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EPIDEMIOLOGICAL AND MICROBIOLOGICAL STUDIES OF CALF DIARRHOEA AND PNEUMONIA IN DEBRE ZEIT, HOLETA AND MUKE TURI DAIRY FARMS, ETHIOPIA

BY

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A thesis submitted to the School of Graduate Studies of Addis Ababa University, Faculty of Veterinary Medicine in partial fulfillment of the requirements for the Degree of Master of Veterinary Science in Tropical Veterinary Microbiology

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ABBREVIATIONS

AAU	Addis Ababa University
AIDS	Acquired Immunodeficiency Syndrome
BCoV	Bovine coronavirus
BHI	Brain heart infusion agar
BPW	Buffered peptone water
BRDC	Bovine respiratory disease complex
BRSV	Bovine respiratory synctial virus
CDC	Center for Disease Control
CI	Cumulative Incidence
CRTD	Chronic Respiratory Tract Disease
dsRNA	Double-stranded RNA
EAggEC	Enteroaggregative *Escherichia coli*
EHEC	Enterohaemorrhagic *Escherichia coli*
EIEC	Enteroinvasive *Escherichia coli*
ELISA	Enzyme Linked Immunosorbent Assay
EMB	Eosine methylene blue
EPEC	Enteropathogenic *Escherichia coli*
ETEC	Enterotoxigenic *Escherichia coli*
Fc	Fragment of Crystallization
FVM	Faculty of Veterinary Medicine
GH	Gonadotrophin hormone
HAI	Hemagglutination inhibition
HR	Hazard Ratio
Ig	Immunoglobulin
IMViC test	Indole, methyle red, voges-proskauer and citrate test
ISO	International Organization for Standardization
LH	Luteinizing hormone
LIA	Lysin Iron Agar
MIC	Minimum Inhibitory Concentration

MKTTn	Muller-Kauffmann tetrathionate with novobiocin
MSc	Master of Science
MSRV	Modified Semisolid Rappaport-Vassiliadis
NAD	Nicotinamide Adenine Dinucleotide
NCCLS	National Committee for Clinical Laboratory Standards
NDC	Neonatal diarrhoea complex
ORT	Oral Replacement fluid Therapy
PAGE	Polyacrylamide gel electrophoresis
PMN	Polymorphonuclear
RV	Rotavirus
SS-agar	*Salmonella-Shigella* agar
STEC	Shiga-like toxin producing *Escherichia coli*
TSI	Triple sugar iron
VNT	Virus neutralization test
VP	Viral Protein
VTEC	Vero toxin producing *Escherichia coli*
XLD	Xylose lysine deoxycholate

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ABSTRACT

A six-month prospective study was conducted in Holeta, Debre Zeit and Muke Turi dairy farms between November, 2007 and April, 2008 with the major objective of determining both the cumulative incidences of neonatal calf diarrhoea (NCD) and pneumonia. In addition, determination of calf mortality rates, identification of agents of NCD, pneumonia and potential risk factors as well as determinations of the antibiotic sensitivity patterns of the bacterial pathogens.

Cluster sampling was used. A total of 429 calves, from seven intensive dairy farms were followed up. The farms were described using a pre-tested questionnaire and personal observations. Study calves in each farm were visited weekly and appropriate test samples collected from diarrhoeic and pneumonic cases.

Antigenic ELISA test was utilized to identify four major enteropathogens (rotavirus, coronavirus, *Cryptosporidium parvum* and *E. coli* K 99). Standard isolation techniques were used to identify *Salmonella* and other strains of *E. coli* from diarrhoiec cases (n=112) and *Pasteurella* species, *Mannhaemia* species and *Haemophilus somnus* from pneumonic cases (n=28). In addition, the antimicrobial susceptibility pattern of the bacterial isolates was performed.

The cumulative incidences (CI) of NCD in Holeta, Muke Turi and Debre Zeit dairy farms were 0.41, 0.63 and 0.37, respectively. While for pneumonia, they were 0.12, 0.09 and 0.07, respectively. Higher CI of NCD was recorded from Farm 1 with 0.65 followed by Farm 3 with 0.63. The least CI was observed in Farm 2 of 0.11. Highest calf pneumonia was observed in Farm 6 followed by Farm 1. No calf pneumonia was observed in Farm 5.

Associations of the potential risk factors for NCD and pneumonia revealed significant results with young ages. Significant positive hazard rates of both diseases were recorded in young age-groups. Other risk factors (sex of calves, breed, etc) were not significantly associated with these diseases.

