiple factors, both infectious and non-infectious, are involved in calf diarrhea outbreaks, which makes disease control on farms difficult (Svensson et al., 2003; Trotz-Williams et al., 2007). Timely prevention and control of calf diarrhea is important to reduce economic losses to producers and improve animal welfare (McGuirk, 2008). Dealing with such a large number of potential etiological agents as well as various management factors, is an ongoing challenge for effective control of enteric disease in newborn calves (Booker et al., 2008; Uhde et al., 2008). Therefore, a thorough investigation should include a study of dry cow management and calving practice as well as calf management (Bazely, 2003; Andrews, 2004).

Studies of dairy herds have indicated that improved environmental management of calving pens and calf housing reduces calf diarrhea incidence and the extent of outbreaks. A system of "all in all out" calf housing, cleaning, steaming and disinfection of calf housing and calving pens, regular disinfection of utensils and adequate straw were identified as important management factors. In a study, calf diarrhea incidence was reduced from 36 to 11% within a year by the introduction of early colostrum feeding and improved housing hygiene (Lance et al., 1992).

The passive immunity acquired from the colostrum and absorbed into the circulation from the gut is the calf's main defense mechanism against *E. coli* diarrhea. Inadequate amounts of immunoglobulins in the colostrum, inadequate intake of the colostrum and inadequate absorption of immunoglobulins from the gut render very young calves susceptible to infection (Groutides and Michell, 1990). Additionally, among calves aged 1 to 4 months old, carriage of VTEC *E. coli* O157 was reduced if the calf had suckled colostrum from the mother or if the calf had stayed more than 2 days with the mother after calving (Rugbjerg et al., 2003).

The eradication and control of salmonellosis on a persistently infected farm is difficult. Fecal or serological sampling of all animals with group and individual samples, and identification and slaughter of carrier animals has been used successfully in voluntary salmonellosis control (Jensen et al., 1994). Combined vaccination, fecal identification and culling of infected animals have also been used with varying success (Davies and Renton, 1992). Intensive care with antitoxin, fluids, antibiotics and anti-inflammatory drugs isnecessary for treatment but frequently unsuccessful (Quinn et al., 2002).

Synergism between proplis and antibiotics for treatment of enterotoxaemia in calves due to *C. perfringens* type A and C show great results (Masoud et al., 2008). Commercial toxoids available for vaccination against *C. perfringens*, is not effective against type E infections (Songer and Miskimmins, 2004). Clostridia mode of action is to produce one or more potent toxins. Therefore,

the best program is obtained by the use of toxoid vaccines, it allow protection to pass to the lamb via the colostrum (Lewis, 2011).

Treatment of calf diarrhea

In case of diarrhoea outbreak in a herd, it is important to attempt to diagnose the infective cause of the disease in order to target further control measures appropriately. Isolation of affected calves, effective treatment with rehydration solutions and provision of dry and warm conditions are vital in the treatment of calf scours. The main aim of treatment is to restore the fluid balance in the animal by rehydration therapy. Oral therapy is the best way of providing rehydration fluids, but this may need to be replaced by intravenous therapy in severe cases where the calf is unable to drink. A return to whole milk feeding is recommended within two days of rehydration therapy to avoid a negative energy balance (Grove-White, 2004). Suckled calves should have limited access to the dam or sucker cow until full recovery has been achieved.

The use of antibiotics in diarrheic calves has been shown to be contraindicated in many studies, due to the further disruption of gut flora, the establishment of carrier states of salmonella-infection, and the development of antimicrobial resistance factors in the enteric flora. Although, very sick calves with *Salmonellosis* may benefit from antimicrobial therapy, most studies have also failed to show any beneficial effect of antimicrobial treatment (Rollin et al., 1986). Some studies show limited efficacy in reducing mortality and morbidity in an outbreak of diarrhoea (Holck et al., 1994).

Antibiotics should only be used for *E. coli* and *Salmonella* infection, after sensitivity test to choose the best drug, as inappropriate use of antibiotics can lead to serious antibiotic resistance problems. Ciprofloxacin and probiotics such as *Lactobacillus acidophilus* isolated from colostrum of goat and mere are highly effective in treatment of infection thus, control of calf scour is based on feeding plenty of colostrum immediately after birth (Abd El-Moez et al., 2010). Ciprofloxacin coated with gold nanoparticles showed high hindrance *in vitro* for the growth of *E. coli* and *S. typhimurium* (Zawrah and Abd El-Moez, 2011). Vaccination is very important in the control of calf scour, vaccines are to protect against *E. coli* and rotavirus. However, it is unlikely to be effective unless used in conjunction with good husbandry (Hirsh and Zee, 1999).

The treatment of *Salmonella* infection in cattle with antibiotics in acute cases is common and may reduce mortalities if initiated early and combined with support therapy. The use of antibiotics metaphylactically in the face of an outbreak is not recommended, due to the high risk of antimicrobial resistance development. Prophylactic antibiotic administration in feed does not appear to have any effect on excretion of *Salmonellae* in calves either in an outbreak, appropriate support therapy for severely affected animals and vaccination can be helpful.

CONCLUSIONS AND RECOMMENDATIONS

Most cases of calf diarrhea are likely to be mixed infections, where more than one of the pathogenic agents is present. The impacts of calf diarrhea could be direct by causing calf deaths and indirect through increased treatment expenses and decreased lifetime productivity and survivorship. Among the bacterial causes of diarrhea in neonatal food animals, *E. coli* and *Salmonella* specie are the most common and economically important but, *C. perfringens*, and *Campylobacter* species have also been identified as causes of enteric diseases in calf diarrhea. Diarrhea in calves is commonly caused by ETEC; more recently, AEEC and STEC have also been identified as causes of diarrhea and dysentery in calves.

Acute diarrheal disease is most common with *S. typhimurium* and systemic disease with *S. dublin* in cattle. *Campylobacter* specie, especially *C. jejuni*, may cause diarrhea in calves. However, newborn calves which have a low level of proteolytic enzymes in gastrointestinal tract can be easily infected by *C. perfringens* type C. Diarrhea in livestock is important because of the public health implications. Numerous infectious agents causing diarrhea in animals are zoonotic and have been associated with food-borne diseases. Inadequate intake of colostrum, poor hygiene, overcrowding in the calf pens, low temperature of the incoming air, contamination of the incoming air, inadequate ventilation, close proximity to adult cows, mixing of different age groups are the major risk factors for calf diarrhea.

In Ethiopia, very few studies were carried out on the identification of the causes of calf morbidity and mortality giving special emphasis on the cause of calf diarrhea in dairy farms of the country. It is important to identify the infectious agents in the outbreaks of calf diarrhea on a farm basis in order to target prevention and control of this disease complex, and implementation of improved calf management practice to reduce the high-level risk factors of calf disease problems. Detailed studies on the extent of calf diarrhea in Ethiopia, on its etiology and risk factors for infection and zoonotic significance of these agents should be initiated.

Conflicts of interest

The authors declare that they have no conflicts of interest.

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