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LIST OF ABBREVIATIONS

AAU	Addis Ababa University
BPLS	Brilliant green phenol red lactose sucrose
BPW	Buffered peptone water
ELISA	Enzyme Linked Immunosorbent Assay
ETEC	Enterotoxigenic *E. coli*
FAO	Food and Agriculture Organization
H_2S	Hydrogen sulfide
IgG	Immunoglobulin G
ILRI	International Livestock Research Institute
IMViC test	Indole, methyle red, voges-proskauer and citrate test
ISO	International Organization for Standardization
µm	Micrometer
mm	Millimeter
MSc	Master of science
MKTTn	Muller-Kauffmann tetrathionate with novobiocin
nm	Nanometer
MH	Muller-Hinton
RVS	Rappaport-Vassiliadis with soya
TSI	Triple sugar iron
XLD	Xylose lysine desoxycholate
XLT4	Xylose lysine tergitol4

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ABSTRACT

A longitudinal prospective observational study on calf diarrhea and mortality in dairy farms in and around Addis Ababa was conducted from October, 2006 to May, 2007 with the objectives of describing incidence of calf diarrhea and mortality and identification of potential pathogens associated with calf diarrhea. A total of 254 calves; 37 from four large dairy farms and a random sample of 217 calves from market oriented smallholder and medium-sized dairy farms were included in the study. Each calf was individually identified and regularly monitored for clinical health problems up to the age of six months. Information on different management aspects were collected by personal observation during the regular visit to farms and from questionnaire survey conducted during the study period. Fecal samples were also aseptically collected from diarrheic calves for laboratory examination to detect entropathogens involved. Rotavirus, Coronavirus, *Escherichia coli* K99 and *Cryptosporidium parvum* were detected using antigen ELISA. *Salmonella* were isolated and identified following ISO 6579 (2002) and Quinn *et al*. (1994). Antimicrobial susceptibility test for *Salmonella* isolates was also done following NCCLS (1997) guidelines. The overall incidences of calf diarrhea and crude mortality found in this study were 33.6% and 11.6%, respectively. Other disease conditions that were diagnosed in calves included navel ill, joint ill, pneumonia, septicemia, congenital loss of vision and other miscellaneous cases. The incidence of calf diarrhea was apparently higher in medium-sized and large dairy farms than smallholder dairy farms while the incidence of calf mortality was higher in smallholder and large dairy farms. Based on laboratory examination, Rotavirus, Coronavirus, *E. coli* K99, *Cryptosporidium parvum* and *Salmonella* were detected from diarrheic calves at rate of 1/36 (2.8%), 3/36 (8.3%), 8/36 (22.2%), 23/36 (63.9%) and 6/36 (16.7%), respectively. The serotypes of *Salmonella* identified were *Salmonella* Typhimurium (4/6), *S*. Dublin (1/6) and *S*. Mishmarhaemek (1/6). Of the serotypes tested for resistance to a panel of 13 antimicrobial agents all isolates of *Salmonella* Typhimurium showed intermediate resistance to one or more drugs. *Salmonella* Dublin was intermediate in resistance to tetracycline, kanamycin and nalidixic acid. All serotypes tested were resistant to erythromycin. In conclusion, the incidence of calf diarrhea and mortality found in this study were high and could affect dairy production through substantial economic losses mainly due to morbidity, cost of treatment, mortality and future productivity. All potential pathogens were detected in diarrheic calves. Some of these pathogens such as

Cryptosporidium and *Salmonella* are zoonotic and hence can serve as source of infection to humans. Control and management of calf diarrhea should focus on reducing exposure to the infectious agents and optimizing the calves' resistance to them.

Keywords: Coronavirus, Rotavirus, *E. coli* K99, *Cryptosporidium parvum, Salmonella*, Calf, diarrhea, dairy farms, incidence, mortality, Addis Ababa, Ethiopia

1. INTRODUCTION

Calf rearing is a practice to provide conditions in which the animal can survive. Good management such as colostrum provision, good nutrition, penning and housing helps healthy production. Overcrowding, poor ventilation, poor hygiene and incorrect nutrition on the other hand aid a disease. Thus the principal factor in the achievement of success or failure is management (Snodgrass *et al*., 1986).

Calves are important assets in that the future of dairy and beef herds depends on rearing healthy calves to replace cows that leave the herd (Hartman *et al*., 1997). In the tropics calf losses have been known to be as high as 50% of the calf crop due to bad management, poor adaptation of exotic dairy breed to the prevailing tropical environment and endemic diseases (Kifaro and Temba, 1990).

Information regarding the association between calf diseases and management has in the past been neglected. However, as the price of calves has risen, it tends to result in greater interest in their rearing (Reynolds *et al*., 1986).

Infectious diarrhea in neonatal animals is one of the most common and economically devastating conditions encountered in the animal agriculture industry (Sevun *et al*., 2005). Calf diarrhea or commonly called calf scours, is a multifactorial disease entity that can have serious financial and animal welfare implications in both dairy and beef herds. It has been estimated that 75% of early calf mortality in dairy herds is caused by acute diarrhea in the pre-weaning period (Roderick and Hovi, 1999).

The occurrence of diarrhea in calves is a result of the complex interactions of three sets of factors: the calf and the dam, the calf's environment, including management, and infectious agents (Radostits and Acres, 1980; Northeim and Simensen, 1985; Cockram and Rowan, 1989; Besser *et al*., 1991; Lance *et al*., 1992).

Four mechanisms have been demonstrated to be important in occurrence of diarrhea in calves. Hyper secretions of ions and water into the bowl, increased osmotic pressure from maldigestion-malabsorption disease caused by damage to enterocytes, increased mucosal permeability due to inflammation and alteration of the intestinal motility (Hunt, 1993).

There are numerous infectious causes of calf diarrhea and as the clinical signs are general, it is not possible to identify or predict a specific cause from the clinical signs observed. The most important infectious causes of diarrhea in calves include, Rotavirus, Coronavirus, enterotoxigenic *E. coli*, *Cryptosporidium* and *Salmonella* species (Reynolds *et al*., 1986; Snodgrass *et al*., 1986; Abraham *et al*., 1992). Mixed infections with more than one of the causative agents are common. All the recognized infectious causes of calf scours are commonly found within the cattle rearing environment and *Cryptosporidium* and Rotavirus are thought to be present in all herds. It is not possible to eradicate these infections from farms and therefore the risk of infection is always present (Snodgrass *et al*., 1986). Some microbial pathogens associated with calf diarrhea such as *Salmonella* and *Cryptosporidium parvum* are also pathogenic to humans (Lorenzo *et al*., 1995; D'Aoust, 1997).

In Ethiopia, although diarrhea is an important cause of calf morbidity and mortality, studies done to quantify the magnitude of the problem and to determine the underlining causes are scarce. Few studies undertaken were done on dairy farms owned by government and research centers and are less relevant to dairy farm set-up in the country. Therefore, the present study was undertaken with the following objectives: -

- to describe the incidences of calf diarrhea and mortality
- to identify the infectious agents associated with calf diarrhea
- to determine the serotypes of *Salmonella* and their antimicrobial resistance patterns to the commonly used antimicrobials

2. LITERATURE REVIEW

2.1. The calf and special features of its body system

Calf refers to the age group of young cattle from birth to six or nine months (West, 1995). The term calf, in less intensive system of production, may generally include cattle older than the age indicated in the above definition. The proportion of calves weaned before six months of age increase from less intensive to more intensive system of production (ILRI, 1996).

Calves have some special features in their body system that have relevance in disease occurrence and accordingly requires special attention in management. Those that have particular importance are the poorly developed defense mechanism and a dynamic digestive system that has to evolve from milk digestion system to a solid feed digestion. This makes calves particularly susceptible to diseases. In normal development, in which calf grows with its mother, nature has a correcting mechanism (Bath *et al*., 1985).

A newborn calf has poorly developed defense mechanism. The normal flora is not well established and calves are born with no or extremely low blood immunoglobulin concentrations to combat infection. Yet the calf must survive in highly contaminated environment (Bath *et al*., 1985; Wereme *et al*., 2001).

In the digestive system of newborn calves there are certain alterations. These include delayed acid secretion from stomach for several days, similarly delay in the development of pancreatic function thus; acid and trypsin digestion of protein is not active. On the other hand, rate of immunoglobulin absorption by the calf intestine begins declining shortly after birth and the intestinal lining is closed to further immunoglobulin absorption into the blood by about 24 hours after birth (Cunningham, 1992). This process of declining rate of immunoglobulin absorption in the calf's intestine is referred to as closure. Preweaned calves are considered as monogastrics. The abomasum in this age group, in contrast to adult ruminant, makes up to 70% of the total stomach volume (Radostits *et al*., 1994).

2.2. The care of young calves

2.2.1. Management of the calving cow

Prior to parturition, the calving cow should be sheltered. This has three major advantages: First, the cow and her calf will obtain maximum protection from predators at this particular vulnerable stage in their lives; second, the practice allows a strong dam-offspring bond formation; third, calf delivery into a relatively uncontaminated environment (Selman, 1981).

The use of indoor calving accommodation is not usually necessary in tropical areas and is rarely advisable. Indoor calving may lead to high neonatal losses due to infection build-in in calving pens and interference factors which adversely affect early suckling and significant colostral globulin absorption. If absolutely unavoidable, indoor calving should occur in clean, well-littered, well-lighted, loose boxes with surveillance to avoid mismothering and to insure early suckling (Selman, 1981; Quigley, 1997).

2.2.2. Management of the newborn calf

Once on its feet, a newborn calf almost immediately initiates teat-seeking activities. Calf's teat-seeking activity is largely directed upwards towards the highest part of the dam's underbelly. In most beef cows and in dairy heifers, this is usually where the teats are located and early suckling is possible. However, in a large proportion of mature dairy cows the highest point of the underbelly is the xiphoid region; teat-seeking is thus directed anteriorly (i.e., away from the teats) and first suckling is delayed. Therefore, it is essential that animal attendants and veterinarians should not assume that by leaving a cow and calf together in loose box, suckling will inevitably ensue. Consequently, whenever possible, and when faced with a neonatal calf diarrhea problem, careful attention is necessary to ensure early suckling. If for any reason the calves are not groomed by their dams it is important for attendants to dry them off as soon as possible. Many experienced cattle attendants advise spraying or otherwise dressing the navels of newborn calves with an antibiotic preparation as soon as possible after birth to prevent umbilical infections (Selman, 1981).

2.2.3. The importance of colostrum

Colostrum is the thick secretion which is normally present in a cow's udder around the time of parturition. It has long been accepted that an early feeding of colostrum is essential if a calf is to survive the neonatal period without serious illness. A great deal of work involving a vast number of different techniques has shown that the "protective factor" in the colostrum is immunological and that the colostrum is rich source of immonoglobulins (antibodies) if only for a limited period (i.e., a day or two) after calving. It is also a rich food source with laxative properties (Selman, 1981; Wereme *et al*., 2001).

The newborn calf is normally born devoid of significant amount of immunoglobulins and is totally dependent upon acquiring these from colostrum (Bath *et al.,* 1985). For a limited period only, its intestinal epithelial cells are capable of absorbing intact immunoglobulin molecules (and many other macromolecules, also) (Wereme *et al*., 2001). If due attention is paid to factors which are of prime importance in respect to immunoglobulin absorption in very young calves, it is possible to bring about maximal absorption rates, and hence high post-colostral serum immunoglobulin concentrations, thereby giving a calf maximum chance of survival. The most important factors to be considered in this context are (i) the timing of the first feed of colostrum, (ii) the mass of immunoglobulin consumed and (iii) whether or not a calf receives its colostrum in the presence of its dam. Breed factors also exist but are not usually considered to be of practical significance (Selman, 1981).

From an immunological view point, further colostrum feeding is not necessary since intestinal absorption of intact immunoglobulin molecules is unlikely to occur after 24 hours. One school of thought, however, suggests that further feeding is beneficial; immunoglobulins may exert a "local' effect on the intestinal epithelium. Colostrum is also a highly nutritious product which does not harm and probably does a little good even when fed at a late stage. If for any reason a calf dam has no colostrum, deep-frozen colostrum or colostrum from another calving cow (Selman, 1981) and more recently, colostrum replacers which are essentially produced by the newly established colostrum industry (Wereme *et al*., 2001) may be used.

2.2.4. Feeding and management to weaning

Dairy calves are sometimes reared by single or more commonly, multiple suckling systems. However, this is applicable to small scale enterprises; more often, this type of calf is reared artificially. Dairy calves may be reared indoors, outdoors, or on the "shelter-and-run" principle. In each of these systems calves may be housed in single pens, in small numbers in large pens or in large loose-housed group of 20 or more animals. There is some evidence to show that calves reared in groups are more socially adjusted than calves reared in single pens. On the other hand, certain infectious diseases spread more easily through group-housed calves and it is far easier for them to develop unpleasant and potentially damaging habits such as intersucking and urine drinking (Kung *et al*., 1997).

It is essential that calves be provided with a dry resting area and if they are housed, the building should be airy and well-ventilated but relatively drought free. If large groups of calves are housed together, size matching is important to minimize bullying. The other major consideration is hygiene. It is very important to ensure that adequate alternative accommodation is available so that indoor pens can be depopulated regularly and disinfected, and outdoor pens and paddocks can be moved or rotated around other parts of the farm. Control of vermin is another important point (Selman, 1981).

Despite the relatively higher cost involved, many dairy farmers prefer to feed raw cow's milk during the preweaning period; milk is convenient, home-produced and in regular supply; in addition, if it is fed fresh and undiluted, there is little that can go wrong with it (Selman, 1981).

2.3. Calf diarrhea in dairy farms

Calf diarrhea is the passing of abnormally high amounts of fluid in the feces. This may be due to excessive passing of fluid into the intestine from the body or the failure to absorb a sufficient quantity of fluid from the contents of the intestine during the digestion process. As a result, the nature of the feces can vary from a pasty texture to pure liquid. Depending on the severity of disease, blood and products of inflammation resembling the lining of the gut can be passed in the

feces. If the calf is able to drink equivalent volume of fluid to that being lost in the feces it is likely to remain bright and alert. If the calf is not able to do this, it will suffer a net loss of fluid from the body and become dehydrated. This can be noted clinically as slightly "sunken eye" if severe can lead to collapse, circulatory failure and death. In addition, in some cases a metabolic acidosis may occur more rapidly than dehydration. In such cases the calf may be unable to stand and be severely depressed before severe signs of diarrhea are seen. Failure to recognize this state and seek veterinary attention will result in the death of the calf (Scott *et al*., 2004).

