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# Isolation, Identification and Antimicrobial Susceptibility Patterns of Salmonella and Co-Infection with Cryptosporidium from Diarrheic and Non-Diarrheic Calves in and Around Haramaya District and Harar Town Dairy Farm, Eastern Ethiopia

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## Abstract

**Background:** Calf diarrhea is a major health problem in young cattle and results in considerable economic losses to the dairy industry worldwide. Salmonella is an important bacterial cause of calf diarrhea and is often associated with antimicrobial resistance and co-infection with other enteric pathogens such as Cryptosporidium. This study aimed to determine the prevalence of Salmonella, its antimicrobial susceptibility patterns, and its co-infection with Cryptosporidium in diarrheic and non-diarrheic calves in Eastern Hararghe, Ethiopia.

**Methods:** A cross-sectional study was conducted from November 2023 to May 2024 in intensive and extensive dairy farms in and around Haramaya and Harar towns, Eastern Hararghe. Fecal samples were collected from 384 calves and examined for Salmonella using standard bacteriological techniques and for Cryptosporidium using parasitological methods. Antimicrobial susceptibility testing was performed using the disk diffusion method. Data were analyzed using SPSS version 20, and logistic regression and chi-square tests were applied to assess associations between risk factors and infection.

**Results:** Salmonella was isolated from 63 of 384 calves, giving an overall prevalence of 16.4%. The prevalence was significantly higher in diarrheic calves (38.8%) than in non-diarrheic calves (6.7%). Multivariable logistic regression analysis showed that management system (OR = 2.9, P = 0.002), age category (OR = 4.5, P < 0.001), and fecal consistency (OR = 8.7, P < 0.001) were significantly associated with Salmonella infection. Co-infection with Cryptosporidium was detected in 63.5% (40/63) of Salmonella-positive calves and was significantly associated with age, fecal consistency, body condition score, and management system (P < 0.05). Multiple antimicrobial resistance was observed in 95.2% of the isolates. High resistance was recorded against nalidixic acid (95.2%), amoxicillin (80.9%), and cefoxitin (73.0%), while all isolates were susceptible to ciprofloxacin (100%) and most to chloramphenicol (87.3%).

**Conclusions:** The study demonstrated a high prevalence of Salmonella infection and a substantial level of multidrug resistance among isolates from calves in the study area. Management system, age, and fecal consistency were important risk factors for infection, and co-infection with Cryptosporidium was common among Salmonella-positive calves. Improved farm management practices, rational use of antimicrobials, and integrated control strategies targeting multiple enteric pathogens are essential to reduce calf diarrhea and its impact on dairy production.

**Keywords:** salmonella; antimicrobial resistance; cryptosporidium; calf diarrhea; risk factors

## Introduction

### Background of The Study

Ethiopia, located in Eastern Africa, is predominantly an agricultural country, where animal production is practiced across all ecological zones (Tegegne and Crawford, 2000). The total cattle population is estimated at about 53.99 million, of which 9.77% are under six months of age and 7.64% are between six months and one year (CSA, 2013). Indigenous breeds account for 98.95% of the cattle population, while hybrid and exotic breeds represent approximately 0.94% and 0.11%, respectively (CSA, 2013).

The health of replacement calves is a critical component of dairy farm profitability, as heifer calves represent the foundation of future milking herds (Fox, 2007; Razzaque et al., 2009). High calf morbidity and mortality result in substantial economic losses due to treatment costs, reduced lifetime productivity, impaired survivorship, and limitations on herd expansion and genetic improvement (Mellado et al., 2014). Calf diseases arise from complex interactions among management practices, environmental conditions, infectious agents, and host factors (Klein-Jöbstl et al., 2014).

Neonatal calf diarrhea (NCD) is among the most common and economically devastating diseases of young calves worldwide (Cho and Yoon, 2014). It is a multifactorial syndrome involving infectious agents as well as non-infectious factors related to calf immunity, nutrition, housing, hygiene, and management (Izzo et al., 2011). Due to this multifactorial nature, effective control of NCD remains challenging (Cho and Yoon, 2014). Infectious diarrhea is the leading cause of morbidity and mortality in neonatal dairy calves and is commonly associated with viruses (rotavirus and coronavirus), protozoa (*Cryptosporidium parvum*), and bacteria, particularly enterotoxigenic *Escherichia coli* (ETEC) and *Salmonella* spp. (Izzo et al., 2011).

Although co-infections are frequently observed in diarrheic calves, single-agent infections may also occur. The prevalence of these pathogens varies with farm location, management practices, and herd size (Cho and Yoon, 2014). Identification of causative agents during outbreaks is essential for implementing targeted preventive strategies, including vaccination and biosecurity measures, and for identifying risk factors and sources of infection (Izzo et al., 2011).

*Salmonella enterica* colonizes the intestinal tract of both adult cattle and calves; however, infection is most commonly observed during the first three months of life and often results in severe clinical disease (Fossler et al., 2005). The most prevalent serovars in cattle are *S. enterica* serovar Typhimurium and serovar Dublin, which typically cause acute enteritis and systemic disease, respectively (Cho and Yoon, 2014). Diarrhea associated with salmonellosis is often watery or mucoid and may contain blood and fibrin (Fossler et al., 2005). Infected calves may shed the organism intermittently for prolonged periods, and up to 5% of recovered animals may become asymptomatic carriers (Jay, 2003; Cho and Yoon, 2014). These carriers serve as reservoirs of infection for other animals and humans, highlighting the importance of detecting *Salmonella* in fecal samples for both clinical diagnosis and epidemiological control (Warnick et al., 2003).

Cryptosporidiosis is also a major cause of morbidity in neonatal calves (Szonyi et al., 2012). Although the disease is often mild and self-limiting, it may lead to severe diarrhea, dehydration, and mortality, particularly in cases of co-infection with other enteric pathogens (Santin et al., 2008; Wang et al., 2010). Infected calves can shed large numbers of infective oocysts for 3-14 days, including during asymptomatic periods (Santin et al., 2008). Clinical signs include

watery or bloody diarrhea, malabsorption, dehydration, and poor growth (Wang et al., 2010).

*Cryptosporidium* oocysts are highly resistant to environmental conditions and many commonly used disinfectants, enabling prolonged survival under a wide range of temperatures (-22°C to 60°C). The infectious dose for neonatal calves may be as low as 17 oocysts, making control on farms particularly difficult (Thomson et al., 2017). The disease is usually self-limiting when adequate colostrum immunity is present, calves are maintained under good hygienic conditions, and supportive therapy is provided, especially in the absence of concurrent infections (Thomson et al., 2017).

Current treatment strategies for neonatal calf diarrhea rely primarily on fluid therapy and antimicrobial agents. However, the indiscriminate use of antimicrobials has contributed to the emergence of antimicrobial resistance, particularly among *Salmonella* isolates from pre-weaned dairy calves. This growing resistance poses a significant challenge to effective disease management and public health (Izzo et al., 2011).

### Statement of The Problem

Diarrhea (scouring) is a complex multifactorial disease and the leading cause of neonatal calf mortality during the first weeks of life, with risk decreasing as calves age. *Salmonella* spp. is among the major bacterial pathogens responsible for calf diarrhea and cause substantial economic losses due to high morbidity and mortality in the cattle industry worldwide. In addition to *Salmonella*, several other infectious and non-infectious agents, including rotavirus, *Escherichia coli*, *Giardia duodenalis*, and *Cryptosporidium* spp., contribute to diarrheal disease in young livestock. Co-infections involving multiple pathogens are common, yet little is known about the interactions between these agents and their effects on disease severity and outcomes.