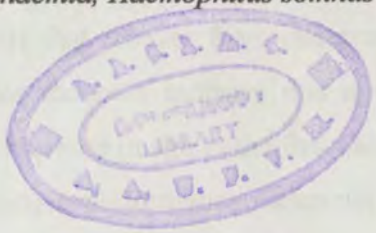
The ELISA results showed that, *C. parvum* was prevalent in the majority of calves with diarrhoea followed by rotavirus, coronavirus and *E. coli* K 99. The bacterial isolates were 91.96 % of *E.*

coli other than the strain K 99 and 24.11 % of *Salmonella* species. Nasal swab samples taken for isolation of *Pasteurella* and *Mannhaemia* species revealed 42.85 % positive reactors for *Pasteurella* and *Mannhaemia* species. No *Haemophilus somnus* was isolated.

Antimicrobial sensitivity test was performed for all the bacterial isolates. Norfloxacillin and gentamycin were highly sensitive and clindamycin the least sensitive. The susceptibility to other antimicrobial agents varied by different bacterial isolates, but resistance to all antimicrobials used were observed in most of the isolates.

In conclusion, NCD is a serious problem among dairy calves followed by pneumonia. Both of them were influenced by the age of calves. These diseases have complex etiopathogenesis. Therefore, further epidemiological and microbiological studies of NCD and pneumonia with aims of designing and implementing appropriate prevention and control strategies are strongly recommended. In addition, the higher rate of antimicrobial resistance necessitates the strategic use of the drugs.

Key words: Neonatal calf diarrhoea, Calf pneumonia, rotavirus, coronavirus, *Cryptosporidium parvum, Escherchia coli, Salmonella, Pasteurella, Mannhaemia, Haemophilus somnus*



1. INTRODUCTION

The future development of any dairy production relies, among other things, on successful programmes of raising calves and heifers for replacement (Bath *et al.*, 1985). In addition, the health management practices of replacement animals are important components of the total herd productivity. The latter can be negatively impacted by poor growth of calves, limited opportunity for genetic selection due to high mortality of replacement animals and reduced milk yield from these animals that experience chronic illness during growth of calves to adult cows and increased veterinary costs (McGuirk and Ruegy, 2004).

Amongst all animals present in dairy farms, the highest morbidity rates generally occur prior to weaning (McGuirk and Ruegy, 2004). Risk factors for morbidity in young dairy calves have been investigated over many years. Diarrhoea and respiratory illness have been incriminated as the most important risks for morbidities in calves in many ecological niches among many countries (Svensson *et al.*, 2006).

Diarrhoea and pneumonia are among the major causes of economic losses in livestock industry associated with decreased production, high levels of morbidity and mortality and increased veterinary and labor costs (Langoni *et al.*, 2004; Trotz-Williams *et al.*, 2007). Chronically, the fibrosis and loss of functional lung capacity in animals that recover from pneumonia have negative impact on daily live weight gains. Thus, for the beef industry this means long finishing times. Whilst, for the dairy industry, it means increase in age at the first calving and, subsequent negative effects on production and reproductive performance parameters (Potter, 2007).

Calf diarrhoea is usually a multi-pathogen syndrome (Godden, 2007). This is because of the complex polymicrobial disease syndrome that includes bacteria, viruses and protozoa. These act singly or in combination to produce the outbreaks of calf diarrhoea. Among bacteria, enterotoxigenic *Escherichia coli* (ETEC) and *Salmonella* are the common and the most economically important agents. The viral agents include rotavirus and coronavirus. *Cryptosporidium* species are one of the most important protozoal agents (Khan and Khan, 1991; Naciri *et al.*, 1999; Garcia *et al.*, 2000; Achá *et al*, 2004; Godden, 2007). All these

micro-organisms are responsible for the vast majority (75-95%) of enteric infections in neonatal calves worldwide (de la Fuente *et al.*, 1998; de la Fuente *et al.*, 1999; Achá *et al.*, 2004). In addition to these infectious agents, environment, management (Khan and Khan, 1991; Langoni *et al.*, 2004), nutritional factors such as copper deficiency (Picco *et al.*, 2004) and physiological risk factors like hormonal imbalances (Bru¨ckmann *et al.*, 2000) could influence the severity and outcome of the disease.