2.3.1. Economic importance

Diarrhea in young calves causes substantial economic losses mainly due to morbidity, cost of treatment and mortality. Calf diarrhea is the commonest disease in young calves and is the greatest single cause of death (Gitau *et al*., 1994; Sivula *et al*., 1996a; Busato *et al*., 1997; Heinrichs and Radostits, 2001). It accounts for approximately 75% of the mortality of dairy calves under three weeks of age (Blowey, 1990). An animal that has been sick as a calf often has other health problems later in life which in turn may result in a decrease in productivity. Morbidity due to calf diarrhea causes direct cost for treatment and nursing, affects days at first calving, dairy herd survivorship and future productivity. Economic losses resulting from calf hood morbidity and mortality can be easily recognized, but the effect of morbidity on future health and performance, which may constitute a loss of even greater importance, is difficult to estimate. For example, heifers that had been treated for diarrhea were 2.9 times more likely to calve after 900 days of age than other heifers (Waltner-Toews *et al*., 1986a).

Calf diarrhea has a multifactorial etiology, resulting from an interaction between the calf, its environment, nutrition and infectious agent. Several such agents may cause diarrhea, but it should be emphasized that solving these problems on a herd basis, requires the identification of poor management practices rather than focusing solely on the causative agents involved. Pursuing a specific etiological diagnosis is, however, necessary in order to determine the antibiotic sensitivity of the bacteria involved; allow for the implementation of specific therapeutic and prophylactic measures; and if feasible, institute a vaccination program. A dairy farm system could employ a strategy that will reduce calf mortality and improve calf performance by

controlling diseases. In a good management practices, annual mortality of calves under one month of age can be reduced to below 3 - 5% and first calving age around 24 months (Heinrichs and Radostits, 2001).

2.4. Major microbial causes of calf diarrhea

Bovine neonatal diarrhea is a multifactorial disease. In addition to the influence of varied environmental, managemental and nutritional factors, the infectious agents capable of causing diarrhea in calves include viruses (Coronavirus or Rotavirus), bacteria (*Salmonella*, pathogenic strains of *E. coli*) and protozoa such as *Cryptosporidium parvum* (Waltner-Toews *et al*., 1986b; Reynolds *et al*., 1986; Snodgrass *et al*., 1986; Abraham *et al*., 1992).

2.4.1. Bovine rotavirus

Bovine rotavirus (BRV) belongs to the family Reoviridae. Bovine rotaviruses are nonenveloped wheel-shaped viruses with double-stranded RNA genome (Saif, 1992b). The outer capsid forms nearly spherical icosahedron; it consists of glycoprotein VP7 from which dimmers of VP4 extend. The outer capsid and middle capsid, which is composed of VP6, are dissociated readily from the core, which is composed of three proteins, VP1, VP2 and VP3 (Murphy *et al*., 1999b).

The classification of the member viruses of the genus Rotavirus is based on genotypic and serologic analyses. There are six groups of the genus Rotavirus. Variance in the group-specific capsid antigen on VP6 is used to define major groups. Of the six groups, Group A Rotavirus is the most prevalent and clinically important that contains several serotypes of different virulence (Murphy *et al*., 1999b). Another source of antigenic variation among Group A BRV is the serotype-specific antigens, present on outer capsid proteins (VP4 and VP7) of the virus. These antigens induce neutralizing antibodies and are important for development of immunity (Saif, 1992b).

Disease caused by Rotavirus is usually seen only in young animals, 1 to 8 weeks of age, but only rarely during the first week of life. Infection by Rotavirus leads to the destruction of epithelial

cells of the apices of villi in the small intestine. With virulent stains of Rotavirus, the loss of enterocytes exceeds the ability of the intestinal crypts to replicate; hence, the villi height is reduced with a consequent decrease in intestinal absorptive area and intestinal digestive activity leading to diarrhea (Aiello, 1998). Villi become covered with cuboidal epithelial cells that migrate from the crypts. These cells secrete reduced level of disaccharidases such as lactase and are less able to carry out glucose-coupled sodium transport. Undigested lactose in the milk due to this reduced lactase secretion promotes bacterial growth and exerts a further osmotic effects; both mechanisms contribute to the diarrhea (Murphy *et al*., 1999b).

2.4.2. Bovine coronavirus

Bovine coronavirus (BCV) belongs to the family Coronaviridae. Bovine coronavirus is second to Rotavirus as a cause of calf diarrhea. Bovine coronavirus is an enveloped pleomorphic virus with RNA genome (Murphy *et al*., 1999a; Saif, 1992a). It contains two layers of peplomers. The virion is composed of four major structural proteins, including the matrix protein (E1), the nucleocapsid protein (N), the peplomer protein (E2) and the hemagglutinin (E3). This latter protein accounts for the ability of BCV to hemadsorb rat, mouse and hamster erythrocytes. Both the E2 and E3 protiens elicit neutralizing antibodies and may be important for induction of immunity (Saif, 1992a).

Coronaviruses have the largest genome of all RNA viruses and replicate by a unique mechanism, which results in a high frequency of recombination (Lai and Holmes, 2001). Bovine coronavirs causes enteritis in 3 to 21 days old calves, but clinical infections can occur in calves up to 3 months of age. Bovine coronavirus infection of the upper respiratory tract have also been described, but with few clinical signs of respiratory disease, although intranasally inoculated calves also developed diarrhea and shed BCV in the feces (Saif, 1992a).

The pathogenesis of BCV enteritis is similar to that of Rotavirus diarrhea. The BCV infects villous enterocytes principally of the distal small intestine and superficial and crypt enterocytes in the colon. Loss of these mature absorptive enterocytes leads to a malabsorptive diarrhea (Murphy

et al., 1999a; Saif, 1992a). The virus also replicates in the epithelium of upper respiratory tract and occasionally the lungs (Saif, 1992a).

Infections with BCV occur as enzootics and 90 to 100% of dairy cows tested are seropositive for BCV. Within a herd, reservoirs of infection may be clinically infected calves or chronically infected calves or cows (Saif, 1992a). Bovine coronavirus was the predominant pathogen detected from diarrheic calves in one study in Ethiopia (Abraham *et al*., 1992).

2.4.3. *Escherichia coli*

All calves become colonized within a few hours of birth with many varied strains of *E. coli*. This constantly changing population of organism inhabits the calf's intestine for life and is entirely normal and healthy (Quinn *et al*., 2002). Some strains of *E. coli* have the ability to adhere to the intestinal wall and produce toxins that cause scours. An example of this is *E. coli* K99, which is referred to as an enterotoxigenic *E. coli* K99 (ETEC). This strain is capable of causing disease in calves of less than one week of age (Reynolds *et al*., 1986; Abraham *et al*., 1992). The duration of clinical disease is limited to few days. The organism causes little in the way of damage to the intestine, but leads to rapid fluid loss. Other strains of *E. coli* have very occasionally been shown to cause calf scours. Diagnostic laboratories do not routinely attempt to identify such strains (Scott *et al*., 2004).

Typing of *E. coli* is based on the somatic (O), flagellar (H) and capsular (K) antigens. The somatic antigens are lipopolysaccharide in nature and located in the cell wall; carbohydrate side chains determine the specificity of these antigens. The flagellar antigens are protein in nature and the capsular antigens are composed of polysaccharides. Proteinaceous fimbrial (F) antigens act as adhesions facilitating attachment to mucosal surfaces (Quinn *et al*., 2002).

Escherichia coli causes two common diseases of newborn calves. Collisepticemia in which the bacteria invade the systemic circulation and internal organs, and enteric collibaccilosis in which the bacteria are localized in the lumen and mucosal surface of the small intestine. ETEC usually

interact positively with other pathogens like Rotavirus and *Cryptosporidium* to cause diarrhea in calves (Snodgrass *et al*., 1982; Runnel *et al*., 1986).

The virulence factor of pathogenic strains of *E. coli* includes capsule, endotoxin, structures responsible for colonization, enterotoxin and other secreted substances (Quinn *et al*., 2002).

Capsular polysaccharides interfere with the phagocytic uptake and antibacterial effectiveness of the complement system. The role of endotoxin includes pyrogenic activity, endothelial damage leading to disseminated intravascular coagulation, and endotoxic shock. Fimbrial adhesions allow attachment to mucosal surfaces in the small intestine. The most significant adhesions in strains of *E. coli* producing disease in domestic animals are K88 (F4), K99 (F5), 987P (F6) and F41. An adhesin, termed intimin, appears to be necessary for the binding of enteropathogenic *E. coli* to enterocytes. (Quinn *et al*., 2002).

The pathological effects of infection with pathogenic *E. coli*, other than those attributed to endotoxin, derive mainly from the production of enterotoxins, verotoxins or cytotoxic necrotizing factors. Unlike enterotoxins which affect only the functional activity of enterocytes, verotoxins and cytotoxic necrotizing factors can produce demonstrable cell damage at their sites of location. Alpha-haemolysin, although often a useful marker for virulence in certain strains of *E. coli*, does not appear to contribute directly to their virulence but is closely linked with the expression of other virulence factors. Siderophores, iron-binding molecules such as aerobactin and enterobactin, are synthesized by certain pathogenic *E. coli*. When these substances are available, iron level in the tissues are low, while they may contribute to bacterial survival (Quinn *et al*., 2002).

Some strains of *E. coli* produce a substance called colicin that kills competing strains. The producing strains are immune to the action of this chemical but reproduce at a reduced rate because some of their metabolic energy is devoted to its production (Chao and Levin, 1981).

2.4.4. *Salmonella*

Salmonella has been widely reported in cattle, and infected animals may shed the organism in their feces without showing any clinical signs of disease. There are over 2500 different *Salmonella* serotypes, and all are considered pathogenic to humans. Serotyping is based on the Kaufmann and White scheme in which somatic (O) and flagellar (H) antigens are identified. Occasionally, capsular (Vi) antigens may be detected. In a modification of this scheme, two species are proposed, *S*. enterica and *S*. bongori (D'Aoust, 1997; Quinn *et al*., 2002; OIE, 2004). Relatively few serotypes are associated with cattle and of these ones, *Salmonella* enterica subspecies enterica serotype Dublin (*S*. Dublin) and *S*. enterica subspecies enterica serotype Typhimurium (*S*. Typhimurium) are the most common (McEvoy *et al*., 2003).

Salmonella serotypes especially *Salmonella* Typhimurium and *S*. Dublin and occasionally others cause diarrhea in calves of 2 - 12 weeks of age (Aiello, 1998). The virulence of *Salmonella* relates to their ability to invade host cells, replicate in them and resist both digestions by phagocytes and destruction by the complement component of the plasma (Quinn *et al*., 2002). The disease is seen as profuse diarrhea with septicemia and death may occur within 24 hours. Chronic forms of the disease are also common, in which the affected calf simply has pasty dung and unthriftness. At the far end of the spectrum, some calves may carry the infection without suffering any adverse effects (Aiello, 1998).

2.4.5. *Cryptosporidium*

This is a protozoan parasite which causes scours in many domestic species including man. *Cryptosporidium parvum* is the most common species found in calves and man, and is transmitted readily to several newborn species of mammals by the fecal oral route (Scott *et al*., 1995). *Cryptosporidium parvum* has emerged recently as a prevalent enteropathogen affecting humans and many mammalian species throughout the world. Most reports have cited *C*. *parvum* concomitantly with other enteropathogens especially with Rotavirus and Coronavirus (Lorenzo *et al*., 1995). There have also been reports of outbreaks of diarrhea where *C*. *parvum* has been the only pathogen isolated (Scott *et al*., 1995).

This protozoan parasite mainly infects the intestinal tract and rarely the respiratory tract of animals and people. The damage caused by *C. parvum* infection is similar to that caused by Rotavirus and the disease is more severe and lethal when complicated with other enteropathogens such as *E. coli*, *Salmonella*, Rotavirus, Coronavirus infections, and immunocompromised individuals (Arslan *et al*., 2001). The source of the parasite is thought to be either the adult cows which acts as carriers without showing signs of the disease or infected scouring calves passing the parasite in their feces. The infectious dose of the organism is very low and therefore hygiene is very important for disease control. The disease process is slightly more protracted than for the viral infections and recovery therefore takes a few days longer and can be expected to be completed by ten days (Snodgrass *et al*., 1986).

Infection with *Cryptosporidium* is more commonly reported in calves under 1 month of age, and affected calves may shed large numbers of infective oocysts in the feces. While infection is generally self-limiting, fatalities associated with cryptosporidiosis have been reported (Trotz-Williams *et al*., 2005).

The parasite adheres to the apical surface of enterocytes in the distal small intestine and the colon. This result in loss of microvilli, decreased mucosal enzyme activity with villous blunting and fusion leading to reduced villous surface absorptive areas, and inflammatory changes in the submucosa (Heath, 1992). Diarrhea due solely to *Cryptosporidium* species is often mild and self-limiting, although the severity may be related to the general strength of the calf and to the intensity of challenge with the organism (Corwin, 1992).

2.5. Determinants of calf diarrhea

2.5.1. Calf factors

The most important non-specific calf risk factors for calf diarrhea are related to breed of the calf, age of the calf, vigor and health of the calf at birth. A difference in susceptibility of calves to diseases is often observed among different breeds. Taurine breeds and their crosses are generally more susceptible to diseases in tropical climates. Susceptibility of calves to diseases also differs

among calves of temperate dairy breeds (Olsson *et al*., 1993; Agerholm *et al*., 1993). The age of the calf is one of the most important factor affecting morbidity and mortality. Virtala *et al*. (1996) reported that the peak occurrence of diarrhea and crude mortality are at the second week of life of calves.

Several factors affect the health and vigor of calves immediately after birth. Calves born from dams with inadequate nutrition at late pregnancy or affected with prolonged anorexia, fever, or septicemia may be weak. Dystocia or prolonged parturition affects the calf's survival (Sivula *et al*., 1996b). Research findings have indicated that metabolic, respiratory and mixed acidosis develop frequently at birth in calves, as a result of prolonged or difficult labor and dystocia. This acidosis at birth may have detrimental effect on colostral immunoglobulin absorption (Besser *et al*., 1990; Drewry *et al*., 1999).