In Ethiopia, calf morbidity and mortality associated with diarrhea remain major challenges to dairy production. Despite this, coordinated surveillance systems and comprehensive assessments of the etiological complexes involving bacterial pathogens and protozoan parasites are limited. In particular, information on the isolation, identification, and antimicrobial susceptibility patterns of *Salmonella* spp., as well as their co-association with *Cryptosporidium* infection in diarrheic calves, is scarce. Therefore, research focusing on these pathogens is essential for improving disease control

strategies and reducing economic losses during the critical early months of calf life.

## Objectives

**General Objectives:** The overall objective of this study is to isolate and identify *Salmonella* spp., determine their antimicrobial susceptibility patterns, and assess their co-association with *Cryptosporidium* spp. in diarrheic and non-diarrheic calves.

## Specific Objectives

- To isolate and identify *Salmonella* spp. from diarrheic and non-diarrheic calves.
- To determine the antimicrobial susceptibility patterns of *Salmonella* isolates.
- To assess the co-occurrence of *Salmonella* spp. and *Cryptosporidium* spp. in diarrheic and non-diarrheic calves.
- To identify risk factors associated with *Salmonella* infection in calves.

## Literature Review

### Bovine Salmonellosis

#### Etiology

Salmonellae are Gram negative, short plump shaped rods, nonperforming, no capsulated, aerobic and facultative anaerobic organisms and classified under the family Enterobacteriaceae (OIE Manual, 2006). There are 2 recognized species within the genus: *S. enterica* and *Salmonella bongori*. *S. enterica* can be further divided into 6 subspecies, *S. enterica* subspecies *enterica* being the most relevant in dairy cattle (Smith, 2009). Ninety-nine percent of human and animal infections are caused by the subspecies *enterica*. Within this subspecies more than 2 600 serotypes have been described, based on serological reactions to cell wall lipopolysaccharide (O), flagellar (H) and capsular (VI) antigens (Sanderson & Nair, 2012).

*Salmonella* serotypes have different abilities to infect and cause disease, and are sometimes referred to as host generalists (causing infections in many hosts e.g. *S. Typhimurium* and *S. Enteritidis*), host-adapted (primarily associated with one host e.g. *S. Dublin* in cattle and *S. Choleraesuis* in pigs), and host-restricted (associated with one host only e.g. *S. Typhi* in man and *S. Gallinarum* in poultry) (Sanderson & Nair, 2012). Many serovars can infect cattle; *Typhimurium* and *Dublin* serovars are the most common. *S. typhimurium* is frequently associated with enteric disease in calves less than 2 months of age, and *S. Dublin* is associated with young and adult cattle and

is more invasive than *S. typhimurium* (Mohler et al., 2009).

Salmonellae are chemoorganotrophic, with an ability to metabolize nutrients by the respiratory and fermentative pathway. An important characteristic of the *Salmonella* bacteria is that they are able to grow and multiply outside living host organisms, thus having greater survival chances than otherwise (Gray and Fedorka-Cray, 2002).

#### Epidemiology

The epidemiology of *Salmonella* is complex which often makes control of disease difficult. Epidemiological pattern of prevalence of infection and incidences of disease differ greatly between geographical area depending on climate, population density, land use farming practice, food harvesting and processing technologies and consumer habits. In addition, the biology of serovar differs so widely that *Salmonella* infection or *Salmonella* contamination are inevitably complex (Radostitis et al., 2007).

*Salmonella* *Enteritidis* is the most prevalent species followed by *S. typhimurium* which are a worldwide distribution. Change in the relative frequency of serotypes can be observed over a short period of time. Some times within one or two years only limited number of serotypes is isolated from man or animals in a single region or country and the predominance of one or other can vary over a time. Some serotypes like *S. enteritidis* and *S. typhimurium* are found worldwide in contrast to *S. weltevreden* which seems to be confined to Asia (Kemal, 2014). Sibhat et al., (2011) found the serovars Newport, Anatum and Eastbourne to be the most prevalent in Ethiopia. Salmonellae have a wide variety of domestic and wild animal hosts (Acha and Szyfres, 2001).

#### Pathogenesis

In both calves and adults, those factors that determine pathogenicity and whether or not clinical disease is seen include virulence of the serotype, dose of inoculum, degree of immunity (passive or adaptive) or previous exposure of host to the serotype, and other stressors currently affecting the host (Mohler et al., 2009). The most detailed studies of the pathogenesis of bovine salmonellosis infection come from the literature describing enteric infection via the oral route, mainly in calves (Mohler et al., 2008). Once ingested, *Salmonella* attaches to mucosal cells and is capable of destroying enterocytes. Attachment is increased if gastrointestinal stasis is present or the

normal flora has been disturbed or is not yet established, as is the case in neonates (Smith, 2009). The basic mechanism underlying *Salmonella* virulence includes the ability to invade the intestinal mucosa, multiply in lymphoid tissues, and evade host defense systems, leading to systemic disease. For *Salmonella* pathogenesis, the organism should be capable of invading Intestinal epithelial cells, surviving within macrophages, and causing enteropathogenicity (Reis BP et al., 2003). *Salmonella* colonizes M-cells, enterocytes, and tonsillar tissues following lymphoid tissue (e.g., tonsillar tissue) infection, *Salmonella* easily spreads throughout the whole body by invading mononuclear cells and phagocytes (Holt PS, 2000). *Salmonella* pathogenicity island 1 (SPI-1) and SPI-5 are known to influence the type III secretion system, and are mainly responsible for *Salmonella*-induced diarrhea in calves. SPI2 is involved in the second type III secretion system and is responsible for intracellular survival of the organism (Reis BP et al., 2003).

The virulence mechanisms of salmonellae are, therefore, composed of their ability to invade the intestinal mucosa, locate to and multiply within the lymphoid tissues, and to evade host defense mechanisms. Enterocolitis caused by *Salmonella* spp. is due to inflammation with subsequent maldigestion and malabsorption, and to a lesser extent from secretory mechanisms (Smith, 2009). Inflammation in the colon leads to the commonly observed fresh blood in the feces of both adults and calves. The diarrhea caused by *Salmonella* spp. is principally mediated by the host inflammatory reaction to the infection. To establish infection, enteropathogenesis such as *Salmonella* must first be able to overcome those host factors that resist colonization of the gut, principle among these being a fairly dense gut microbiota, which secrete a variety of bacteriocins, antibiotics, and colicins that hinder enteropathogen growth (Stecher, 2015).

### Clinical Signs

One or more of the syndromes may occur simultaneously in an outbreak of salmonellosis in a herd, or even in a single affected animal. *Salmonella* Dublin is more often the cause of septicemia than *Salmonella* Typhimurium. In Calves clinical disease is most common 2-6 week of age. Calves are more likely than adult animals to suffer from septicemic infections and may develop per acute, acute or chronic salmonellosis. The per acute disease is usually a septicemic condition, it is often fatal and calves may

die suddenly without premonitory sign. Some, however, develop enteritis and diarrhea in addition to septicemia. When the course of septicemia is protracted, sign of hepatitis, pneumonia, meningoencephalitis, polyarthritis and osteomyelitis may develop (Luciana et al., 2012).