The respiratory disease complex (RDC) is one of the most common and devastating diseases in the cattle industry. It has multi-pathgenic agents. In calf RDC, a variety of micro-organisms are involved (Tegtmeier *et al.*, 1999). The major bacterial pathogens are *Mannheimia haemolytica*, *Pasteurella multocida*, and *Haemophilus somnus* (also referred to as *Histophilus somni*) (Derosa *et al.*, 2000; Norcia *et al.*, 2004; Berghaus *et al.*, 2006; Corbeil *et al.*, 2006; Katsuda *et al.*, 2007). The major viral pathogens associated with this disease are, bovine viral diarrhoea virus, bovine herpes virus 1, parainfluenza 3, and bovine respiratory synctial virus (BRSV) followed by one or more bacterial infections (Berghaus *et al.*, 2006: Corbeil *et al.*, 2006; Yeşilbağ and Güngör, 2008). Management and environmental conditions are highly related to bovine respiratory diseases, (Derosa *et al.*, 2000; Yeşilbağ and Güngör, 2008). *H. somnus* can cause pneumonia alone or in conjunction with the above viruses in cattle (Berghaus *et al.*, 2006).

Previous study by Abraham *et al.* (1992) on Neonatal Calf Diarrhoea (NCD) indicated bovine enteric coronavirus as the major infectious cause of NCD in some Ethiopian dairy herds, rotavirus and K99 ETEC also contributing to morbidity, either alone or as mixed infections. Studies were also conducted by Simachew (1998), Tadesse (2004), Temesgen (2004) and Abebe (2005) concerning the causes of calf diarrhoea but all had emphasized mainly on the bacterial and protozoal causes. Nevertheless a study undertaken by Abraham *et al.* (1992) and Demissie (2007) included the viral and bacterial causes of calf diarrhoea. But no study has been conducted about the major bacterial as well as viral causes of calf diarrhoea together with calf pneumonia in intensive dairy farms in Debrezeit, Holeta and Muke Turi.

Considering the complex etiology of diarrhoea and its economical importance in Ethiopian cattle industry, prevalence and etiology should be studied in all agro ecological zones. Therefore, this study was conçeived with the following objectives:

- To determine the cumulative incidences of calf diarrhoea and pneumonia for studying calf morbidity and mortality in Debrezeit, Holeta and Muke Turi dairy Farms;
- To investigate the potential risk factors of calf diarrhoea and pneumonia
- To determine the prevalence of the five major enteropathogens: rotavirus, coronavirus, *Cryptosporidium parvum,* Enterotoxigenic *Escherchia coli* (ETEC) and *Salmonella* species in diarrhoeic calves;
- To determine the prevalence of *Pasteurella*, *Mannhaemia* and *Haemophilus somnus* species from pneumonic calves
- To Identify the antimicrobial susceptibility patterns of *Salmonella* species, *E.coli, Pasteurella* species, *Mannhaemia* species and *Haemophilus somnus* isolates.

2. LITERATURE REVIEW

2.1. Calf Diarrhoea

2.1.1. The clinical picture and effects of Neonatal calf diarrhoea

Neonatal calf diarrhoea (NDC) is characterized by diarrhoea (scouring), progressive dehydration and death (Bicknell and Noon, 1993). Scour is the passing of abnormally high amounts of fluid in the faeces than is normally observed in calves for 2 days or more. 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. Depending on the severity of the disease, a watery yellow, gray or greenish diarrhoea containing varying amounts of mucus, which may be tinged with blood, can be passed in the faeces (Bicknell and Noon, 1993; Mason and Caldow, 2005). Soiling of the hindquarters and tail with the diarrhoeic feces is common (Bicknell and Noon, 1993; Svensson *et al.*, 2006).

At first, diarrhoeic animal may appear alert and normal. But after sometime becomes anorexic depressed, weak, and unable to stand. Dehydration occurs due to fluid loss resulting from severe diarrhoea that is characterized by sunken eyes and dry skin. If the disease is untreated, dehydration progresses, electrolyte (ions of body salts such as sodium, potassium, chloride, and bicarbonate) loss occurs and eventually the calf dies. Body temperature varies, depending to some extent on the causative agents involved. A subnormal temperature in the terminal stages of the disease is prevalent (Bicknell and Noon, 1993).

The normally solid fecal mass is formed by absorption of water from the liquid intestinal content by the cells lining the large intestine. Diarrhoea or scouring occurs when the capability of the intestine to absorb fluid is impaired. Interference with this absorptive function of the intestine may occur in two ways. Damage to the cells lining the intestine may result from cell destruction by certain infectious agents, resulting in loss of the digestive and absorptive capability of the intestine as well as inflammation. Other infectious agents produce toxins that cause the cell lining of the intestine to produce fluid rather than absorb it. Diarrhoea, dehydration and electrolyte loss occur in both instances and have especially severe effects in the newborn animal (Bicknell and Noon, 1993).