2.5.2. Environmental and managemental risk factors

Farm Personnel, feeding management, housing, herd size and season of the year are among the most important environmental and managemental risk factors related to incidences of calf diarrhea. Fewer calf losses were observed on farms where the owner managed the calves than on farms where employees performed the duties (Britney *et al*., 1984). Similarly, low calf morbidities were seen in herds owned by older and more experienced managers (Heinrichs and Radostits, 2001).

Wittum *et al*. (1994) indicated that calves with inadequate blood colostral immunoglobulin concentration in 24 hours of birth were at greater risk of neonatal calf diarrhea. The transfer of passive immunity via colostrum from dam to the neonatal calf is believed to be affected by several factors including age at first feeding, volume and concentration of the immunoglobulin in the colostrum fed, birth weight, method of feeding, seasonal influence, stress, use of colostral supplements, etc. (Drewry *et al*., 1999).

As to seasonal variation, a study by Gay *et al*. (1983) showed that the level of colostral immunoglobulin in calves' serum was higher during summer than winter. There is also evidence

that mastitis during dry period of cows decreases the concentration of IgG, particularly IgG1 in the colostrum of the next lactation (Komine *et al*., 2000). If a transition from liquid pre-weaned feed to solid weaned calf feed is not done carefully, the calf will get dietary stress and be susceptible for different diseases (Blowey, 1990).

Different calf housing types have been associated with different rates of calf morbidity and mortality. Morbidity and mortality rates are usually higher in calves housed indoors than outdoors. This is often attributed to a combination of inadequate control of thermal environment, poor air quality, undesirable relative humidity, inadequate exchange of air and poor sanitation (Blowey, 1990). A study by Olsson *et al*. (1993) indicated that keeping calves in single pen decreased incidence of enteritis as compared to group pens.

Herd size also influences the incidence of infectious diseases. In small herd sizes enough time may elapse between successive births, which will reduce the concentration of infectious agents in the calf-rearing environment. Time available to observe and care for calves is also one factor. Garber (1994) found higher prevalence of *Cryptosporidium* associated with large herd size in dairy farms.

Season is important in areas where the climate goes to either extremity. In areas where winter temperature is very cold there will be more mortality of calves that are born in this season (Aiello, 1998). In tropical areas high mortality was reported in hot dry season which could arise from shortage of feed in dry season (Hailemariam *et al*., 1993).

Other environmental and managemental risk factors that affect calf morbidity and mortality include: dam preventive practices by vaccination, the sanitation of calving area, perinatal care, grazing level (whether zero grazing, partial grazing or total grazing), level of herd production, practice of prophylactic antibiotics, weaning age, separation or mixing of calves etc. (Bruning-Fann and Kanene, 1992; Lance *et al*., 1992; Olsson *et al*., 1993).

2.6. Management of calf diarrhea

2.6.1. Specimens

An etiological diagnosis may be missed due to the incorrect and inadequate collection, storage and/or transportation of specimens to the laboratory for examination. Fecal samples, not rectal swabs, and specimens collected at necropsy (if applicable), should be submitted. At least 5 g of feces each from affected untreated calves and from clinically normal, age-matched animals is required. The feces of the diarrheic calves must be collected as soon as possible after the onset of diarrhea. It is usually pointless to even attempt a diagnosis on fecal samples from only one or two calves. Samples must be stored at 4^0C on ice, not frozen, and submitted in separate sterile containers (Quinn *et al*., 1994).

If an affected, untreated calf is available for necropsy, specimens required for bacteriological and pathological examination should include small and large intestines (preferably ileum and colon), mesenteric lymph node, spleen, liver and brain. Feces must be collected from the rectum as outlined above. A post mortem examination on one or more affected, preferably untreated calves submitted live to the laboratory, can be a valuable aid in arriving at a diagnosis. This is often not acceptable to the farmer, particularly when valuable animals are involved. Information gained by doing a thorough necropsy may be beneficial to detect agents not found by fecal examination, assist in identifying the portal of entry of pathogens (e.g. umbilical), evaluate the extent to which bacteria have disseminated, and rule out concomitant disease (Quinn *et al*., 1994; Scott *et al*., 2004).

Because of the nature of the pathogens causing calf scours, there is no a simple relationship between the presence of a pathogen in a sample and the diagnosis of disease due to that pathogen. The problem is three-fold (Reynolds *et al*., 1986; Snodgrass *et al*. 1986; Donkersgoed *et al*., 1999; Scott *et al*., 2004).

Firstly, not all affected calves may excrete the pathogen (Donkersgoed *et al*., 1999). This means that, no matter how sensitive the test for the pathogen is, a single negative result does not mean that that pathogen is not causing the disease. Thus a single sample cannot rule out disease.

The second problem is that most pathogens can also be found in healthy calves. For example studies on Rotavirus have found that on average 15 - 20% of normal calves can excrete Rotavirus (Reynolds *et al*., 1986) though up to 50% of normal calves may excrete the pathogen (Scott *et al*., 2004). Thus finding a pathogen does not mean that it is causing disease.

The third problem is that most diarrhea outbreaks are multifactorial. Snodgrass *et al*. (1986) reported that 75% of outbreaks were associated with two or more potential pathogens. Thus again the result from a small number of samples may not accurately reflect the true cause. Testing at least four diseased calves and if possible testing a similar number of healthy calves can generally overcome all these problems (Scott *et al*., 2004). However, the cost of this is often prohibitive.

2.6.2. Tests performed at laboratory for diagnosis

Bovine neonatal gastroenteritis is a multifactorial disease. It can be caused by viruses (Coronavirus or Rotavirus), by bacteria (*Salmonella*, pathogenic strains of *E. coli*) or by protozoa such as *Cryptosporidium parvum*. The diagnosis of the etiological agent of diarrhea can be performed only in the laboratory because the clinical signs do not suffice to distinguish between these different microorganisms. It is possible to identify these agents by means of different techniques, including culture (Quinn *et al*., 1994) for bacteria, negative staining electron microscopy (Scott *et al*., 2004) for viruses, and fluorescent staining (Garber, 1994) for *Cryptosporidium parvum*. However, these techniques are labor intensive, impractical and time consuming. These classical techniques have rapidly been replaced by the ELISA technology because of its simplicity and limited laboratory equipment requirements. The sensitivity and specificity of the ELISA technique for detecting these pathogens is at least as good as that of the more classic techniques, and the results are very similar. The ELISA technique is rapid and reliable and is particularly suited to the analysis of large numbers of samples.

Cultural isolation of the organisms from fecal sample is the usual method of diagnosis of *Salmonella* and *E. coli* from diarrheic calves. On blood agar, the colonies of *E. coli* are grayish, round and shiny with a characteristic smell. Colonies may be hemolytic or non-hemolytic. On MacConkey agar colonies are bright pink. The colonies of *E. coli* have a metallic sheen appearance on eosin methylene blue (EMB) agar. On the IMViC tests *E. coli* shows indole positive, methyl red positive, Voges-Proskauer negative and citrate utilization negative. Hydrogen sulfide (H_2S) production and urease activity are negative, and lysine decarboxylation is positive for *E. coli* (Quinn *et al*., 2002).

On brilliant green phenol red lactose sucrose (BPLS) agar, colonies of *Salmonella* and medium are red showing alkalinity. On xylose lysine desoxycholate (XLD) agar, colonies are red (alkaline) with a black center, indicating hydrogen sulfide (H_2S) production. IMViC tests for *Salmonella* show indole negative, methyl red positive, Voges-Proskauer negative and citrate utilization positive. Urease activity is negative and lysine decarboxylation is positive for *Salmonella* (Quinn *et al*., 2002).

Colonies showing characteristics of *Salmonella* and *E. coli* will then be tested for biochemical reactions and serotyped (Quinn *et al*., 1994; Quinn *et al*., 2002). Serotyping of *Salmonella* and *E. coli* species is especially important in advising on vaccination as a prophylactic measure (Scott *et al*., 2004).

Identification of *Cryptosporidium* is routinely performed for diagnosis (Radostits *et al*., 1994). Identification of *Cryptosporidium* is usually done with acid-fast staining of fecal floatation smears. Commercially available ELISA tests have also been developed that have sensitivity and specificity than stained fecal floatation smears (Hendrix, 1998).

Several dipstick- style ELISA tests for Rotavirus (Murphy *et al*., 1999b), and Coronavirus and *E. coli* K99 (Quinn *et al*., 1994) from feces are available which are suitable for use in the practical laboratory.

Viral isolation in the diagnosis of calf diarrhea is not routinely done, as enteric viruses are difficult to grow. On the other hand, the advantages of electron microscopic examination of fecal specimens include a rapid response and the possibility of identifying more than one virus (Scott *et al*., 2004).

2.6.3. Treatments

Generalized treatments: These include intravenous fluids and oral rehydration fluids, and should be administered whatever the cause of scours and are aimed at correcting any dehydration and acidosis that may have occurred and minimizing intestinal damage (Scott *et al*., 2004).

Probiotics that favorably alter the intestinal microflora balance, inhibit growth of harmful bacteria, promote good digestion, boost immune function and increase resistance to infection can also be fed (Smirnov *et al*., 1993; Mel'nikova *et al*., 1993; Namur *et al*., 1998).

Specific treatments:

• *Rotavirus and Coronavirus:* No specific therapy is available for these pathogens, as no antiviral drugs are available. Antibiotics are not indicated in viral infections, but are often used in severely affected calves that are suspected of having a secondary bacterial infection in addition to the viral disease. If the herd has an extended calving pattern, then it is suggested vaccination should be considered for cows greater than one month from calving. If necessary pregnancy diagnosis should be carried out on those cows still to calve to target the vaccine appropriately. For those cows that are within a month of calving various immunological products are available in paste form. It should be emphasized that these will only provide short-term protection and these products should not be used as an alternative to vaccination (Scott *et al*., 2004).

• *Salmonella and Escherichia coli infections:* Antibiotics should only be used in cases involving bacterial enteritis and must be prescribed by veterinarians. When a bacterial isolate is obtained which may be of significance, antibiotic sensitivity testing should be carried out to enable appropriate antibiotic selection (Scott *et al*., 2004).

The concern with the use of antibiotics in *Salmonella* and *E. coli* infections is that they have the potential of predisposing to colonization with other organisms, release of endotoxins, increasing levels and duration of excretion by carriers and of selecting for antibiotic-resistant strains (Farris *et al*., 1979; Scott *et al*., 2004).

• *Cryptosporidium:* Halufiginone is now available as an aid to treatment and prevention of *Cryptosporidium* infection in calves and can be prescribed by veterinarians. The drug reduces the severity of disease in individual calves and suppresses the output of oocysts reducing the risk of spread of disease. On a group basis, the drug works best when used to prevent further scours cases due to *Cryptosporidium* after the initial diagnosis has been made. As symptoms of halofuginone toxicity may occur at only twice the therapeutic dose, it is necessary to adhere strictly to the recommended dosage and not to treat severely dehydrated calves (Scott *et al*., 2004).

2.6.4. Control and prevention

Generalized control and preventive measures: Many husbandry measures will reduce the risk of diarrhea and such measures carry little in the way of additional costs. This focuses on reducing exposure to the infectious agents and optimizing the calves` resistance to them. Calf should be born in a clean well-bedded environment and adequate colostrum should be fed at an early stage. As the placenta is impermeable to protein, the calf is born with little immunity to diseases. This is passively acquired following birth by suckling of colostrum (Blood and Radostits, 1989). Calves should stay with their dams until 24 hours after calving. Absorption of colostrum varies in individual calves, but the maximum ability to absorb lasts six to eight hours after birth. It then declines or no direct intake occurs after 24 to 36 hours (Reynolds *et al*., 1986; Snodgrass *et al*., 1986; Bath *et al*., 1985). Colostrum feeding should continue in the second and third day after birth. The immunoglobulins in this period, though not absorbed will provide some local protective action against bacteria and other organisms in the digestive tract (Bath *et al*., 1985).

Diarrheic calves should always be isolated from healthy calves during the course of diarrhea and for several days after recovery. Calf rearing houses should be vacated and cleaned out on a

regular basis; an all-in all-out management system, with thorough cleaning and several weeks of drying between batches of calves should be used (Radostits *et al*., 1994). Avoiding overcrowding of calves and establishing the optimum stocking density is also required (Radostits *et al*., 1994; Soulsby, 1982).

Specific control and preventive measures: Vaccination of the cow herd using one of the vaccines against Rotavirus, Coronavirus and *E. coli* K99 is recommended in herds where calf scours is a recognized problem. The judgment as to whether this is required or not is best made on the basis of a risk assessment carried out by veterinarians (Scott *et al*., 2004). Pregnant cows are usually vaccinated with inactivated strains one to two months before calving (Baker, 2004). Vaccination against salmonellosis may be used to offer some protection. This vaccine appears to work through reducing the numbers of infectious organisms the cow passes rather than through the colostrum (Scott *et al*., 2004).

2.7. Calf diarrhea in Ethiopia

The most frequently reported causes of morbidity and mortality in dairy calves in Ethiopia include calf diarrhea, calf pneumonia, gastrointestinal parasites, skin diseases, etc (Gryseels and de Boodet, 1986; Yemam and Brannag, 1996; Amoki, 2001; Yosef *et al*., 2002; Mekonen *et al*., 2001). These studies also showed that calf diarrhea and calf pneumonia as leading causes in younger calves and gastrointestinal parasites in older ones that turn out to pasture.

However, there are very few works done to identify specific agents involved in disease syndromes mentioned above. Abraham *et al*. (1992) identified specific infectious agents associated with neonatal calf diarrhea in Ethiopian dairy calves. They found bovine enteric Coronavirus, Rotavirus group A and K99 enterotoxigenic *E. coli* independently or in combination in diarrheic calves. *Salmonella* and *Cryptosporidium* were reported in diarrheic calves from smallholder dairy farms by Temesgen (2004).