Acute enteric salmonellosis is the most common syndrome of salmonellosis encountered in calves. Following an incubation period, which may vary from one to five days, calves develop high fever, inappetence, lethargy, diarrhea, and frequently a serious nasal discharge followed by a slight cough and scouring may only occur terminally. The feces of affected calves have foul smelling, putty-like consistency, and contained large amount of mucous, sloughed mucosa and flecks of blood. At this time body temperature may be normal or subnormal. The mortality rates are 5 to 10% and may reach as high as 75% in purchased calves (Wray and Davies, 2000).

Chronic salmonellosis in calves is characterized by unthriftiness, long and scruffy hair coats and stunting. Diarrhea is not always present and sign of chronic pneumonia with persistent coughing may occur. The calves that survive either per acute, acute or chronic disease may develop pneumonia, meningoencephalitis, purulent polyarthritis, and osteomyelitis of the vertebrae and bones of distal parts of limbs, resulting in lameness, paresis or even almost complete paraplegia. Dry gangrene of the skin of the lower limbs and the tips of ear and tail, resembling ergotism, is rarely encountered (Alemu, 2007).

Salmonellosis is very variable and, in some animals, particularly in very young animal's rapid multiplication occurs both in intestine and systemically associated with poor absorption of specific immunoglobulin G from colostrum or with calves receiving insufficient or no colostrum (L. Plym et al., 2006). More severe symptom may occur in people who are at high risk like those extreme age groups (the young because their immune system are immature and the elderly because the immune system are declining), person with decreased gastric acidity (because gastric acid is the first line of defense for the ingested *Salmonella*), person with altered gastric intestinal bacteria (including those taking broad spectrum antibiotics, purgatives or who have had bowel surgery) and person taking opiate drug in which the bowel movement is decreased. In these highest risk groups of people *Salmonella* may invade beyond the gastrointestinal tract (GIT) to cause severe systematic illness (Kemal, 2014).

## Source of Infection and Transmission

The dominating route of infection is fecal to oral (La Ragione et al., 2013). Cattle herds constitute an integrated part of the environment (Wales & Davies, 2012). Surface water, pastures, and forage may be contaminated by slurry, sewage sludge, or irrigation water containing salmonella bacteria (Ruzante et al., 2010). The bacteria can survive and multiply in the environment, manure, and effluents under suitable conditions (Lahti, 2010).

For transmission between herds, purchase of infected animals is an important factor (van Schaik et al., 2002). Mixing of animals at dealers or on transports also pose a risk. Indirect spread between herds occurs, e.g. through shared pastures (van Schaik et al., 2002), spread of manure from other herds (Veling et al., 2002b), and shared equipment and visitors. Rodents, arthropods, birds and wild animals can serve as vectors, and occasionally as reservoirs, for salmonella (Wales & Davies, 2012).

Spread within herds largely depends on management routines and herd hygiene (Belluco et al., 2015). On-farm feed storages are often open and exposed to contamination by rodents, wildlife and even manure. For *S. Dublin*, the calving area has been identified as an important source of spread (Fossler et al., 2005b; House & Smith, 2004) due to excretion of bacteria from the cow or other adult cattle, leading to infection of the susceptible calves. Even though the dominating route of infection is oral, for *S. Dublin*, other less likely transmission routes identified are aero genic, intrauterine, and conjunctival. Occasionally, calves can also be infected by ingestion of milk from cows excreting salmonella into the milk (La Ragione et al., 2013).

Most *Salmonella* infection in farm animals are likely to acquire from animals of the same species, especially in the case of the host adapted serovars. In adult cattle there are important differences in the behavior of *S. Dublin* and *S. typhimurium*. Those animals which recover from *S. Dublin* infection may become persistent excretors, shedding up to 10<sup>6</sup> organisms per gram of feces daily. Other herd may harbor infection and excrete the organisms only when stressed particularly at parturition. Aerosol transmission has long been suggested as a means by which *Salmonella* may be transmitted and experimental infection of calves by aerosol has been reported recently. In addition, pasture contamination results when flooding occurs and there are many reports of clinical

case in adult cattle arising from grazing recently flooded pasture (Kemal, 2014).

## Diagnosis

Diagnosis is based on the isolation of the organism either from tissues collected aseptically at necropsy or from feces, milk, blood, rectal swabs or environmental samples (Acha and Szyfres, 2001; OIE, 2000). When infection of the reproductive organs or conceptus occurs, it is necessary to culture fetal stomach contents, placenta and vaginal swabs and, in the case of poultry, egg contents. However, salmonellosis is particularly difficult to determine in clinically normal carrier animals.

*Salmonellae* may be isolated by a variety of techniques, which may include pre-enrichment in non-selective medium to resuscitate sub-lethally damaged salmonellae, enrichment media that contain inhibitory substances to suppress non-*Salmonella* organisms, and selective plating agars to differentiate salmonellae from other Enterobacteriaceae (OIE, 2000; ISO 6579, 1998).

Various biochemical and serological tests can be applied to the pure culture to provide a definitive confirmation of an isolated strain. *Salmonellae* possess antigens designated somatic (O), flagellar (H) and virulent (Vi), which may be identified by specific typing sera, and the serovar may be determined by reference to the antigenic formulae in the Kauffman-White scheme. A number of serological tests, such as the serum agglutination test (OIE, 2000) and indirect enzyme-linked immunosorbent assays have been developed for diagnosis of *Salmonella*. Serological tests are useful for the identification of infected herds but are inadequate for the identification of persistently infected animals (OIE, 2000).

## Treatment and Control

In animal treatment supportive treatment with intravenous fluid is necessary for patients that have anorexia, depression, significant dehydration. Individual patient may be treated aggressively following acid base and electrolyte assessment. Oral fluid and electrolyte may be somewhat helpful and much cheaper than IV fluid for cattle demand to be mildly or moderately dehydrated. The effectiveness of oral fluid may be somewhat compromised by malabsorption and maldigestion in salmonellosis patient but still should be considered useful. Cattle that are willing to drink can have specific electrolyte (NaCl, KCl) added to drinking water to help correcting electrolyte (Kemal, 2014).

The implementation of broad prophylactic strategies that are efficacious for all *Salmonellae* may be required in order to overcome the diversity of *Salmonella* serovars present on farms, and the potential for different serovars to possess different virulence factors (Mohler et al., 2009). Early treatment is essential for septicemic salmonellosis but there is controversy regarding the use of antimicrobial agent for intestinal salmonellosis. Oral antibiotic may alter the intestinal micro flora and interfere with competitive antagonism and prolong shading of the organism. There is also a concern that antibiotic resistance strain of *Salmonella* selected by oral antibiotic may subsequently infect human. Antibiotic such as ampicillin or cephalosporin led to lyses of bacteria with release of endotoxin. NSAID may be used to reduce the effect of endotoxemia (Davison, 2005).

Vaccination of calves at 1-3 weeks of age with a modified aromatic defendant *S. Dublin* bacterium have detectable anti-lipopolysaccharide immunoglobins after immunization. Safe live oral vaccine against *S. Typhimurium* and *S. Dublin* has been constructed and shown to control protection against experimental infection with virulent wide type strain of the organisms. A virulent *S. Choleraesuis* vaccine is efficacies experimentally against salmonellosis due to *S. Dublin* in calves to protect young calves. The best program is to vaccinate the cow during pregnancy which will give passive protection to calves for 6 weeks (Radostitis et al., 2007).