2.1.2. Major microbial causes of calf diarrhoea

Viral causes

Rotavirus

Rotaviruses are the most frequent agents associated with diarrhoea in children and domestic animals. Rotaviruses belong to the family Reoviridae, genus rotavirus. The complete virion is 70 nm in diameter and is characterized by a triple protein capsid, and its genome consists of 11 double-stranded RNA (dsRNA) segments. Rotavirus antigenic properties (group, subgroup and serotype) are determined by the capsid proteins. Rotavirus has been classified into seven major groups (A-G) according to its genomic RNA electrophoretic pattern; namely an electropherotype or group-specific antigen on VP6. Most of the strains isolated from human beings and animals belong to group A rotavirus but groups B and C are occasionally found. The external capsid proteins Viral Protein 4 and 7 (VP4 and VP7) induce neutralizing antibodies, which determine the binary classification of rotavirus into serotypes. VP4 is a protease-sensitive protein and variants of this protein are called P serotypes of group A rotaviruses. VP7 is a glycoprotein, which determines G serotypes. Several studies have found a correlation between different rotavirus genotypes and infected animal species, but interspecies transmission has also been described (Fukai *et al.*, 1999; Murphy *et al.*, 1999; Barreiros *et al.*, 2004).

NCD caused by rotavirus is frequently found in different countries regardless of technology, management or sanitary procedures. Rotavirus infections are a common cause of neonatal diarrhoea in many mammalian and avian species. Rotavirus has an important aetiological role in the neonatal calf diarrhoea and mainly found in faeces of diarrhoeic calves up to 3rd week of life (Khan and Khan, 1991; Parreno *et al.*, 2001; Barreiros *et al.*, 2004). In addition to clinical rotavirus infections, subclinical infections are also common in calves. Coronavirus, Cryptosporidium and enterotoxigenic *E. coli* (ETEC) are together with rotavirus the four major enteropathogens associated with NCD worldwide. These organisms are responsible for the vast majority (75 to 95%) of enteric infections in neonatal calves worldwide (Fukai *et al.*, 1999; Garcia *et al.*, 2000).

Pathogenesis of Rotavirus

The virus especially attacks the epithelium of small intestine of young calves. It replicates in intestinal epithelial cells near the tips of villi. Infected cells are desquamated, which results in a failure of digestion and absorption of nutrients. As epithelial cells are lost from the tips of villi, the desquamated cells are replaced by cuboidal, then flattened squamous epithelial cells. Some villi may remain denuded and stroma of villi becomes internally infiltrated with leukocytes. Later on these findings were confirmed by electron microscopy (Khan and Khan, 1991; Mason and Caldow, 2005).

Coronavirus

Bovine coronavirus (BCoV) is a member of the family Coronaviridae, order Nidovirales. The genome of coronaviruses consists a single stranded, capped and polyadenylated positive-sense RNA molecule of approximately 26 to 30 kb in length. Coronaviruses are mostly spherical in shape, enveloped with a diameter ranging between 120 and 150 nm, and possess a helical nucleocapsid. Despite their association to different clinical pathologies, BCoV isolates apparently belong to a single serotype on the basis of virus neutralization test (VNT) and hemagglutination inhibition (HAI) tests using polyclonal hyperimmune sera. However, data obtained from serological findings using *anti*-HE and *anti*-S Monoclonal Antibodies (MAbs) suggest the existence of different subgroups of BCoV, each containing EBCoV and WDBCoV strains responsible for neonatal calf diarrhoea, winter dysentery or chronic shedding in adult cattle. Antigenic and genomic variations have also been reported among EBCoV and RBCoV strains (Murphy *et al.*, 1999; Ge´linas *et al.*, 2001).

BCoV isolates are generally recognized as being associated with enteric diseases of newborn calves (neonatal calf diarrhoea) and winter dysentery or chronic diarrhoea in adult cattle. Incidence of coronavirus in neonatal calf diarrhoea is slightly lower than rotavirus (Ge´linas *et al.*, 2001; Mason and Caldow, 2005).

Pathogenesis of Coronavirus

The virus has an affinity for epithelial cells of the villi of the small intestine. Replication of the virus in these cells is accompanied by loss of epithelium and blunting of the villi, which results

in a failure of digestion and absorption of nutrients. In the colon, surface epithelial cells are also attacked, with loss of surface cells and cystic dilation and accumulation of cellular debris in underling crypts (Khan and Khan, 1991; Mason and Caldow, 2005).