3. MATERIALS AND METHODS

3.1. Study area

The study was carried out in dairy farms of the urban and peri-urban areas of Addis Ababa. Addis Ababa is the capital city, which is situated at latitude of $9^0$3' and $38^0$43' east. It lies in the central highlands of Ethiopia with an altitude of 2500 meters above sea level. The annual average maximum and minimum temperatures are 26^0C and 11^0C, respectively, with an overall average of 18.7^0C. Highest temperature is reaching in May. The main rainy season extends from June to September. Addis Ababa has a relative humidity varying between 70% to 80% during the rainy season and 40% to 50% during the dry season. The area receives an average of 1800 mm rainfall annually (NMSA, 2003). Microbial analysis of the sample was conducted in the Microbiology Laboratory of the Faculty of Veterinary Medicine, Addis Ababa University, Debre Zeit, Ethiopia.

3.2. Study population

Crossbred dairy calves up to 6 months of age were the sampling units for the study. All calves from dairy farms in and around Addis Ababa constituted the study population. There were a number of market oriented smallholder and medium-sized dairy farms. Large dairy farms were few in number. Farm classification as smallholder (< 5 heads of dairy cow), medium-sized (6 - 50 heads of dairy cow) and large (>50 heads of dairy cow) dairy farms was based on previous works in the urban and peri-urban production systems (Lemma *et al*., 1999). All dairy farms kept crosses of Holstein and local animals.

3.3. Study design

The study was longitudinal prospective observational study that extended for 6 months from October 2006 to April 2007. The sampling units (calves) were identified individually and monitored through out the study period. The questionnaire survey was also conducted during the study period.

3.3.1. Sampling procedure and sample size

A representative random sample of calves from market-oriented dairy farms was selected for the study. Considering individual dairy farms as a cluster, cluster sampling method was used to select calves from dairy farms.

The sample size for estimating disease problems using simple random sampling methods was calculated according to Martin *et al*. (1987).

$n = \frac{(Z_{\alpha/2})^2 P_{exp} (1-P_{exp})}{d^2}$, where

n = sample size

$Z_{\alpha/2}$ = confidence level

P_{exp} = expected prevalence

d = precision level

Using 20% expected incidence rate of calf diarrhea, confidence level of 95% and required absolute precision of 5%; a sample size of 246 calves was determined. Selection of the small and medium-sized dairy farms was done by lottery system. When a selected farm did not have calf or calves eligible for the study (having calves under three months of age or pregnant cows with due calving date in the next three months), it was replaced by another farm mostly from neighboring. Totally 254 calves, 37 from four large dairy farms, 103 calves from 57 smallholder dairy farms and 114 calves from 29 medium-sized dairy farms were used for the study.

3.4. Data collection

3.4.1. Longitudinal study

Monitoring dairy farms for calf diarrhea was carried out for 6 months from October 2006 to April 2007. For the purpose of this study calf was defined as young cattle less than 6 months of age. For monitoring purpose, all calves in the selected farms that were under three months of age at

the beginning of the follow up period and those born in the subsequent three months were individually identified and monitored through out the follow up period. The calves were withdrawn from the follow up when they completed their 6 months of age. In this way each calf was monitored at least for three months unless censured by death.

Individual records were prepared when a calf joins the study cohort. The record was used to record genealogy of the calf, events surrounding the birth of the calf, routine management practices provided to the calf and health problems encountered during the monitoring. The sample of calf card format is shown in Annex 2. In the actual monitoring work, calves under three months of age were regularly visited every two weeks and monthly thereafter. The main activities accomplished during the regular visit were: -

. Clinical examination of calves for any health problem that included physical examination of calves for body temperature, respiratory rate and pulse rate when abnormality was suspected.

. Observation of different calf management aspects like cleanness of the calf house and feeding practices.

. Asking calf attendants the occurrence of health problem incidents between the visits and recording of the history of the calf health problem that would enable the investigator infer the possible cause and thus assist diagnosis.

. Collecting fecal samples from active cases of diarrhea in calves.

In addition to regular visits, emergency visits in response to calls from farms for any calf health problems were there.

In addition to calf diarrhea, calf morbidities encountered during the monitoring period were the following:

Pneumonia: When frequent coughing observed with or with out respiratory discharges and fever.

Septicaemic condition: Any condition characterized by depression, anorexia and fever.

Navel ill (omphalitis): Swelling of umbilical cord, which is painful when palpated.

Joint ill (arthritis): Enlargement of joints in any one or all limbs.

Congenital problems: Any problems that was acquired inborn.

Miscellaneous cases: Different health problems that could not be grouped in any one of the other groups mentioned before.

3.4.2. Cross-sectional study based on questionnaire

A questionnaire designed to collect information on farm characteristics, calf management techniques including periparturient care, feeding and housing and previous histories of calf diarrhea and other health problems was administered. The sample of questionnaire format is shown in Annex 1.

3.5. Identification of infectious agents associated with calf diarrhea

3.5.1. Sample collection

The diagnosis of the microbial pathogens associated with diarrhea can be made only in the laboratory because it is not possible to differentiate between the different causative agents on the basis of the clinical symptoms. For this reason a fresh fecal sample of approximately 30 grams was collected from untreated diarrheic calves directly from rectum soon after onset of diarrhea. The sample was placed in a separate sterile container, kept at an ice-cold condition and transported to laboratory on the same day of collection. At the time of sampling, the name of the farm, date of sampling, consistency and color of the feces, and the age, breed, and tag number of the calves were recorded for each calf on a recording format. Samples were collected to examine

them for Rotavirus, Coronavirus, *Escherichia coli* strain K99, *Cryptosporidium parvum* and *Salmonella*.

3.5.2. Detection of Rotavirus, Coronavirus, *E. coli* K99 and *Cryptospordium parvum*

Antigenic ELISA diagnostic kit which is a direct test for fecal material (Bio-X Easy Digest®; Bio-X, Belgium) was used for the detection of Rotavirus, Coronavirus, *E. coli* K99 and *Cryptosporidium parvum* according to the instructions of the manufacturer as follows:

Fecal samples were diluted volume by volume into the ready to use dilution buffer. This was a qualitative dilution only, which must allow the pipetting of fecal suspensions. Any gruds were discarded by natural decantation for about 10 minutes. Then, the microtitration plate was taken out of its wrapper and the diluted samples were pipetted into the wells at the rate of 100 µl taking care to change pipettes between two different samples. The arrangement of samples on the plate was set according to the number of fecal samples tested and the four valences for each sample. The ready to use positive and negative controls were also distributed over the plate as well (one well per valence tested). The plate was then incubated at room temperature for one hour. After incubation, the contents of the microplate were emptied by flipping it over sharply over a sink. The microplate was tapped upside down over against a piece of clean absorbent paper to remove all the fluid. All the used wells were then filled with the washing solution (prepared by diluting it 1:20 with distilled water) using a washing bottle, and then the wells were emptied once more by turning the plate over above the sink. This washing operation was repeated two more times, taking care to avoid the formation of bubbles in the microwells.

After the plate has been washed three times, the ready to use conjugates were added into the wells at the rate of 100 µl per well directly from the bottles and incubated at room temperature for one hour. After this incubation, the plate was washed three times as described above; 100 µl of the ready to use chromogen solution which contains tetramethylbenzidine (TMB) was then added to each well on the plate and incubated at room temperature for one hour without covering.

Interpretation of results: The results were interpreted following the guidelines of the manufacturer of the kit. Blue color developments indicate positive reactions and the intensity of color development were compared with positive and negative controls.

3.5.3. Detection of *Salmonella*

Isolation and identification of *Salmonella* was carried out based on procedure made available by international organization for standardization (ISO 6579, 2002) and Quinn *et al*. (1994). About 10 -25 grams of feces was inoculated into buffered peptone water (BPW) in a ratio of 1 gram of sample to 9 ml of BPW. The mixture was homogenized and then incubated at 37^0C for 18 to 24 hours. From the incubated culture, 0.1 ml was inoculated into a tube containing Rappaport-Vassiliadis with soya (RVS) broth and was incubated at 42^0C for 18 to 24 hours. Another 1 ml of the same culture was inoculated into 10 ml of Muller-Kauffmann tetrathionate with novobiocin (MKTTn) broth and was incubated at 37^0C for 18 to 24 hours. From these selective enrichments, subcultures were made at 24 hours to selective plate media Xylose lysine desoxycholate (XLD) and xylose lysine tergitol4 (XLT4) and incubated at 37^0C for 24 hours. Presumptive colonies were further subcultured on Rambach agar. Finally colonies that exhibited typical reactions of *Salmonella* were cultured on brain heart infusion (BHI) agar and sent to AFSSA, France, for serotyping.

Antimicrobial susceptibility test: For antimicrobial susceptibility test, the National Committee for Clinical Laboratory Standards (NCCLS) (1997) guidelines were followed throughout the agar dilution testing procedure, and interpretation of results as susceptible, intermediate and resistant. Briefly, the isolates were first cultured on nutrient agar and then grown to 0.5 - 1 McFarland density in tryptone soya broth and plated onto Muller Hinton (MH) agar plates. The list of antimicrobials used, their symbols and concentrations to classify an isolate as resistant, intermediate or susceptible are shown on Table 1.

Table 1. Antimicrobials used, their symbols, concentrations and zone size interpretation

Antimicrobials (disc content in μg unless otherwise stated)	Symbol	Diameter of zone of inhibition to nearest mm		
		Resistant		Susceptible
		≤	Intermediate	≥
Amoxicillin (10)	AML	13	-	18
Ampicillin (10)	AMP	13	-	17
Chloramphenicol (30)	CHL	12	13 - 17	18
Erythromycin (15)	ERY	13	14 - 22	23
Gentamycin (10)	GEN	12	13 - 14	15
Kanamycin (30)	KAN	13	14 - 17	18
Nalidixic acid (30)	NAL	13	14 - 18	19
Norfloxacin (10)	NOR	12	13 - 16	17
Polymyxin B (300 units) *	POB	8	9 - 11	12
Streptomycin (10)	STR	11	12 - 14	15
Sulfamethoxazole/ trimethprim (25)	SXT	10	-	16
Trimethoprim (5)	TMP	10	11 - 15	16
Tetracycline (30)	TET	14	15 - 18	19

*: Not included in NCCLS chart; FDA approved performance standards for Antimicrobial Discs obtained from drug manufacturers

Source: NCCLS (1997) and HiMedia Laboratories Pvt. Limited.

3.6. Data management and analysis

3.6.1. Describing incidence of calf diarrhea and mortality

As animals in this longitudinal study were recruited at different times and were followed for different periods of time, incident rate (true rate) was used in describing calf diarrhea. Incidence rate was calculated by dividing the number of case of interest to the number of calf days at risk.

Number of calf days at risk was found by adding the number of days at risk of obtaining a new case in each calf in the study period. Incidence was also calculated for mortality.

In the calculation to describe incidence rate of calf diarrhea, a calf recovered from diarrhea was considered to be at risk for the same type of illness as far as the second illness occurred after complete recovery (complete disappearance of clinical signs) of the preceding one. Similarly, two or more cases of the same disease condition were considered as different cases in calculating the incidence of that disease condition as far as the second occurred after the disappearance of the clinical sign of the first. Nevertheless, in this case the days in which the calf stayed ill were not counted as days at risk.

3.6.2. Data analysis

Data processing was done by Microsoft Excel (2002). Regarding calf morbidity and mortality, previous incidence reports varied with the period covered during the follow up, the methods used in measuring rates (incidence density (true rate) or cumulative incidence (risk rate)) and the unit of study used (herd or calf). Therefore, to facilitate the comparison of the results with other studies, incidence density (true rate) was derived into risk rate using the formula,

Risk rate = $1 - e^{-\text{true rate}}$ (Martin *et al*., 1987).

4. RESULTS

4.1. Incidence of calf diarrhea and mortality

Six months monitoring of 254 dairy calves for clinical health problems was conducted to determine the incidence of calf diarrhea and mortality. The results of this study revealed that the incidence of calf diarrhea in the first 6 months of calf hood was 33.6%. From disease conditions diagnosed during the monitoring, calf diarrhea was the leading cause of calf morbidity. Crude mortality rate of 11.6% was also recorded (Table 2).

Table 2. The incidence (true rate and risk rate) of calf diarrhea and mortality

			Incidence rate	
Disease condition	N	Calf days at risk	True rate/6 calf months at risk	Risk rate (%)
Diarrhea	49	21551	0.409	33.6
Crude mortality	15	21882	0.123	11.6

N = number of cases

The average age for the occurrence of diarrhea and crude mortality was 42 days and 45 days, respectively for smallholder dairy farms, and 25 days and 9 days, respectively for large dairy farms. Thirty-nine days was the average age both for the occurrence of diarrhea and crude mortality in medium-sized dairy farms. In general, about 16% of the total cases of diarrhea and 26.7% of the total cases of mortality were occurred in the first week of life.

The principal cause of death was diarrhea directly accounting for the 6 cases of the total 15 deaths. Five (1 from smallholder, 2 from medium-sized and 2 from large dairy farms) of these calves died showing the classical pattern of acute calf diarrhea (dehydration and depression). One (from smallholder dairy farms) calf died of unthriftness after recovering from diarrhea. Other

causes of death recorded include bloat from smallholder dairy farms (1), pneumonia from smallholder and medium-sized dairy farms (2), grain engorgement from smallholder dairy farms (1), navel ill complication from medium-sized dairy farms (1) and congenital anomaly from large dairy farms (1). Three calves (2 from smallholder and 1 from large dairy farms) died of weakness at the time of birth.

When calf diarrhea and crude mortality were compared by farm types, the incidence of calf diarrhea was apparently higher in medium-sized and large dairy farms than smallholder dairy farms while the incidence of calf mortality was higher in smallholder and large dairy farms (Table 3 and Figure 1). In Tables 2 and 3 incidence rates were derived into risk rates to facilitate the comparison of results with other studies.