Generally, it is agreed that supportive therapy and good nursing are important. These include oral or parenteral re-hydration, correction of electrolyte balance and stabilization of acid base equilibrium. Both live and attenuated vaccines produced from rough strain in bacteria commercially. There is some evidence that inactivated bacterins can induce a lower level of protection (Danielle, 2006).

## **Cryptosporidiosis in Bovine**

### **Etiology and Epidemiology**

Cryptosporidia are protozoan parasites historically classified as belonging to phylum Apicomplexa, class Coccidea, together with e.g. *Eimeria*, *Isoospora* and *Toxoplasma*. This classification was based on similarities in life cycles, such as invasion of host epithelial cells (Fayer, 2008). *Cryptosporidium parvum* affects many species of mammals that include human and it has considerable economic significance in farm animals, especially in the ruminant (Ryan and

Hijawi, 2015). Modern molecular methods are used to analyze the genetic profile of this parasite and new sub-species and genotypes of the parasite have been identified. Currently, 28 species have been recognized (Hotchkiss et al., 2015).

The prevalence of *C. hominis* and *C. parvum* varies in different regions of the world. Indeed, *C. hominis* by far more prevalent in North and South America, Australia and Africa, while *C. parvum* causes more human infections in Europe, especially in the UK. The only documented report of *Cryptosporidium* infection in Ethiopia is by that of Wudu (Wudu, 2004) who reported 6.7% prevalence in calves after a longitudinal study conducted to determine the cause of calf morbidity and mortality. However, full account on the epidemiology of the parasite and the species encountered were not provided (Learmonth et al., 2004).

*Cryptosporidium* oocyst is resistant to many unfavorable environmental conditions and can be well preserved under the cold and wet environment surrounding the calving grounds (Hoar et al., 2001). The presence of high concentrations of the parasite when cattle are overcrowded is also related to higher rates of infection. Another risk factor identified for increasing probability of calves shedding oocyst is frequent bedding changes as personnel and equipment used for removal of the bedding can actually become a vehicle for spreading the infection (Sischo et al., 2000).

### **Risk Factors**

There is a significant association between age and risk of infection with *Cryptosporidium*. *Cryptosporidiosis* due to *C. parvum* is predominantly a problem of neonate animals with maximum rate of excretion of oocysts between the age of 4 and 21 days. According to (Nguyen et al. (2007), age appeared to be an important factor that influences the occurrence of the *Cryptosporidium*. The calves under 3 months are at higher risk of infection compared to the older ones. The higher prevalence in this age group can be attributed to the fact that these age groups are highly susceptible to the disease because of the immature immune system of the animal at this age. Kvac et al., (2006) explained that the animal is becoming resistant with age due to the immune development through time. Although exceptions occur, older animals generally develop poor infections, even when unexposed previously to this parasite (Xiao et al., 2004) age-related resistance, unrelated to prior exposure, has been observed in lambs but not calves

(Radostits et al., 2006). The common occurrence of cryptosporidiosis in young animals reflects their susceptibility to infection with a low number of oocysts and common exposure to oocysts (Fayer, R. and L. Xiao, 2008).

Oocysts are resistant to most disinfectants and can reportedly remain viable for about 18 months in a cool, damp or wet environment, can survive for several months in soil and slurry, but are susceptible to desiccation and temperatures above 60°C. The infectivity of the oocysts can be destroyed by ammonia, formalin, freeze-drying and exposure to temperatures below 0°C (32°F) and above 65°C (149°F). Ammonium hydroxide, hydrogen peroxide, chlorine dioxide, 10% formalin saline and 5% ammonia are effective in destroying the infectivity of the oocysts. The infectivity of oocysts in calf feces is reduced after 1-4 days of drying (Radostits et al., 2006).

### Diagnosis

Several methods exist to detect *Cryptosporidium* in fecal samples. Among them the most common method is microscopy for the detection of oocysts. Fecal samples can be examined directly on slides or after concentration either by flotation or sedimentation to remove fecal debris or to concentrate the number of oocysts; the detection of oocysts in animals with low numbers of oocysts is facilitated (Fayer and Xiao, 2007).

Visualization of *Cryptosporidium* oocysts by microscopy most commonly done by direct smear and without any staining and by the modified Ziehl-Neelsen stain under light microscopy, whereby the oocysts stain purple with blue background. Immunofluorescence staining techniques using monoclonal antibodies against the oocyst wall antigen under epifluorescence microscopy are also useful. Immunofluorescent antibody-based (IFA) procedures have a high sensitivity, but still the easier and cheaper traditional staining methods such as the Ziehl-Neelsen stain are widely used, despite their lower sensitivity (Caccio and Widmer, 2014).

A differential diagnosis list for cryptosporidiosis includes *Giardia*, *Isospora*, *microsporidia*, *Salmonella*, rotavirus and other diarrheal diseases. Cryptosporidiosis can be diagnosed by finding *C. parvum* after a fecal flotation in either sucrose or zinc sulfate solutions. The mature oocysts are 4-5 µm in diameter and contain four thin, flat, motile sporozoites. *Cryptosporidium parvum* can also be detected through an acid-fast staining. Oocysts are not

shed continuously and repeated sampling may be necessary. The oocysts appear red after the staining. Immunofluorescence can also be used to detect *Cryptosporidium* in feces. Finally, cryptosporidiosis can be detected by stained biopsy/necropsy specimens or fresh intestinal scrapings (CFSP H, 2011).

## Materials and Methods

### Study Areas

The study was carried out in two selected areas of eastern Hararghe Ethiopia, which are Haramaya and Harar towns. Haramaya is located in Oromia Regional State of Eastern Hararghe Zone, at 500 km East of Addis Ababa, the capital city of Ethiopia and 14 km from Harar town to west (HADB, 2009). Geographically, Haramaya district is situated at 410 511 5811 N latitude and 9002411011S longitude. The area is located at 2000 m altitude above sea level and receives 492 mm cube average annual rain fall ranging from 118-866 mm cube with a bimodal rain fall of short rain season ranging from February to May and long rain season ranging from June to September. The rainfall reaches peak in mid-August and April. The max and min temperatures are 24 0c and 90c respectively. Haramaya district has livestock population of 114062 cattle, 109765 goats and 730745 sheep (HWAO, 2018). Harar town is found at 525 Km East of Addis Ababa the capital city of Ethiopia. The town is located at 42.04 - 42.22 °E Longitude and 100-250 °N Latitude. It has an average altitude of 1780 m above sea levels and average temperature of 22.65 °c. The annual rain fall on average is 700 mm (HRAO, 2018).

### Study Animals

The study was conducted on diarrheic and non-diarrheic calves which were managed under intensive and extensive management systems in study areas. The study animals include both sexes male and female aged up to one year. All selected farms were visited before and during the study period which enable us to observe health status, body condition and management systems. Any calve that was in antimicrobial treatment regiments (i.e., for diarrhea case) during the data collection period was excluded from sampling.

### Sample Size Determination and Sampling

The sample size required for the current study was determined according to Thrusfield (2007) with the expected prevalence of 50%, 5% absolute precision at 95% confidence level as follows:

$$n = \frac{1.96^2 \times P_{exp}(1 - P_{exp})}{d^2}$$

Where  $n$  = required sample size;  $P_{exp}$  = expected prevalence,  $d$  = desired absolute precision.

Therefore, 384 calves were selected from the target population in the study areas.

### Study Design

Cross sectional study design was employed from November 2021 to May 2022 to achieve the objective of the study in the study area.