Table 3. Incidence (risk rate) of calf diarrhea and mortality by farm types

Disease condition	Risk rate (%)		
	Smallholder dairy farms	Medium-sized dairy farms	Large dairy farms
Diarrhea	18.1	42.3	41.7
Mortality	13.1	8.6	16.5

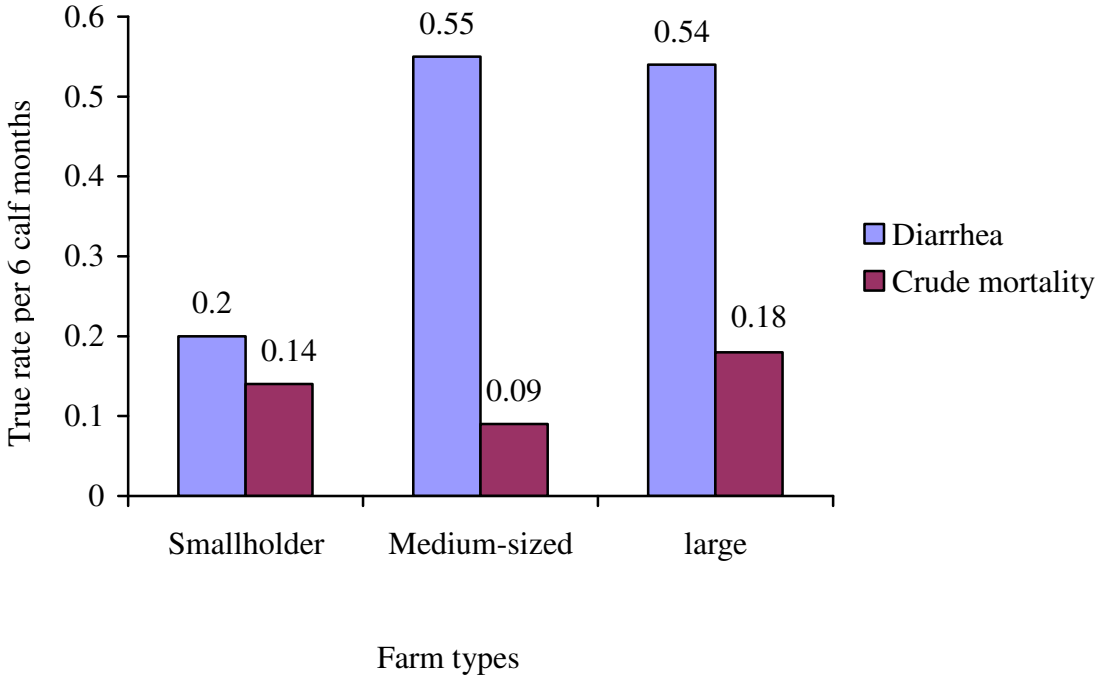

Figure 1. True rate per 6 calf months of calf diarrhea and crude mortality by farm types

4.2. Agents associated with calf diarrhea

Laboratory examination of 36 fecal samples from 49 cases of diarrhea that occurred during the study period was done to identify pathogens associated with calf diarrhea. Examination of samples was done for Rotavirus, Coronavirus, *E. coli* K99, *Cryptosporidium parvum* and *Salmonella*.

Of the total 36 (6, 24 and 6 samples from smallholder, medium-sized and large dairy farms, respectively) samples examined, 1 (2.8 %) was positive for Rotavirus, 3 (8.3 %) were positive for Coronavirus, 8 (22.2 %) were positive for *E. coli* K99, 23 (63.9 %) were positive for *Cryptosporidium parvum*, and 6 (16.7%) were positive for *Salmonella* (Table 4).

Table 4. Frequency of occurrence of calf pathogens in the feces examined

Enteropathogen	Number positive (%)
Rotavirus	1 (2.8)
Coronavirus	3 (8.3)
E. coli K99	8 (22.2)
C. parvum	23 (63.9)
Salmonella	6 (16.7)
Coronavirus + *C. parvum*	1
E. coli K99 + *C. parvum*	4
E. coli K99 + *Salmonella*	1
C. parvum + *Salmonella*	2
Coronavirus + *E. coli* K99 + *C. parvum*	1

Of the 6 samples obtained from smallholder dairy farms one was positive for Coronavirus and 4 were positive for *Cryptosporidium parvum*. Of the 24 cases sampled from medium-sized dairy farms 1, 2, 6, 15 and 6 samples respectively were positive for Rotavirus, Coronavirus, *E. coli* K99, *Cryptosporidium parvum* and *Salmonella*. From the total of 6 diarrheic samples examined from large dairy farms, 2 and 4 samples were positive for *E. coli* K99 and *Cryptosporidium parvum*, respectively (Table 5).

Table 5. Frequency of occurrence of calf pathogens in the feces examined by farm types

Farm types	Number of samples	Number (%) of samples positive for:				
		Rotavirus	Coronavirus	*E. coli* K99	*C. parvum*	*Salmonella*
SH*	6	-	1 (16.7)	-	4 (66.7)	-
MS*	24	1 (4.2)	2 (8.3)	6 (25.0)	15 (62.5)	6 (25.0)
L*	6	-	-	2 (33.3)	4 (66.7)	-
Total	36	1 (2.8)	3 (8.3)	8 (22.2)	23 (63.9)	6 (16.7)

*SH: Smallholder dairy farms; *MS: Medium-sized dairy farms;*L: Large dairy farms

Most of the samples positive for *E. coli* K99 and *Salmonella* were from calves that were less than one month old suffering from acute diarrhea.

From the total 36 samples examined, 1 (2.8%) was positive for Coronavirus, *E. coli* K99 and *C. parvum*, 8 (22.2%) were positive for two pathogens, 22 (61.1%) were positive only for one pathogen and 5 (13.9%) were negative for any of the pathogens tested. Of the 8 samples positive for two pathogens, 1 was positive for both Coronavirus and *C. parvum*, 4 were positive for both *E. coli* K99 and *C. parvum*, 2 were positive for both *C. parvum* and *Salmonella* and 1 was positive for both *E. coli* K99 and *Salmonella*. *Cryptosporidium parvum* was the only pathogen detected on 15 (41.7%) of diarrheic calves and concurrently with other pathogens in 8 (22.2%) of diarrheic calves (Table 4). In general, 86.1% of the fecal samples examined were positive for one or more of the pathogens tested (Figure 2).

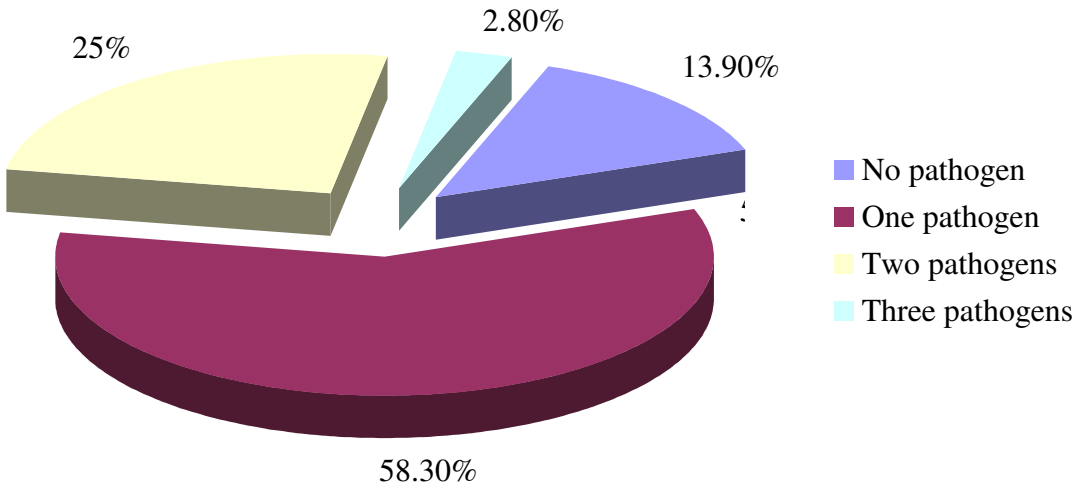

Figure 2. Simultaneous detection of enteropathogens in the fecal samples examined

4.3. Serotyping and antimicrobial resistance of *Salmonella*

In the present study, three different *Salmonella* serotypes were identified from the six *Salmonella* isolates. Four isolates were *Salmonella* Typhimurium, and the remaining two were *S.* Dublin and *S.* Mishmarhaemek.

All *Salmonella* serotypes tested were resistant to erythromycin. Two isolates of *S*. Typhimurium were intermediate in resistance to streptomycin. One isolate of *S*. Typhimurium showed intermediate resistance to streptomycin and polymyxin B, and another isolate of *S*. Typhimurium showed intermediate resistance to polymyxin B. *Salmonella* Dublin showed intermediate resistance to tetracycline, kanamycin and nalidixic acid. Resistance was not observed to amoxicillin, ampicillin, chloramphenicol, gentamycin, norfloxacin, sulfamethoxazole/ trimethprim and trimethoprim (Table 6).

Table 6. Resistance pattern of *Salmonella* isolates to the tested antimicrobial agents

Salmonella serotype	Resistant	Intermediate
Salmonella Typhimurium*	ERY	STR
Salmonella Typhimurium*	ERY	STR
Salmonella Typhimurium*	ERY	POB
Salmonella Typhimurium*	ERY	STR, POB
Salmonella Mishmarhaemek	ERY	-
Salmonella Dublin	ERY	TET, NAL, KAN

* Serotypes from different isolates

4.4. Description of farms based on questionnaire result and observation

All the farms included in this study kept crossbred animals (Holstein x local breeds) under intensive system of production. All farms were completely stall-fed and raise their own replacement stock. In most farms, male calves were sold soon after birth. Navel treatment during birth of calves was practiced in none of the farms. Only 48% of the dairy farmers had knowledge of the advantage of colostrum over ordinary milk and only 35.6% of them did know the optimum time to feed colostrum to calves. About 45.6% of the smallholder dairy formers had knowledge about the importance of colostrum and 38.6% of them did know the optimum time to feed colostrum. About 51.7% of the medium-sized dairy farmers had knowledge about the importance

of colostrum and only 24% of them did know the optimum time to feed colostrum. Three of the 4 large dairy farmers did know the importance and optimum time to feed colostrum. All study farms fed whole milk for calves two times daily. No special starter feed was used in any of the farms rather same feed given to cows were used for calves. Age to introduce non-milk feed and weaning age varied from farms to farms and weaning age was lower for male calves. Smallholder dairy farms either house their calves in cow barns, in a separate calf house or in groups when calves are more than one. Most of the medium-sized and two large dairy farms use group calf pens to house their calves. Only one medium-sized and one large dairy farms use individual calf box. One large dairy farm uses raised individual calf cage. Most of these dairy farms provide bedding material for the calves only during the first few weeks of life.

In most of the medium-sized and large dairy farms there were part time employed veterinarians and/or animal health technicians to deal with health aspect of the farm. All smallholder dairy farms either call private veterinary practitioners or brought sick animals to nearby government owned veterinary clinics, whenever they face health problems. Very few dairy farms practiced dam vaccination to protect future calf morbidities and mortalities. Of the 90 dairy farms interviewed 39 (43.3%) of them mentioned calf morbidity as one of the health problems in their farms of which calf diarrhea was number one in 17 (43.6%) dairy farms. Calf morbidity was the first complaint for 21 (36.8%) smallholder and 14 (48%) medium-sized dairy farms. All the 4 large dairy farms complained calf morbidity as a major problem in their farms. The rest of the farms complained reproductive problem, pneumonia, bloat or mastitis. For the calf deaths experienced, 5 smallholder, 7 medium-sized and 3 large dairy farms complained diarrhea as the cause of death.

5. DISCUSSION

In this study, the small number of fecal samples collected (36 samples from a total of 49 diarrheic cases recorded) was due to problems of getting diarrheic calves on time for sampling, and this might have influenced the prevalence of pathogens detected.

5.1. Incidence of calf diarrhea and mortality

In the present study, in and around Addis Ababa, the 33.6% incidence rate of calf diarrhea found for 6 months has considerably agreed with reports by Shiferaw *et al*. (2002) in Ethiopia, however, it is lower than 51.7% reported for market oriented specialized dairy production (MOSDP) and higher than 11.7% reported for mixed crop livestock production (MCLP) for similar period by Amoki (2001). The 33.6% incidence rate of calf diarrhea and 11.6% crude mortality found in the current study were lower than incidences reported by Temesgen (2004).

The 11.6% crude mortality found in this study was in agreement with the 12% mean calf mortality rate in smallholder dairy production in sub-Saharan Africa (Otte and Chilonda, 2002) and even with that of Europe which was reported in the ranges of 9 to 13 % (Heinrichs and Radostits, 2001). On the other hand, the present finding was lower than the 25% and 50% reported by Sisay and Ebro (1998) and Hassan and Brannag (1996), respectively.

In the present study, the numbers of calves per farm were small and the farmers can easily monitor calves and take measures to avoid calf health problems. Moreover, farmers in Addis Ababa have relatively more access to animal health information. This could be one of reasons to find relatively lower incidences of diarrhea and mortality than other reports in the country (Hussein, 1998; Lemma *et al*., 2001; Temesgen, 2004).

Calf diarrhea was the predominant calf health problem followed by arthritis and omphalitis in the current study. Diarrhea was also the leading cause of mortality in the study herds. The finding of calf diarrhea as predominant calf health problem and leading cause of mortality was in agreement with reports of Lemma *et al*. (2001) and Hussein (1998) in Ethiopia. Unlike reports of Olsson *et*

al. (1993), Debanth *et al*. (1995) and Sivula *et al*. (1996a), who reported pneumonia as second important disease complex that affect calf health, arthritis and omphalitis were found to be second important disease complex in the current investigation. On the other hand, there are studies which found pneumonia as the leading cause of calf mortality (Rao and Nagarcenkar, 1980; Agerholm *et al*., 1993; Shiferaw *et al*., 2002). Calf diarrhea as a leading health problem in growing dairy farms is a common finding and the incidence in this study suggests the significance of poor hygienic handling of feeding utensils and calf house observed during the study. In addition, only few farms were aware of the optimal time for colostrum feeding and this could greatly contribute to the high incidence of calf diarrhea in those herds. Amoki (2001) indicated high percentage of failure of passive immunity transfer in the central highland of Ethiopia. On the other hand, the relatively lower cases of pneumonia encountered in this study might be due to the small calf herd size per farms. Stocking density has strong correlation with environmental stress that exposes calves to respiratory problems; it was observed that a 50% decrease in stocking density was increasing the ventilation rate by 20 times there by decreasing the risk of pneumonia (Blowey, 1990).