### Isolation and Identification of Salmonella

**Pre-enrichment in Non-Selective Media:** In a first step, samples were diluted in a pre-enrichment medium, followed by homogenization in a stomacher for 1 minute. The reason for stomaching is that *Salmonella* spp. is not homogeneously distributed in faeces, especially when only low numbers of *Salmonella* spp. are shed (Funk et al., 2000). Processed samples in appropriate amount of buffered peptone water (BPW) (1:9) were incubated for  $18\text{h} \pm 2\text{h}$  at  $37\text{°C} \pm 1\text{°C}$ .

**Enrichment in Selective Media:** Enrichment in selective liquid media was done by transferring 0.1 ml of culture obtained from the non-selective pre-enrichment media to a tube containing 10ml of Rappaport vassiliads *Salmonella* enrichment broth then incubated at  $42\text{°C}$  for  $24 \pm 3\text{hrs}$  (ISO 6579; 1998; ISO 6579; 2002).

**Plating Media:** Xylose-lysine Deoxycholate agar (XLD) and Brilliant Green Agar (BGA) plates were used for plating out and identification purpose. A loop full of inoculums from Rappaport vassiliads *Salmonella* enrichment broth Culture was streaked on to XLD and BGA agar plates and the Table 1 Table 2 inoculated plates were incubated at  $37\text{°C}$  for  $24 \pm 3\text{h}$ . After proper incubation, plates were examined for the presence of typical *Salmonella* colonies. Typical colonies of *Salmonella* grown on XLD medium produce hydrogen sulphide ( $\text{H}_2\text{S}$ ) and have black ( $\text{H}_2\text{S}$ ) centre and a lightly transparent zone of reddish color due to the color change of the indicator while on BGA medium they become Red due to non-lactose-fermenting. *Salmonella*  $\text{H}_2\text{S}$  negative variants (e.g. *S. Paratyphi A*) grown on XLD agar are pink with a darker pink center where as lactose- positive *Salmonella* are yellow with or without blackening (ISO 6579; 2002).

**Biochemical Tests:** For confirmation, at least five presumptive colonies were selected from every selective plating media. Pure cultures obtained from

nutrient agar were used for biochemical confirmation. Triple sugar iron agar (TSI) slants were inoculated from pure culture by streaking the slant and stubbing the butt incubated at  $37\text{°C} \pm 1\text{°C}$  for  $24 \pm 3\text{h}$  loosely capped to maintain aerobic condition and to prevent excessive production of  $\text{H}_2\text{S}$  production. Typical *Salmonella* culture in TSI agar show alkaline (red) slants, and acid (yellow) butts with gas (bubbles) formation and (in about 90% of the cases) formation of hydrogen sulphide (blackening of the agar). Alkaline reaction (purple color) both in the slant and the butt superimposed with  $\text{H}_2\text{S}$  after incubation indicates a typical positive reaction for *Salmonella* in lysine iron agar (ISO 6579; 2002). Pure isolates were inoculated on urea agar and Simmons's citrate agar by streaking the slant. Both of the inoculated tubes were incubated at  $37\text{°C} \pm 1\text{°C}$  for  $24 \pm 3\text{h}$  (ISO 6579, 2002) and change in the incubated media were interpreted for *Salmonella* after the end of incubation following the guide line. Positive reaction in urea changes the color of phenol red to rose pink and latter to deep cerise due to liberation of ammonia as the result of splitting of urea. However, typical *Salmonella* colonies do not hydrolyse urea; therefore, the medium remain yellow (negative test) (ISO 6579; 2002). *Salmonella* colonies on Simmons's citrate agar produce alkaline products using the medium as the sole carbon source hence deep blue color indicates positive reaction. The pure isolates were also inoculated in to tryptose soya broth (TSI) to determine the ability of an organism to spilt amino acid tryptophan to form the compound indole. For this an inoculated broth culture was incubated for  $37\text{°C} \pm 1\text{°C}$  for  $24 \pm 3\text{h}$  and Kovac's reagent was added. In positive reaction pink colored ring is formed on the top of the broth due to reaction of indole with Kovac's reagent. However typical *Salmonella* colonies due not hydrolyze amino acid tryptophan; therefore, the medium remain unchanged after addition of appropriate reagent (ISO 6579; 2002). Finally, pure cultures were inoculated in to MR- VP Medium (Buffered glucose broth) (glucose phosphate broth) incubated at  $37\text{°C} \pm 1\text{°C}$  for  $24 \pm 3\text{h}$  and broth culture in each incubated tube is divided in to two halves. One half is used for methyl-red test and the other is for Vogus-Proskauer test. Alpha-naphtol (5%) and potassium hydroxide (40%) were added to the medium prepared for vages-proskauer test to know where the organism utilize glucose though butylene glycol pathway and produce acetoin. If acetoin is present, the medium will turn

pink-reddish in color. Methyl red reagent is added in another half. If the organism uses the mixed acid fermentation pathway and produces stable acidic end-products, the acids will overcome the buffers in the medium and produce an acidic environment in the medium. When methyl red is added, if acidic end products are present, the methyl red will stay red. Typical *Salmonella* isolates are VP negative and MR positive (ISO 6579; 2002).

#### Examination of Feces for *Cryptosporidium* Oocysts:

*Cryptosporidium* oocysts will be detected by modified Ziehl-Neelsen staining method. Preparation of staining solutions and procedures will be according to the recommendations given by (OIE, 2016). In this method a thin smear will be prepared directly from fresh fecal samples and allowed to air dry. Then the slides are fixed with absolute methanol for 3 minutes and stained with carbol fuchsin for 15 minutes. The slides then will be washed with tap water and decolorized with acid alcohol (1ml HCl and 99ml of absolute methanol) for 10-15 seconds. After washing the slides with tap water, the slides will be counter stained by 0.4% malachite green for 30 seconds, washed in tap water and allowed to air dry. The slides will then be examined for the presence of oocysts by scanning the slide systematically using the  $\times 40$  objective lens of a bright-field microscope. Confirmation of the presence of oocysts will be under the oil immersion objective lens with 100x magnification. *Cryptosporidium* spp. oocysts will appear red stained discoid or crescent shaped on a pale green background (Hendrix, 1998).

**Antimicrobial Susceptibility Test of *Salmonella*:** The antibiotic susceptibility tests of the *Salmonella* isolates

were performed according to the Clinical and Laboratory Standards Institute guidelines (CLSI, 2012). Each isolate is tested for a series of eight antimicrobials. Amoxicillin (AML) (10  $\mu\text{g}$ ), ampicillin (AMP) (20  $\mu\text{g}$ ), cefoxitin (FOX) (30  $\mu\text{g}$ ), chloramphenicol (C) (30  $\mu\text{g}$ ), streptomycin (S) (10  $\mu\text{g}$ ), nalidixic acid (NA) (30  $\mu\text{g}$ ), ciprofloxacin (CIP) (5  $\mu\text{g}$ ), and Erythromycin E 5  $\mu\text{g}$ . Four to five well isolated colonies grown on nutrient agar were transferred onto tubes containing 5 ml of tryptic soy broth (Oxoid LTD, England). The broth culture was inoculated at 35°C for 4 hrs until it achieves or exceeds the 0.5 McFarland turbidity standard (appendix II). For these tubes which exceeded the turbidity standard, adjustment was made by adding the sterile saline solution to obtain turbidity visually comparable to the standard. Within 15 minutes after adjusting the turbidity of the inoculum's suspension, a sterile swab was immersed in each of dilution suspension and swabbed uniformly over surface of two plates of Muller Hinton agar (Oxoid LTD, England) for each inoculum. The plates were held at room temperature for 30 minutes to allow drying. Using sterile forceps, discs impregnated with known concentration of antimicrobials were dispensed onto the surface of Muller Hinton agar plates. The plates were incubated at 37°C for 20 hrs and examined for zone of inhibition. The diameter of zone of inhibition was recorded to the nearest millimeter, and classified as resistance, intermediate, or susceptible according to published interpretive chart (CLSI, 2012). The type of tested antimicrobials, their concentration in the discs and the zone of inhibition in deciding susceptibility are given in (Table 1).

**Table 1:** Antimicrobials and concentrations used to test susceptibility of *Salmonella* isolates (CLSI, 2012).

Antimicrobials	Disk potency	Resistant	Intermediate	Sensitive
Ampicillin	Amp 10 $\mu\text{g}$	13 or less	14-16	17 or more
Amoxicillin	AMC (20 $\mu\text{g}$ )	13 or less	14-16	17 or more
Ciprofloxacin	CIP 5 $\mu\text{g}$	14 or less	15-17	21 or more
Chloramphenicol	C 30 $\mu\text{g}$	12 or less	13-17	18 or more
Cefoxitin	FOX (30 $\mu\text{g}$ )	41 or less	15-17	18 or more
Erythromycin	E 5 $\mu\text{g}$	13 or less	14-22	18 or more
Nalidixic acid	30 $\mu\text{g}$	13 or less	14-18	19 or more
Streptomycin	10 $\mu\text{g}$	11 or less	12-14	15 or more

**Data Storage, Management and Analysis:** The occurrence of *Salmonella* was determined by descriptive statistics such as frequency and percentage. Chi square ( $\chi^2$ ) analysis was used to measure the association between the different risk factors and occurrence of *Salmonella* in studied

animals. In addition, the multivariable logistic regression analysis was used to calculate the association of variables with the occurrence of *Salmonella*. Before running a multivariable logistic regression analysis, all the variables were first screened for the presence of association using univariable

logistic regression analysis. Then, those having a p-value of 0.25 and less were considered for the multivariable logistic regression. The overall effects of variables on the Salmonella occurrence were reported as statistically significant if p-value is less than or equals to 5 % ( $p < 0.05$ ).

## Results

### The Over All Prevalence of Salmonella

In the present study, Salmonella was recorded in 16.4 % (63/384) of sampled animals. The isolates were higher in Haramaya University farm (24.2%), 8/33 followed by Harar town farms (22.6%), 35/155 and Haramaya District (10.3), 20/195 (Table 2).

**Table 2:** The Over All Prevalence of Salmonella.

Study Area	No. of Animals Examined	Salmonella Positive Animals (%)	$\chi^2$ value	p-value
Haramaya University	33	8 (24.2)	10.944	0.004
Harar Town	156	35 (22.4)		
Haramaya District	195	20 (10.3)		
Total	384	63 (16.4)		

### Salmonella Occurrence and Associated Risk Factors

Chi-square analysis shows that: age category ( $\chi^2=21.211$ ,  $P=0.000$ ), different body condition scores ( $\chi^2=11.551$ ,  $P=0.004$ ), fecal consistency

( $\chi^2=60.741$ ,  $P=0.000$ ) and management systems ( $\chi^2=10.931$ ,  $P=0.000$ ) were significantly associated with the occurrence of salmonella. However, no significant association was observed between the prevalence of Salmonella and sexes ( $\chi^2=0.183$ ,  $p=0.381$ ) (Table 3).

**Table 3:** Chi square analysis of Salmonella and associated risk factors.

Variables*		No. of Animals Examined	No. of animals with Salmonella (%)	Chi Square Analysis	
				$\chi^2$ -Value	p-Value
Management	Intensive	189	43 (22.8)	$\chi^2=10.93$	0.001
	Extensive	195	20 (10.3)		
Sex	Female	175	27 (15.4)	0.183	10.38
	Male	209	36 (17.2)		
Age	6-12 Months	205	52 (25.3)	21.2	0.000
	<6 Months	179	11 (6.1)		
Study Area	Haramaya Town	195	20 (10.3)	10.94	0.004
	Haramaya University	33	8 (23.5)		
	Harar Town	156	35 (22.6)		
Body Condition	Good	91	11 (11.7)	11.55	0.003
	Medium	163	19 (11.8)		
	Poor	130	33 (25.4)		
Fecal Consistency	Diarrheic	116	45 (38.8)	60.74	0.000
	Normal	268	18 (6.7)		

### Regression Analysis of Salmonella Occurrence

Multi-variable logistic regression analysis shows that, Salmonella infection was significantly associated with the management system, fecal consistency and age groups (Table 4). Accordingly, calves under six months of age had a higher likelihood (OR =5.2, 95% CI = 0.4-10.9,  $p < 0.000$ ) of harboring, salmonella

compared to calves of 6 to 12 months of age. Diarrheic animals had harboring Salmonella more frequently than those calves with the normal fecal matter (OR =8.7, 95% CI =0.2-19.2,  $P < 0.000$ ). calves under intensive management system had a higher risk of infection as compared to extensive management system (OR = 2.9, 95% CI=0.7-5.8,  $p = 0.002$ ) (Table 4).

**Table 4:** Regression analysis of Salmonella and associated risk factors.

Variables*	No. of Animals with Salmonella (%)	Univariable Logistic Regression		Multivariable Logistic Regression	
		OR (95% CI)	p-value	OR (95% CI)	p-value

Management	Extensive	20 (10.3)	Ref	0.001	Ref	0.002
	Intensive	43 (22.3)	2.6 (0.7-4.6)		2.9 (0.7-5.8)	
Sex	Male	36 (17.1)	1.1(0.65-0.1.9)	0.67	-	-
	Female	27 (15.5)	Ref		-	
Age	6-12 Months	11 (6.1)	Ref	0.000	Ref	0.000
	< 6 Months	52 (25.3)	4.5 (4.5-9.0)		5.2 (0.4-10.9)	
Study Area	Haramaya District	20 (10.3)	Ref	Ref	-	-
	Haramaya University	8 (24.2)	1.1 (2.3-2.5)	0.91	-	-
	Harar	35 (22.6)	2.5 (1.4-4.6)	0.002	-	-
Body Condition	Good	11 (11.7)	Ref	Ref	Ref	Ref
	Medium	19 (11.8)	1(0.4-5-2.2)	0.995	-	-
	Poor	33 (25.4)	2.5 (0.8-5.3)	0.014	-	-
Fecal Consistency	Normal	18 (6.7)	Ref	0.000	Ref	0.000
	Diarrheic	45 (38.8)	8.7 (0.2-16.1)		9.8(0.2-19.2)	

OR= Odds Ratio; Ref= Reference Variable.

### Salmonella Co-infection with Cryptosporidium Oocyst

In the current investigation from 63 (16.4%) salmonella positive animal; 40 (63.5%) were positive for Cryptosporidium oocyst, which accounts 10.4 % (40/384) out of total sample. The highest co-infection association was recorded in age categories of (< 6 months) (69.2%) than calves 6-12 month (36.4%) ( $\chi^2 = 4.23$ ,  $P = 0.045$ ). Calves with poor body condition (81.8%) significantly higher co-infection than calves with medium (52.6%) and good body condition (27.2%) ( $\chi^2 = 11.97$ ,  $P = 0.003$ ). Statistically

significant difference in prevalence of co- infection with fecal consistency. The result indicates that co-infection was highly in diarrheic calves (79.5%) than calves with normal feces (22.2%) ( $\chi^2=18.52$ ,  $p=0.000$ ). The revealed that, there was strong co-infection association between two management system ( $\chi^2 = 18.73$ ,  $P= 0.000$ ). Calves kept under intensive management system (81.4%) were found to be affected more than those kept under extensive management system (0.25). However, no significant co-infection was observed on sex of the animals ( $\chi^2=0.36$ ,  $P =0.34$ ) (Table 5).