Even though the sampling method and level of analyses used do not allow statistical comparisons of calf health problems between farm types, calf diarrhea was apparently higher in medium-sized and large dairy farms than smallholder dairy farms. Calf mortality, however, was higher in smallholder and large dairy farms. Similarly, Temesgen (2004) and Amoki (2001) indicated higher rates of calf diarrhea in large dairy farms than smallholder dairy farms. However, they reported higher mortality of calves in smallholder farms as compared to large dairy farms. They did not include medium-sized dairy farms in their study. The higher calf diarrhea in medium-sized and large dairy farms can be due to the need for more calf rearing facilities. The indiscriminate and continuous use of the same calf rearing facilities for different calves allows a build up of infectious agents in the calf rearing environment and hence increases the risk of calf diseases. The relatively higher mortality rate in smallholder and large farms indicates poor management of sick calves. As observed during the monitoring period, there was lack of easy access to animal health professionals for smallholders, and luck of responsiveness for large farms.

5.2. Microbial agents associated with calf diarrhea

Laboratory detection of infectious agents involved in calf diarrhea was one of the major tasks of the present investigation. All the common potential enteropathogens that could cause diarrhea in interaction or independently were identified from diarrheic calves in the study herds. These include Rotavirus (2.8%), Coronavirus (8.3%), *E. coli* K99 (22.2%), *C. parvum* (63.9%) and *Salmonella* (16.7%). These pathogens were also isolated from diarrheic calves by many other authors in different parts of the world (Bellinzoni *et al*., 1990; Bendali *et al*., 1999). Although these pathogens can be isolated from healthy calves as well, the excretion rate is higher in diarrheic calves indicating their role as a cause of calf diarrhea (Reynolds *et al*., 1986). The 8.3% Coronavirus detection rate in diarrhea sample is in agreement with the finding of Björkman, (2003). Detection of *C. parvum* at this prevalence rate is comparable to the findings of Trotz-Williams *et al*. (2005).

The detection of Rotavirus in 2.8% and Coronavirus in 8.3% diarrheic samples is lower than the reports of Abraham *et al*. (1992). However, the detection of *E. coli* K99 in 22.2% of the samples is higher than the reports of the same authors. Moreover, in the work of Abraham *et al*. (1992) in dairy farms of the central highlands of Ethiopia no evidence of cryptosporidial infection was found using a differential staining method on fecal smears nor was *Salmonella* excretion detected. Detection of *C. parvum* and *Salmonella* from diarrheic calves at prevalence rates of 63.9 % and 16.7%, respectively, are higher than reports of Temesgen, (2004). These researchers (Temesgen, 2004 and Abraham *et al*., 1992) used differential staining method on fecal smears to detect *C. parvum*. Therefore, the high prevalence for *C. parvum* in the present investigation could be due to the different technique (ELISA which is more sensitive than differential staining) used to detect the pathogen. Of the six *Salmonella* isolates, four of them were from the same farm and they were of the same serotype (*Salmonella* Typhimurium) indicating the presence of a possible source of infection with *Salmonella*.

In the present finding, *Cryptosporidium parvum* was the only pathogen detected in 15 (41.7%) of diarrheic calves and concurrently with other pathogens in 8 (22.2%) of diarrheic calves. de la Fuente *et al*. (1999) also found *Cryptosporidium* as the only pathogen detected on 52.6% of

diarrheic calves and concurrently with other pathogens in 47.4% of diarrheic calves. These findings suggest that *Cryptosporidium* is a major pathogen associated with calf diarrhea at least in the studied herds.

Though the prevalence of some pathogens found in this study was relatively lower than reports from other countries, the present study clearly indicated the presence of all potential pathogens in Ethiopian dairy farms as well. Moreover, in addition to their role in calf diarrhea complex, C*ryptosporidium* and *Salmonella* are potential zoonotic pathogens.

5.3. Serotypes and antimicrobial resistance of *Salmonella*

In the present study, S*almonella* Typhimurium was the most frequent serotype isolated from calves with diarrhea. The detection of S*almonella* Dublin and *S*. Mishmarhaemek in calves with diarrhea in this study indicated their importance in calf diarrhea. S*almonella* Typhimurium was also among the most frequent serotype isolated from calves with diarrhea in a number of studies (Waltner-Toews *et al*., 1986b; Lance *et al*., 1992; Warnick *et al*., 2001). Bellinzoni *et al*. (1990) was also reported *Salmonella* Dublin in calves with diarrhea. The other serotype, *Salmonella* Mishmarhaemek was not commonly reported in connection with calf diarrhea.

Antimicrobial susceptibility test on these serotypes indicated their susceptibility to many of the commonly used antimicrobial agents. This could be due to less frequency of treating diarrheic calves in the study herds. All *Salmonella* serotypes tested were resistant to erythromycin. *Salmonella* Typhimurium showed intermediate resistance to streptomycin and polymyxin B whereas, *S*. Dublin showed intermediate resistance to tetracycline, nalidixic acid and kanamycin. Few studies conducted in Ethiopia indicated a high level of antimicrobial resistance in *Salmonella* serovars isolated from food animals (Alemayehu *et al*., 2003; Molla *et al*., 2003, 2004). Rapid emergence and dissemination of antimicrobial resistant pathogens have become a public health concern worldwide.

In general in most sub-Saharan African countries like Ethiopia where salmonellae and other zoonotic bacterial pathogens are not routinely isolated and identified and their resistances to

commonly used antimicrobials are rarely assessed suggest that the high level of antimicrobial resistance among *Salmonella* strains and other zoonotic pathogens will remain a major health problem (Molla *et al*., 2006). The magnitude of the problem should be reduced at various levels through the prudent use of antimicrobials both in the veterinary and public health sectors.

5.4. Questionnaire findings

From the questionnaire survey, it was clear that calf diarrhea was among the major problems in the study area. Of the 39 (43.3%) farms in which calf morbidity was one of the health problems, calf diarrhea was the leading one in 17 (43.6%) dairy farms. This was in agreement with the findings of Temesgen (2004).

Only 48% of the dairy farmers had knowledge of the advantage of colostrum over ordinary milk and only 35.6% of them did know the optimum time to feed colostrum to calves. The fact that only less than half dairy producers know the importance of colostrum and only few of them know when to feed, show lack of basic knowledge to calf management techniques. Moreover, some farmers wrongly considered colostrum as one cause of diarrhea and restricted calves from having access to enough colostrum as observed during the study period. None of the farmers practiced immediate isolation of sick calves. In many of the farms, diarrheic calves did not receive appropriate treatments.

6. CONCLUSIONS AND RECOMMENDATIONS

In this study attempts have been made to estimate the incidence of calf diarrhea and mortality among calves from smallholder, medium-sized and large dairy farms in and around Addis Ababa, Ethiopia. The infectious agents associated with calf diarrhea were also identified.

Calf diarrhea and mortality rates found in this study were higher than the economically tolerable and that can be achieved through good management. Results of the present study indicated the presence of major pathogens (Rotavirus, Coronavirus, *E. coli* K99, *C. parvum* and *Salmonella*) associated with calf diarrhea in the study herds. Although a number of antimicrobial agents tested were effective against the *Salmonella* serotypes tested, resistance was observed to erythromycin whereas streptomycin, polymyxin B, tetracycline, kanamycin and nalidixic acid showed intermediate resistance. Based on these conclusions, the following recommendations are forwarded:

- Improved calf management practices that could reduce the high level of calf diarrhea in the study herds should be implemented.
- Special emphasis should be given to the time of colostrum feeding, the hygiene of calf house and isolation of sick calves.
- As some of the microbial pathogens detected from calves with diarrhea are also pathogenic to humans, farm personnel and those individuals in contact with calves should take the necessary care to avoid the risk of infection.

7. REFERENCES

Abraham, G., Roeder, P. T. and Roman, Z. (1992): Agents associated with neonatal diarrhea in Ethiopian dairy calves. *Trop. Anim. Hlth. Prod.*, **25**: 239 - 248.

Agerholm, J. S., Bessa A., Krogh, H. V. Christene, K. and Ronshoit, L. (1993): Abortion and calf mortality in Danish cattle herds. *Acta Vet. Scand.*, **34**: 371 - 378.

Aiello, S. E. (1998): Diarrhea in neonatal ruminants (scours). In: The Merck Veterinary Manual. 8th ed. New Jersy: Merck & Co. Inc. Pp 260 - 266.

Alemayehu, D., Molla, B., Muckle, A. (2003): Prevalence and antimicrobial resistance pattern of *Salmonella* isolates from apparently healthy slaughtered cattle in Debre Zeit, Ethiopia. *Trop. Anim. Hlth. Prod.,* **35**: 309 - 319.

Amoki, O. T. (2001): Management of dairy calf in Holeta area, Central highlands of Ethiopia. MSc thesis, FVM, AAU, Debre Zeit, Ethiopia and FVM, FUB, Berlin, Germany.

Arslan, M. Ö., Gicik, Y., Erdogan, H. M. and Sari, B. (2001): Prevalence of *Cryptosporidium* spp. oocysts in diarrheic calves in Kars province, Turkey. *Turk J. Vet Anim. Sci.*, 25: 161 - 164.

Baker, I. D. (2004): Vaccine and vaccination of cattle. In: Bovine Medicine. Eds. Andrews, A. H., Blowey, R. W., Boyd, H. and Eddy, R. G. 2nd ed. Oxford Blackwell Publishing, Pp 1004 - 1018.

Bath, D. L., Dickinson, F. R., Tucker, H. A. and Appleman, R. D. (1985): Raising calves and growing heifers. In: Dairy Cattle: Problems, Practice and Profits, 3rd ed. Lea and Febiger, Philadelphia, USA, Pp 325 - 338.

Bellinzoni, R. C., Blackhall, J., Terzolo, H. R., Moreira, A. R., Auza, N., Mattion, N., Micheo, G. L., La Torre, J. L. and Scodeller, E. A. (1990): Microbiology of diarrhea in young beef and dairy calves in argentina. *Rev. Argent. Microbiol.*, **22**: 130 - 136.

Bendali, F., Bichet, H., Schelcher, F. and Sanna, M. (1999): Pattern of diarrhea in newborn calves in south- west France. *Vet. Res.*, **30**: 61 - 74.

Besser, T. E., Szenio, O. and Gay, C. C. (1990): Decreased colostral immunoglobulin absorption in calves with postnatal respiratory acidosis. *J. Am. Vet. Med. Assoc.*, **196**: 1239 - 1243.

Besser, T. E., Gay, C. C. and Pritchett, L. (1991): Comparison of three methods of feeding colostrum to dairy calves. *J. Am. Vet. Med. Asso.*, **198**: 419 - 422.

Björkman, C., Svensson, C., Christensson, B. and de Verdier, K. (2003): *Cryptosporidium parvum* and *Giardia intestinalis* in calf diarrhea in Sweden. *Acta Vet. Scand.*, **44**: 145 - 152.

Blood, D. C. and Radostits, O. M. (1989): Veterinary Medicine, a text book of diseases of cattle, sheep, pigs, goats and horses, 7th ed. English Language Book Society Bailliere Tindall, Pp 501 - 559.

Blowey, R. W. (1990): The young calf. In: Veterinary Book for Dairy Farmers. 2nd ed. Farming Press Ltd. Ipswich, UK. Pp 15 - 33.

Britney, J. B., Martin, S. W., Stone, J. B. and Curtis, R .A. (1984): Analysis of early calfhood health status and subsequent dairy herd survivorship and productivity. *Prev. Vet. Med.*, **3**: 45 - 52.

Bruning-Fann, C. and Kaneene, J. B. (1992): Environmental and managemental risk factors associated with morbidity and mortality in perinatal and preweaning calves: a review from an epidemiological perspective. *Vet. Bull.*, **62**: 399 - 403.

Busato, A., Steiner, L., Martin, S. W., Shoukri, M. M. and Gaillard, C. (1997): Calf health in cow-calf herd in Switzerland. *Prev. Vet. Med.*, **30**: 9 - 22.

Chao, L. and Levin, B. R. (1981): Structured habitats and the evolution of anti-competitor toxins in bacteria. *Proc. Nat. Acad. Sci.*, **78**: 6324 - 6328.

Cockram, M. S. and Rowan, T. G. (1989): Effects of air temperature, air velocity and feeding level on apparent digestibility, water intake, water loss and growth in calves given a milk substitute diet. *Anim. Prod.*, **48**: 51 - 65.

Corwin, R. M. (1992): Cryptosporidiosis: A coccidiosis of calves. *Comp. Cont. Edu. Pract. Vet.*, **14**: 1005 - 1007.

Cunningham, J. G. (1992): Gastrointestinal physiology. In: Text Book of Veterinary Physiology. W. B. Saunders Campany, Philadelphia, Pp 331.

D'Aoust, J. Y. (1997): *Salmonella* species. In: Food Microbiology: Fundamentals and Frontiers (Eds.), Doyle, M. P., Beuchat, L. R. and Montville, T. J. Washington DC: American Society for Microbiology, Pp 129 - 158.

de la Fuente. R., Buzon, J. A., Ruiz- Santa-Quiteria-garcia, A., Cid, D., Orden, J, A., Gracia, S. R. and Gomez- Bautista, A. (1999): *Cryptosporidium* and concurrent infections with other major enteropathogens in 1 to 30 days old diarrheic dairy calves in central Spain. *Vet. Parasitol.*, **80**: 179 - 185.

Debanth, N. C., Taimur, M. J. and Gburr, E. C. (1995): A retrospective study of calf losses on the central dairy cattle breeding station in Bangladish. *Prev. Vet. Med.*, **24**: 43 - 53.

Donkersgoed, V. J., Graham, T. and Gannon, V. (1999): The prevalence of verotoxins, *Escherichia coli* O157**:**H7, and *Salmonella* in the feces and rumen of cattle at processing. *Can. Vet. J.,* **40**: 332 - 338.

Drewry, J. J., Quigley, J. D, Geiser, D. R. and Matthew, G. W. (1999): Effect of immunoglobulin G absorption in calves. *Am. J. Vet. Res.*, **60**: 609 - 614.