**Table 5:** Salmonella Co-infection association with Cryptosporidium.

Variables*		No. of Animals Positive for Salmonella	No. of Animals Co-Infection with Cryptosporidium (%)	Chi Square Analysis	
				$\chi^2$ -Value	p-Value
Management	Extensive	20	5 (0.25)	18.730	0.000
	Intensive	43	35 (81.4)		
Sex	Male	36	24 (66.7)	0.360	0.34
	Female	27	16 (59.3)		
Age	6-12 Months	11	4 (36.4)	4.230	0.045
	<6 Months	52	36 (69.2)		
Study Area	Haramaya Town	20	5 (0.25)	18.90	0.000
	Haramaya University	8	6 (0.75)		
	Harar Town	35	29 (82.8)		
Body Condition	Good	11	3 (27.2)	11.970	0.003
	Medium	19	10 (52.6)		
	Poor	35	27 (81.8)		
Fecal Consistency	Normal	18	4 (22.2)	18.520	0.000
	Diarrheic	45	36 (79.5)		

\*Means values are based on Fisher's exact test.

### Antibiotics Susceptibility Profile of Salmonella Isolates

All the 63 isolates were tested against eight commonly used antimicrobials. All isolates were resistant at least to one or more antimicrobials. The antibiotic

susceptibility profiles of the isolates showed that; the isolates were 95.2%, 80.9% and 73.0% resistant to Nalidixic acid, Amoxicillin and Cefoxitin, respectively. However, the isolates were found to be susceptible to ciprofloxacin (100 %) and chloramphenicol (87.3%)

(Table 6). Multiple antimicrobial resistances (resistance to two or more antimicrobials) were detected in 95.2% (60/63) of the isolates. A total of seven different antimicrobial resistance patterns were observed. The most frequent MDR pattern consisting three drugs were found against Nalidixic acid,

Amoxicillin and Cefoxitin with a resistance of (95.2%), (80.9%) and (73.0%) respectively. The isolates were found to be resistant to four antimicrobial drugs namely, Streptomycin (46.0%), Erythromycin (25.5%) Ampicillin (23.8%) and Chloramphenicol (7.9%) (Table 7).

**Table 6:** Antibiotic susceptibility result of Salmonella isolates.

Antimicrobials	No. of Isolates (%)	No. of Susceptible Isolates (%)	No. of Intermediate Isolates (%)	No. of Resistant Isolates (%)
Ampicillin	63	38 (60.3)	10 (15.8)	15 (23.8)
Ciprofloxacin	63	63 (100)	0 (0)	0 (0)
Chloramphenicol	63	55 (87.3)	3 (4.7)	5 (7.9)
Cefoxitin	63	17 (26.9)	0 (0)	46 (73.0)
Erythromycin	63	35 (55.5)	12 (19.0)	16 (25.3)
Nalidixic acid	63	0 (0.00)	3 (4.8)	60 (95.2)
Streptomycin	63	25 (39.7)	9 (14.3)	29 (46.0)
Amoxicillin	63	0 (0.00)	12 (19.0)	51 (80.9)

**Table 7:** Multi drug resistance of Salmonella isolates.

Isolates with The Same Pattern	Antimicrobial Resistance Pattern	No. of Antimicrobials Developed Resistance
18	NA, FOX	2
11	NA, FOX, AML,	3
11	NA, FOX, AML, S	4
13	NA, FOX, AML, S, ERY	5
9	NA, FOX, AML, S, ERY, AMP	6
1	NA, FOX, AML, S, ERY, AMP, C	7

AML=Amoxicillin, AMP=Ampicillin, FOX=Cefoxitin, C= Chloramphenicol, S=Streptomycin, ERY = Erythromycin, NA= Nalidixic Acid

## Discussion

In the present study, from a total of 384 diarrheic and non-diarrheic calves, 16.4 % of the isolates was positive for Salmonella. This finding was relatively comparable with the report of (Sunday et al., 2016) (13%) and (Manickam et al., 2017) (18.33%). The current result was higher than that of (Dereje, 2012) (5%) and lower than that of (Abunna et al., 2017) (43.75%) and (Abdullah et al., 2013) (21.9%). The variations in the Salmonella isolates found in the present study in Ethiopia as well as in other countries could possibly be due to variations in climatic conditions, management practices, including hygienic conditions, time of first colostrum feeding and housing system of the farms.

Significant association was observed between the prevalence of Salmonella and faecal consistency; where diarrheic calves had harboring Salmonella more frequently than those calves with the normal faecal matter. Accordingly, animals with diarrheic fecal condition had 8.7 times the chance of harboring Salmonella (OR=8.7, p-value=0.000) than animals with normal fecal matter. This finding is in line with the report of (Moussa et al., 2010) (43.5%)

and (27.69%) in diarrheic and apparently healthy contact calves respectively. The result also shows that the calves with severe diarrhea had highest percentage positivity to bacterial scours. This affirms the virulent ability of the organism to destroy the mucosa of the intestinal lining which affects the absorption and secretion ability of the gastro intestinal system and result in profuse watery foul smelling diarrhea. The study revealed that the occurrence of the Salmonella infection was significantly associated with the age of the calf, indicating the calves under six months of age had a higher risk of infection as compared to the older ones (OR =4.5, p = 0.000). This finding was relatively comparable with the report of (Adeladlew, 2020) and (Temesgen, 2004) that calves aged between 0-3 months were at a higher risk of diarrhea, particularly during the first week of life and the risk decreased with age. This was well explained by (Godden, 2007), (Mellor and Stafferd, 2004) who reported that the structure of the bovine placenta impedes easy acquisition of immunoglobulins by unborn calves during pregnancy and therefore calves are born without circulating protective antibodies, therefore they are more susceptible to various pathogens. The present study

showed that the occurrence of the Salmonella infection was significantly associated with the management system applied indicating that, calves under intensive management system had a higher risk of infection as compared to extensive management system (OR = 2.9,  $p = 0.002$ ). (Charles et al., 2003) indicated that gaps in management includes inadequate nutrition, exposure to severe environment, insufficient attention to the new born calf, or a combination of these factors are often involved in scours outbreaks. The study identified that, Calves with good body condition had high probability of being infected by Salmonella as compared with calves of poor body condition but statistically not significant ( $P=0.995$ ). Similarly, higher infection was recorded in male calves than female, but statistically not significant ( $p=0.670$ ).

In the present study, all Salmonella positive animals were subjected for analysis of co infection association status with Cryptosporidium oocyst. Generally, 63.5% (40/63) of Salmonella positive animals were positive for Cryptosporidium oocyst. A statistically significant co-infection was observed on age < 6 month ( $\chi^2 = 4.23$ ,  $P = 0.045$ ), diarrheic calves ( $\chi^2=18.52$ ,  $p=0.000$ ), poor body condition scores ( $\chi^2 = 11.97$ ,  $P = 0.003$ ) and intensive management system ( $\chi^2 = 18.73$ ,  $P= 0.000$ ). However, no significant co-infection was observed on sex of the animals ( $\chi^2=0.36$ ,  $P =0.34$ ). A strong co-infection was observed on the types of fecal consistency, where, Positive sample were higher in diarrheic calf than non-diarrheic one which was statistically significant ( $\chi^2=18.52$ ,  $p=0.000$ ). This is in accordance with the reports of (Brar et al., 2017), who reports that, 40% of *C. parvum* positive fecal samples were also positive for Salmonella spp. This is could be due to the fact that Cryptosporidium is an enteric parasite that causes the loss of epithelial cells and microvillus of the intestine as well as a reduction in the absorptive surface area of the intestine, resulting in diarrhea (Brook et al., 2008). Lorenz et al., (2011) reported that; this is probably due to free cycling of these bacteria between animals in a limited host environment once the pathogen gets access to the farm in animals kept indoors. It can be inferred that calves reared in extensive management have low risk of infection and are more resistant while those reared in intensive are exposed to causes of infection and less resistant to infection. Reasons for this could be poor hygiene in intensive, feeding animals with contaminated concentrates, hay and forages animal to

make choice of what to eat could also be a contributing factor.

Improper use of antimicrobials in both human and veterinary medicine has contributed to development and dissemination of antimicrobial resistant pathogens (Tajbakhsh et al., 2012). Out of all 63 isolates tested 60 (95.2%) found to be resistant to one or more antimicrobials. This is in agreement with the report of (Abunna et al., 2017) (96.4), higher than the report of (Zewdu, 2009) (32.7%) and lower than the report of (Adeladlew, 2020) (100%) from Bahir Dar. In the current investigation, the resistance profile of ampicillin (23.8%) and cefoxitin (73.0) were lower than reported by (Adeladlew, 2020) (100%). Manickam et al., (2017) reported (75%) resistance to ampicillin which was higher than the result found in this study.

On the other hand, the sensitivity of Salmonella to ampicillin (23.8%) was lower than reports of (Mulaw, 2017) (94.4%) and cefoxitin (73.0%) was higher than reports of (Mulaw, 2017).

(36.6%). Study in Alexandria Egypt (Mohamed et al., 2011) reported that 85.7% of Salmonella species isolated from dairy cattle were sensitive to ampicillin. This result strongly disagrees with the current study, in which 23.8% of the isolates were resistant to ampicillin. Resistance rates to ampicillin were very high when compared to results documented in America (Blau et al., 2005) reported as 4.4 % resistance levels. Similarly, resistance level of Nalidixic acid (95.2%) relatively comparable with result of (Abunna et al., 2017) (85.7%). The resistance profile of amoxicillin (80.9%) was relatively comparable with the study reported by (Abe Kebede et al., 2016) amoxicillin (77%). Therefore the difference in the level of antimicrobial resistance from the previous study might be described from the view of differences in frequency and type of antimicrobials used in an area where the animals originated, or might arise from differences in bacterial culture and antimicrobial susceptibility testing technique (Padungtod and Kaneene, 2006). The difference observed might be also due to differences in the number of antimicrobial drugs tested in the present study and as well as diversity in their usage. In addition, in Ethiopia, the indiscriminate usage of antimicrobials for human as well as animal treatment is increasing from time to time and this might have been contributing to drug resistance. However, the sensitivity of Salmonella isolates to chloramphenicol (87.3%), and ciprofloxacin (100%) agreed with the reports of

(Mulaw, 2017). In present study the Salmonella isolates were most susceptible to Ciprofloxacin (100%). This result was similar with the result reported by (Adeladlew, 2020) (100%) from Bahir Dar.

Multi drug resistances of seven out of the sixty-three Salmonella isolates obtained in this study showed MDR to nalidixic acid (95.2%), amoxicillin (80.9%), cefoxitin (73.3%), streptomycin (46.0), erythromycin (25.3) ampicillin (23.8), and chloramphenicol (7.9) which could be the consequence of indiscriminate use of antibacterial in clinical practice. The possible reason for high rate of AMR level of Salmonella might be due to the increasing rate of irrational use of antimicrobials in the dairy farms, frequent usage both in livestock and public health, use of counterfeit drugs in animal husbandry self-medication due to easy access to antimicrobials without prescription in public health sector and administration of sub therapeutic dose of antimicrobials to livestock for prophylactic or nutritional purpose in food animals (Tadesse Birhanu et al., 2014). Most cases of calf diarrhea are likely to be mixed infection, where more than one pathogenic agent is present. In this study, we recognized the following limitations: The study was a cross-sectional study, which could not determine the causal effect relationships between independent and dependent factors. The study did not involve species identification, molecular characterization and seasonal investigation, which may limit the value of the report.

## Conclusions and Recommendations

Salmonella is one of the health problems of calves in and around Haramaya District and Harar town, West Hararge zone. Out of 384 samples, 63 (16.4 %) tested positive for Salmonella. In the current investigation from 63 (16.4%) salmonella positive animal; 40 (63.5%) were positive for Cryptosporidium oocyst. Our findings confirmed that risk factors such as types of farm management, age, body condition scores and fecal consistency were found to have a significant effect on the occurrence of salmonella and co-infection association. Sixty of the 63 isolates were resistant to two or more antimicrobials. The antibiotic susceptibility profiles of the isolates showed that the isolates were 96.8%, 80.9% and 73.0% resistant to Nalidixic acid, Amoxicillin and Cefoxitin, respectively. However, the isolates were found to be susceptible to ciprofloxacin (100 %) and chloramphenicol (87.3). In general,

pathogenic characteristics of two agents, continuous multidrug resistance patterns of salmonella, absence of effective drug for Cryptosporidium make them difficult to control and this condition makes continuous and persistency infection in the dairy farm. However, Awareness creation, adequate management and hygienic practices are paramount importance in the control and prevention of the disease. Based on the above conclusion the following recommendations are forwarded:

- Better management practices like stream-bank fencing, use all-in, all-out management system, isolate infected animals, cleaning and disinfection activities to limit environmental contamination with fecal matter and use protective materials when handling animals should be implemented.
- Use of effective drugs and treatment of animals by professionals in order to minimize the impact of infection.
- Regular antimicrobial susceptibility tests against Salmonella should be conducted in order to monitor emergence of new MDR strains.
- Veterinarians should be advised to use ciprofloxacin and chloramphenicol in their farms against diarrheal diseases of Salmonella.
- Collaboration is needed among government, professional organizations and interest groups on control and prevention of the disease.
- While the molecular-based study is required to be carried out aiming at species identification and the epidemiology of the salmonella, particular attention should be paid to the control of the disease in the study area.

## Abbreviations

BGA: Brilliant Green Agar; BPW: Buffered Peptone Water; CDC: Center for Disease Control; CFSPH: Center for Food Security and Public Health; CSA: Central Statistical Agency; ELISA: Enzyme Linked Immuno Sorbent Assay; FAO: Food and Agricultural Organization; H: Flagellar Antigen; IMViC: Indole, Methyl Red, Voges-Proskauer and Citrate Test; ISO: International Organization for Standardization; MR: Methyl Red; NCD: Neonatal Calf Diarrhea; O: Somatic Antigen; OIE: Office International des Epizooties; OR: Odds Ratio; TSI: Triple Sugar Iron; VP: Vogus-Proskauer; WHO: World Health Organization; XLD: Xylose-lysine-deoxycholate

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