Farris, A. S., Wierup, M. and Jacobsson, S. –O. (1979): Antibiotic resistance and transferable antibiotic resistance of *Escherichia coli* isolated from calves from modern farm with therapeutic problems and unsatisfactory management conditions. *Nordisk Vet.Med.*, **31**: 20 - 24.

Garber, L. P. (1994): Potential risk factors of *Cryptosporidium* infection in dairy calves. *J. Am. Vet. Med. Asso.*, **205**: 86 - 90.

Gay, C. C., McGuire, T. C. and Parish, S. M. (1983): Seasonal variation in passive transfer of IgG_1 to newborn calves. *J. Am. Vet. Med. Assoc.*, **183**: 566 - 568.

Gitau, G. K., McDermott, J. J., Walter-Toew, K. D., Osuma, J. M. and Muriuki, D. (1994): Factors influencing calf morbidity and mortality in smallholder dairy farms in Kambu district of Kenya. *Prev. Vet. Med.*, **21**: 167 - 177.

Gryseels, G. and de Boodet, K. (1986): Integration of cross breed cows (Boran & Friesian) on smallholder farmer in Debre Zeit area of the Ethiopian highlands. ILCA Highland Program report, ILCA, Addis Ababa, Ethiopia.

Hailemariam, M., Banjaw, K., Gebre Meskel, T. and Ketema, H. (1993): Productivity of Boran cattle and their Friesian cross at Abernosa ranch, Rift valley of Ethiopian. I. Reproductive performance and preweaning mortality. *Trop. Anim. Hlth. Prod.*, **25**: 239 - 248.

Hartman, D. A., Everert, R. W., Slack, S. T. and Warner, R. G. (1997): Calf mortality. *J. Dairy Sci.*, **55** : 576 - 578.

Hassan, Y. and Brannag, E. (1996): Calving performance and mortality in Danish Jersey cattle at Ada Berga state farm, Ethiopia. The proceeding of the 10^{th}conference of Ethiopian Veterinary Association, Addis Ababa, Ethiopia.

Heath, S. E. (1992): Neonatal diarrhea in calves. *Comp. Cont. Edu. Pract. Vet.*, **14**: 995 - 1003.

Heinrichs, A. J. and Radostits, O. M. (2001): Health and production management of dairy calves and replacement heifers. In: Radostits, O.M. (ed.): Herd Health, Food Animal Production Medicine, 3rd ed. Philadelphia, W.B. Saunders Company, Pp 333 - 395.

Hendrix, C. M. (1998): Diagnostic Veterinary Parasitology. 2nd ed. St. Luis: Mosby, Pp 281 - 282.

HiMedia Laboratories Pvt. Limited.

Hunt, E. (1993): Diarrheal disease of neonatal ruminants. In: Howard, J. L. (ed.): Current veterinary therapy, food animal practice. 3rd ed.: W. B. Saunders Company, Philadelphia, Pp 103 - 111.

Hussein, N. (1998): A study on calf mortality at Adamitulu livestock research center. Proceeding of 5th conference of Ethiopian society of animal science (ESAP) 15-17 May 1997, Addis Ababa, Ethiopia. Pp 157 - 162.

ILRI (International Livestock Research Institute) (1996): ILRI Annual project report 1995, Addis Ababa, Ethiopia.

ISO (International Organization for Standardization) (2002): Microbiology of food and animal feeding stuff - horizontal method for detection of *Salmonella*. ISO, 6579, Geneva.

Kifaro, G. C. and Temba, E. A. (1990): Calf mortality and culling rates in two dairy farms in Iringa region, Tanzania. In: Proceedings of Tanzanian Societyof Animal Production, Tanzania, Pp 138 - 146.

Komine, Y., Abe, S., Asai, A., Itagak, M., Wantanbe, D., Komine, K. and Kumagai, K. (2000): Induction of infectious diarrhea in newborn calves fed dysplasia colostrum of mother cows suffering from mastitis during dry period. *Anim. Sci. J.*, **71**: 279 - 285.

Kung, L. Jr., Demarco, S., Siebenson, L. N., Joyner, E., Haenlein, G. F. W. and Morris, R. M. (1997): An evaluation of two management systems for rearing calves fed milk replacer. *J. Dairy Sci*. **80**: 2529 - 2533.

Lance, S. E., Miller, G. Y., Hancock, D. D., Bartlett, P. C., Heider, L. E. and Moeschberger, M. L. (1992): Effects of environment and management on mortality in preweaned dairy calves. *J. Am. Vet. Med. Asso*., **201**: 1197 - 1202.

Lemma, M., Kassa, T. Azage, T. (1999): Major health problems of dairy cattle in market oriented urban and peri-urban production systems in the central highlands of Ethiopia. In:

Proceedings of the seventh annual conference of Ethiopian Society of Animal Production, May 26 - 27, 1999, Addis Ababa, Ethiopia, Pp 353 - 363.

Lemma, M., Kassa, T., Tegegne, T. (2001): Clinically manifested major health problems of crossbred dairy herds in urban and periurban production systems in the central highlands of Ethiopia. *Trop. Anim. Hlth. Prod.* **33**: 85 - 93.

Lai, M. M. C. and Holmes, K. V. (2001): Coronaviridae; The Virus and Their Replication. In: Field Virology, 4th ed., Philadelphia, Pp 1163 - 1185.

Lorenzo, M. J., Ben, B., Mendez, F., Villacorta, I. and AresMazas, M. E. (1995): *Cryptosporidium parvum* oocyst antigen recognized by sera from infected asymptomatic adult cattle. *Vet. Parasitol.*, **60**: 17 - 25.

Martin, S. W., Meek, A. H., Willeberg, P. (1987): Veterinary Epidemiology: Principle and Methods. Iowa, Iawa Stata University Press.

McEvoy, J. M., Doherty, A. M., Sheridan J. J., Blair, I. S. and McDowell, D. A. (2003): The prevalence of *Salmonella* species in bovine fecal, rumen and carcass samples at a commercial abattoir. *J. Appl. Microbiol.*, **94**: 693 - 700.

Mekonen, L., Tesfu, K. and Azage, T. (2001): Clinically manifested major health problems of crossbred dairy herds in urban and periurban production system in the Central highlands of Ethiopia. *Trop. Anim. Hlth. Prod.*, **33**: 85 - 93.

Mel'nikova, V. M, Gracheva, N. M., Belikov, G. P., *et al.*, (1993): The chemoprophylaxis and chemotherapy of opportunistic infections. *Antibiotiki i Khimioterapiia*, **38**: 44 - 8.

Molla, B., Mesfin, A. and Alemayehu, D. (2003): Multiple antimicrobial resistance pattern of *Salmonella* serotypes isolated from chicken carcass and giblets in Debre Zeit and Addis Ababa, Ethiopia. *Ethiop. J. Health Dev.* **17**: 131 - 149.

Molla, B., Salah, W., Alemayehu, D., Mohammed, A. (2004): Antimicrobial resistance pattern of *Salmonella* serotypes isolated from apparently healthy slaughtered camels (Camelus dromedarius) in eastern Ethiopia. Berliner und Münchner Tierärztliche Wochenschrift,. **117**: 39 - 45.

Molla, B., Berhanu, A., Muckle, A., Cole, L., Wilkie, E., Kleer, J. and Hildebrandt, G. (2006): Multidrug resistance and distribution of *Salmonella* serovars in slaughtered pigs. *J. Vet. Med.*, **53**: 28 - 33.

Murphy, F. A., Gibbs, P. J., Hurinek, M. C. and Studdert, M. J. (1999a): Coronaviridae. In: Veterinary Virology, 3rd ed. Sandiago: Academic Press, Pp 495 - 508.

Murphy, F. A., Gibbs, P. J., Hurinek, M. C. and Studdert, M. J. (1999b): Reoviridae. In: Veterinary Virology, 3rd ed. Sandiago: Academic Press, Pp 391 - 409.

Namur, A. P., Morel, J. and Bickel, H. (1998): Compound Animal Feed and Feed Additives. Live stock production science. Elsevier Science Publisher. B. V., Netherlands, **18**: 208.

NCCLS (National Committee for Clinical Laboratory Standards) (1997): Performance standards for antimicrobial disc and dilution susceptibility tests for bacteria isolated from animals and humans. Approved Standard. NCCLS Document M31-A, NCCLS, Villanova, PA.

NMSA (National Meteorological Service Agency) (2003): Rainfall and temperature data, Addis Ababa, Ethiopia, NMSA.

Northeim, K. and Simensen, E. (1985): An epidemiological study of factors affecting serum IgG levels in dairy calves. *Nordisk Vet. Med.*, **37**: 121 - 135.

OIE. (2004): Salmonellosis. In: Manual of Standards for Diagnostic Tests and Vaccines. 5th ed. Pp 195 - 210.

Olsson, S. O., Viring, S., Emanuelsson, U. and Jacobsson, S. O. (1993): Calf disease and mortality in Swedish dairy herds. *Acta Vet. Scand.* **34**: 263 - 269.

Otte, M. J. and Chilonda, P. (2002): Cattle and small ruminant production system in sub-Sharan Africa – A systemic review. Rome, FAO.

Quigley, J. D. III (1997): Raising replacement heifers from birth to weaning. Proceedings of the 1997 Western Canadian Dairy Seminar, Red Deer, Alberta, Canada.

Quinn, P. J., Carter, M. E., Markey, B. K. and Carter, G. R. (1994): Clinical Veterinary Microbiology. London: Wolfe Publishing, Pp 13 - 16.

Quinn, P. J., Markey, B. K., Carter, M. E., Donnelly, W. J. C., Leonard, F. C. and Maguire, D. (2002): Enterobacteriaceae. In: Veterinary Microbiology and Microbial Diseases. London: Blackwell Science, Pp 106 - 123.

Radostits, O. M. and Acres, S. D. (1980): The prevention and control of epidemics of acute undifferentiated diarrhea of beef calves in Western Canada. *Can. Vet. J.*, **21**: 243 - 249.

Radostits, O. M., Blood, D. C. and Gay, C. C. (1994): Veterinary Medicine. A Text Book of the Diseases of Cattle, Sheep, Goats and Horse; 8th ed. Bailliere Tindall, London, Pp 107 - 156.

Rao, M. K. and Nagarcenkar, R. (1980): Calf mortality in crossbred dairy cattle. *Trop. Anim. Hlth. Prod.*, **12**: 137 - 144.

Reynolds, D. J., Morgan, J. H., Chanter, N., Jones, P. W., Bridger J. C., Debney, T. G. and Bunch, K. J. (1986): Microbiology of calf diarrhea in Southern Britain. *Vet. Rec.*, **119**: 34 - 39.

Roderick, S. and Hovi, M. (1999): Animal health and welfare in organic livestock systems: Identification of constraints and priorities. A report to MAFF, University of Reading.

Runnel, P. L., Moon, H. W. Mattew, P. J. (1986): Effect of microbial and host variables on the interaction of Rotavirus and *E. coli* infections in genetobiotic calves. *Am. J. Vet. Res.*, **47**: 1542 - 1546.

Saif, L. J. (1992a): Coronavirus. In: Veterinary Diagnostic Virology. (Eds.) Castro, A. E. and Heuschele, W. P.; A practitioners guide, Mosbly-Year Book, Inc, Pp 92 - 97.

Saif, L. J. (1992b): Rotavirus. In: Veterinary Diagnostic Virology. (Eds.) Castro, A. E. and Heuschele, W. P.; A practitioners guide, Mosbly-Year Book, Inc, Pp 126 - 130.

Scott, C. A., Smith, H. V., Mtambo, M. M. M. and Gibbs, H. A. (1995): An epidemiological study of *Cryptosporidium parvum* in two herds of adult beef cattle. *Vet. Par.*, **57**: 277 - 287.

Scott, P. R., Hall, G. A., Jones, P. W. and Morgan J. H. (2004): Calf diarrhea. In: Bovine Medicine. Eds. Andrews, A. H., Blowey, R. W., Boyd, H., Eddy, R. G. 2nd ed. Oxford Blackwell Publishing, UK, Pp 185 - 214.

Selman, I. E. (1981): The care of young calves, neonatal calf diarrhea, the calf pneumonias. In: Current topics in veterinary medicine and animal science, Disease of cattle in the tropics. Martitins Nijhoff publisher, London, Pp 543 - 565.

Sevun, F., Simsek, A. and Uslu, U. (2005): Massive *Cryptosporidium parvum* infection associated with an outbreak of diarrhea in neonatal goat kids. *Turk J. Vet. Anim. Sci.*, **29**: 1317 - 1320.

Shiferaw, Y., Yohannes, A., Yilma, Y., Gebrewold, A. and Gojjam, Y. (2002): Dairy husbandry and health management at Holleta. Proceeding of the 16thconference of the Ethiopian Veterinary Association. Addis Ababa, Ethiopia. Pp 103 - 119.

Sisay, A. and Ebro, A. (1998): Growth performance of Boran and their Semmintal cross calves. Proceeding of 6thnational conference of the Ethiopian society of animal production (ESAP). Addis Ababa, Ethiopia. Pp 157 - 162.

Sivula, N. J., Ames, T. R., Marsh, W. E. and Werdin, R. E. (1996a): Descriptive epidemiology of morbidity and mortality in Minnesota dairy heifer calves. *Prev. Vet. Med.*, **27**: 155 - 171.

Sivula, N. J., Ames, T. R. and Marsh, W. E. (1996b): Management risk factor for morbidity and mortality in Minnesota Dairy Heifers. *Prev. Vet. Med.*, **27**: 173 - 182.

Smirnov, V. V., Reznik, S. R., V'iunitskaia, V. A, *et al*., (1993): The current concepts of the mechanisms of the therapeutic-prophylactic action of probiotics from bacteria in the genus *Bacillus*. *Mikrobiolohichnyi Zhurnal*., **55**: 92 - 112.

Snodgrass, D. R., Smith, M. L. and Krautis, F. L. (1982): Interaction of rotavirus and Enterotoxigenic *Escherichia coli* in conventionally reared dairy calves. *Vet. Microbiol*., **7**: 51 - 56.

Snodgrass, D. R., Terzolo, H. R., Sherwood, D., Campbell, I., Menzies, J. D. and Synge, B. A. (1986): Etiology of diarrhea in young calves. *Vet. Rec*., **119**: 31 - 34.

Soulsby, E. J. L. (1982): Helminths, Arthropods and Protozoa of Domesticated Animals, 7th ed. Bailliere Tindall, UK, Pp 594 - 664.

Temesgen, W. (2004): Calf morbidity and morbidity in dairy farms in Debre-Zeit & its environs. MSc thesis, FVM, AAU, Debre Zeit, Ethiopia.

Trotz-Williams, L. A., Javie, B. D., Martim, S. W., Leslie, K. E. and Peregrine, A. S. (2005): Prevalence of *Cryptosporidium parvum* in fection in souther Ontario and its association with diarrhea in neonatal dairy calves. *Can Vet J*., **46**: 349 - 351.

Virtala, A. M., Mechor, G. D., Grohn, Y. Y. and Erb, H. N. (1996): Morbidity from non-respiratory disease and mortality in dairy heifers during the first three months of life. *J. Am. Vet. Res*., **208**: 2043 - 2046.

Waltner-Toews, D., Martin, S. W. and Merk, A. H. (1986a): Effect of calfhood disease on survivorship and age at first calving. *Can. J. Vet. Res*., **50**: 314 - 317.

Waltner-Toews, D., Martin, S. W. and Merk, A. H. (1986b): Epidemiological study of selected calf pathogens on Holstein dairy farms in Southern Ontario. *Can. J. Vet. Res*., **50**: 307 - 313.

Warnick, L. D., Crotton, L. M., Pelzer, K. D., Hawkins, M. J. (2001): Risk factors for clinical Salmonellosis. *Prev. Vet. Med*., **49**: 259 - 275.

Wereme, A. N., Strabel, M., Grongnet, J. and Piot, M. (2001): Immunoglobulin G absorption from pooled maternal colostrum, commercial powder and freeze-dried colostrum by newborn calves. *Anim. Res.*, **50**: 315 - 323.

West, G. (1995): Blacks Veterinary Dictionary, 18th ed. Black Publisher Ltd., London, UK, Pp 98.

Wittum, T. E., Salmon, M. D. and King, M. E. (1994): Individual animal and maternal beef calves in Colorado, USA. *Prev. Vet. Med.*, **19**: 1 - 14.

Yemam, H. and Brannag, E. (1996): Calving performance and mortality in Danish Jersey cattle at Ada Berga state farm, Ethiopia. In: Proceedings of the 10th Conference of Ethiopian Veterinary Association, Addis Ababa, Ethiopia.

Yosef, S., Aster, Y., Zelalem, Y., Alemu, G. and Yohannes, G. (2002): Dairy husbandry and health management at Holeta. In: Proceedings of the 16th Conference of Ethiopian Veterinary Association, Addis Ababa, Ethiopia, Pp 103 - 119.

8. ANNEXES

Annex 1. Questionnaire format

1. Farm identification

   Owner's name……

   Address: Kebele ……., House no ……., Telephone no …….

2. Farm description

   2.1. Owner / manager educational status

      a) Illiterate

      b) Read and write

      c) Elementary school

      d) High school graduate

      e) Professional

      If professional a) Related to animal production b) Unrelated to animal production

   2.2. Herd size: Cows ……. , Bulls ……, Heifers …….

      Male calves ……., Female calves ……

   2.3. Breed and age of animals kept…….

   2.4. Age of the farm…….

   2.5. The farm as a source of income: a) Primary income b) Secondary income

3. Management data

   3.1. Calf caretakers (attendants)

      3.1.1. Ownership: a) Owner (family member) b) Hired

      3.1.2. Sex: a) Male b) female

      3.1.3. Experience: a) $\leq$ 5 years b) > 5 years

   3.2. Periparturient care

      3.2.1. Calving facilities: a) Calving pen b) The same barn

      3.2.2. Navel treatment: a) Practiced b) Not practiced

      3.2.3. Awareness of importance of colostrum to neonates: a) Yes b) No

         If yes method of feeding: a) Suckling b) Hand feeding

         Time of first feeding: a) < 6 hours b) 6-24 hours c) > 24 hours

Duration of feeding: a) For 24 hours b) 24 hours to 4 days c) > 4 hours

If hand feeding, source of feeding: a) Dam b) Another cow

3.3. Feeding

3.3.1. Type of feeding: a) Milk b) Milk replacer

3.3.2. Amount of milk / milk replacer given daily per unit of body weight

3.3.3. Frequency: a) Once per day b) Twice per day c) Three times per day

3.3.4. Type of supplementary feed and quantity per unit of body weight:

a) Grazing b) Concentrates c) Hay

3.3.5. Weaning age……

3.4. Housing

3.4.1. Housing: a) Separate pen b) Together with cows in the same barn c) Other

If separate pen: a) Individual pen b) Group pen

3.4.1. Bedding: a) Present b) Absent

If present what is the bedding material and how frequently it is changed…….

a) > Once per week b) Once per week c) < Once per week

4. Experience on calf health problems; and prevention and control of the problems

4.1. Major health problems for the farm …….

4.2. Number of calves the farm lost due to diarrhea during the last one year…….

4.3. Disease or disease syndrome responsible for sickness and health of calves in order of importance……

4.4. Measures taken to treat sick calves……

4.5. Measures taken to prevent disease problems…….

Annex 2. Calf card format

1. Genealogy and periparturient care
   1.1. Date of birth: Month ……., Day ……..
   1.2. Time of birth: a) Night b) Day
   1.3. Site of birth: a) The same cow barn b) Calving pen
   1.4. Condition of birth: a) Normal delivery b) Dystocia
   1.5. Sex: a) Male b) Female
   1.6. Parity of the dam: a) First parity b) Second parity and above
   1.7. Exotic blood level: a) < 50% b) 50 – 75% c) > 75%
   1.8. Navel disinfection: a) Yes b) No
   1.9. Chemical used in navel disinfection …….
   1.10. Time of colostrum ingestion: a) Before 6 hour b) 6 - 12 hour c) > 24 hour
   1.11. Method of colostrum feeding: a) Hand feeding b) Suckling
   1.12. The number of days the calf stayed with the dam …….
   1.13. Total amount of colostrum ingested in liter……
2. Feeding and housing
   2.1. Type of liquid feed given to calves: a) Milk b) Milk replacer
   2.2. Time of introduction of extra diet ……
   2.3. Type of extra feed ……
   2.4. Amount of liquid diet before introduction of extra feed ……
3. Health problems encountered during the monitoring period
   3.1. Type of health problem encountered…….
   3.2. Date ……..

Annex 3. Media used and preparations for the isolation and identification of *Salmonella*.

1. Buffered peptone water (BPW) (AES loboratoire, Cedex, France)

Composition (g/l): Peptone from casein 10.0; Sodium chloride 5.0; Di-sodium hydrogen phosphate 3.5; Potassium dihydrogen phosphate 1.5.

Preparation: Dissolved 20 grams of this medium in one liter of distilled water and sterilize by autoclaving at 121^0C for 15 minutes.

2. Rappaport-Vassiliadis with Soya (RVS) (Titan Biotech, Raj, India)

Composition (g/l): Magnesium chloride (anhydrous) 13.58; Sodium chloride 7.2; Soya peptone 4.5; Potassium dihydrogen phosphate 1.26; Di- potassium hydrogen phosphate 0.18; Malachite green 0.036.

Preparation: Suspend 26.75 grams of the powder in 1 liter of purified water. Gently heat to dissolve the medium completely. Dispense 10 ml amounts into tubes and sterilize by autoclaving at 115^0C for 15 minutes.

3. Muller-Kauffmann tetrathionate with novobiocin (MKTTn) broth (Oxoid, Hampshire, England)

Composition (g/l): Meat extract 0.9; Peptone from meat 4.5; Yeast extract 1.8; Sodium chloride 4.5; Calcium carbonate 25; Sodium thiosulphate 40.7; Ox bile, dried 4.75; Potassium iodide 5; Iodine 4; Brilliant green 0.01.

Preparation: Suspend 82 grams of the powder in one liter of distilled water, heat briefly to boil cool rapidly. Don't autoclave. Potassium iodide solution 20ml/l and 0.015 solution of brilliant green (10 ml/l), dispense into test tubes taking care to suspend any precipitate evenly. Preparation

of the iodine/potassium iodide solution is potassium iodide 5 grams; iodine 4 grams; distilled water 20 ml. The ready-to-use broth should be prepared and used on the same day.

4. Xylose lysine deoxycholate (XLD) agar (AES laboratoire, Cedex, France)

Composition (g/l): Yeast extract 3.0; L-Lysine hydrochloride 5.0; Xylose 3.75; Lactose 7.5; Sucrose 7.5; Sodium desoxycholate 1.0; Sodium chloride 5.0; Sodium thiosulphate 6.8; Iron (III) ammonium citrate 0.8; Phenol red 0.08; Agar 16.5.

Preparation: Suspended 57 grams of the powder in one liter of distilled water, bring to the boil with frequent agitation to dissolve completely, mix well and pour into Petri dishes.

5. XLT4 agar (Difco, Becton Dickinson, France)

Composition (g/l): Proteoose peptone No.3 16.0; Yeast extract 5.0; L-Lysine 5.0; Xylose 3.75; Lactose 7.5; Sacchrose 7.5; Ferric ammonium citrate 0.8; Sodium thiosulfate 6.8; Sodium chloride 5.0; Phenol red 0.08; Agar 18.0.

Preparation: Suspend 59 gram of the powder in 1 liter of purified water. Add 4.6 ml XLT4 Agar Supplement. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder. Avoid overheating. Do not autoclave. Test samples of the finished product for performance, using stable, typical control cultures. Final pH 7.4 $\pm$ 0.2.

6. Rambach agar (Merck, Darmstadt, Germany)

Composition (g/liter): Peptone 8.0; sodium chloride 5.0; sodium desoxycholate 1.0; chromogenic mix 1.5; propylene glycol 10.5; agar-agar 15.0.

Preparation: i. One vial of liquid mix was added to 250 or 1000 ml distilled water and mixed by swirling until completely dissolved (The water quantity is dependent on the respective pack size.) ii. One vial of nutrient-powder was added and mixed by swirling until completely suspended.

iii. The medium was heated in a boiling water bath until totally dissolved, while carefully shaking from time to time.
iv. The medium was cooled as fast as possible in a water-bath (45-50^{0}C). During this procedure (max. 30 minutes) it gently shook from time to time and poured in to plates.
v. In order to prevent any precipitate or clotting of the chromogenic-mix in the plates, Petri dishes were placed on a cool surface during pouring procedure.

7. Brain heart infusion agar (Difco, Becton Dickinson, Claix, France)

Composition (g/liter):
Infusion from calf brains 200.0, infusion from beef heart 250.0, proteose peptone 10.0, dextrose 2.0, sodium chloride 5.0, disodium phosphate 2.5, and agar 14.0.

Preparation: Suspend 52 grams in 1 liter distilled water and heat to boiling to dissolve completely. Sterilize in the autoclave for 15 minutes at 121^{0}C.

8. Nutrient agar (Oxoid, Hampshire, England)

Composition (g/liter):
"Lab-Lemco" powder 1.0; yeast extract 2.0; peptone 5.0; sodium chloride 5.0; agar 15.0.

Preparation: Suspend 28g in 1 liter of distilled water. Bring to boil to dissolve completely. Sterilize by autoclaving at 121^{0}C for 15 minutes.

9. Tryptone soya broth (Oxoid, England)

Composition (g/liter): Pancreatic digest of casein 17.0; Pancreatic digest of Soya bean meal 2.0; Sodium chloride 5.0; Di-basic potassium phosphate 2.5; Glucose 2.5.

Preparation: Dissolve 30.0 g in one liter of distilled water and distributed into final containers, sterilize by autoclaving at 121^{0}C for 15 minutes.

10. Muller-Hinton Agar (BBL®, Becton Dickinson, USA)

Composition (g/l): Meat infusion 5.0; Casein hydrolysate 17.5; Starch 1.5; Agar-agar 13.

Preparation: Dissolve 34.0 g in 1 litre of distilled water, boil to homogenize it, autoclave at 115^0C for 10 min, cool to 50^0C and dispense on to sterile petridishes.

9. CURRICULUM VITAE

PERSONAL DATA

Name	Dagnachew Demissie
Date of Birth	April 20, 1978
Place of birth	Aleltu
Sex	Male
Citizenship	Ethiopian
Marital status	Single
Language Proficiency	Amharic,Oromifa and English
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EDUCATIONAL BACKGROUND

1984 - 1989.	Rufa Bido Primary School, Wonoda (Certificate)
1990 - 1991	Aleltu Elementary and Junior Secondary School, Mikewa (Certificate)
1992 -1995	Sendafa Senior Secondary School, Sendafa (Certificate)
1996 - 1997	Addis Ababa University, Faculty of Veterinary Medicine, Debre Zeit (Diploma)
2000	Addis Ababa University, Faculty of Science, Addis Ababa (freshman courses)
2001 - 2005	Addis Ababa University, Faculty of Veterinary Medicine, Debre Zeit (Courses of general Veterinary medicine) (DVM)
2006 - 2007	Addis Ababa University, Faculty of Veterinary Medicine, Debre Zeit (Master of Veterinary Science in Veterinary Microbiology)

Research paper

Prevalence and distribution of enterohaemrrhagic *Escherchia coli* O157: H7 and *Salmonella* serotypes isolated from selected samples in Debre Zeit and Addis Ababa, Ethiopia. DVM thesis (2005), FVM, AAU, (Unpublished)

Other papers

Hazard analysis critical control point (HACCP) system: Principles and application. Seminar paper (2004).
Microbial causes of calf diarrhea. Seminar paper (2006).

Additional trainings and certificates

Computer skills: Basic computer application software courses (Certificate).
HIV/ AIDS and reproductive health peer education training (Certificate).

Work experience

Animal health assistant from September 1999 - September 2000 in east Wollega zone, Nekemt.

Hobbies

Reading, Music, Sports

SIGNED DECLARATION

This thesis is my original work, has not been presented for a degree in any other university and that all sources of material used for the thesis have been duly acknowledged.

Name Dagnachew Demissie

Signature

Date of submission 25 - 06 - 2007

This thesis has been submitted for examination with our approval as university advisors.

Dr. Bayleyegn Molla (Associate Professor) _____

Dr. Kelay Belihu (Assistant Professor) _____