Bacterial causes

Escherichia coli

Escherichia coli belongs to the family enterobacteriaceae, a gram-negative rod shaped bacteria, which is a natural inhabitant of the large intestine and lower small intestine of all mammals. It has capsular (K) antigens, cell wall or somatic (O) antigens, flagellar (H) and fimbrial (F) antigens. The O, H and K antigens can be used to serotype strains of *E.coli* and the numbers of the antigens it bears designates each serotype (Quinn *et al.*, 1994).

Different strains of *E. coli* are prevalent in diarrhoeic calves. Diarrhoeal diseases of farm animals and / or man are frequently due to infection by one or the other pathotypes of *Escherichia coli*: Enterotoxigenic *E.coli* (ETEC), Vero- or Shiga-like toxin producing *E.coli* (VTEC or STEC), Necrotoxigenic *E.coli* (NTEC), Enteropathogenic *E.coli* (EPEC), Enterohaemorrhagic *E.coli* (EHEC), Enteroaggregative *E.coli* (EAggEC), attaching and effacing *E. coli* (AEEC) and Enteroinvasive *E.coli* (EIEC) (Khan and Khan, 1991; China *et al.*, 1998; Law and Chart, 1998; Orden *et al.*, 1998; Holland *et al.*, 1999; Orden *et al.*, 1999; Mason and Caldow, 2005; Nagy and Fekete, 2005).

Enterotoxigenic *E.coli* is an important and global cause of severe, watery diarrhoea in the offspring of some animal species such as newborn (suckling) calves and suckling and weaned pigs. Mostly K 99+ antigen is possessed by *E. coli* found involved in NCD. In man ETEC is recognized as one of the most frequent (sometimes fatal) causes of childhood diarrhoea in the developing countries, and as an important causative agent of traveller's diarrhoea. This suggests that many similarities can be found in the pathogenesis of ETEC infections of animals and man (Marsolois *et al.*, 1978; Achá *et al.*, 2004; Nagy and Fekete, 2005; Wani *et al.*, 2006; Persson *et al.*, 2007). The diarrhoea caused by ETEC in calves mainly occurs during the first two weeks of life and even some reports are available that the highest frequency of *E. coli* occurs in calves younger than 3 days old. It produces enterotoxic and septicemic colibacillosis

in young calves (Marsolois *et al.*, 1978; Khan and Khan, 1991; de la Fuente *et al.*, 1998; Achá *et al.*, 2004).

Pathogenesis of *E.coli*

All calves become infected within a few hours of birth with many varied strains of *E. coli*. This constantly changing population of organisms inhabits the calves' intestines for life and is entirely normal and healthy. Some strains of *E coli* however have adherence antigens on the surface to adhere to the intestinal wall and produce toxins that cause scour examples of these are *E coli* K88, K99 or others, which are enterotoxigenic *E coli*. This strain is only capable of causing disease in calves in the first four days of life. The duration of clinical disease is limited to a few days. The organism causes little in the way of damage to the intestine, but leads to rapid fluid loss (Marsolois *et al.*, 1978; de la Fuente *et al.*, 1998; Achá *et al.*, 2004). ETEC has fimbrial adhesins (primarily hair-like appendages called fimbriae or pili) K88, K99 or others. The production of these colonization factors correlates with enterotoxin (proteins or peptides) production. These strains cause the majority of cases of neonatal colibacillosis (Quinn *et al.*, 1994).

ETEC bacteria are known to adhere to the small intestinal epithelium without inducing significant morphological changes, and to secrete enterotoxins that alter the functions of enterocytes by increasing secretion and reducing absorption. This loss of fluid causes the principal sign (diarrhoea) and often leads to dehydration and high rate of death in the neonatal calves. In addition to adhesive and enterotoxic virulence factors, pathogenesis also involves host factors, the most important of which are receptors for adhesins and for enterotoxins. Species specificity, which is a general characteristic of ETEC infections, is largely due to the presence of specific adhesin receptors in only one or in a limited spectrum of animal species. Therefore, animal ETEC strains do not represent real hazards to man and cannot be regarded as zoonotic in contrast to strains of VTEC/ STEC or EHEC (Khan and Khan, 1991; Nagy and Fekete, 2005).

EPEC stains do not appear to produce enterotoxins or shiga-like toxins but they can cause enteritis and diarrhoea by other mechanisms (Quinn *et al.*, 1994).

EIEC strains adhere to cells of the distal small intestine, invade the enterocytes and deeper layers of the intestinal mucosa. They reach the lymphatic system where there is multiplication. The death of some *E. coli* cells occurs and endotoxin is released. The virulence factors such as capsules, adhesions, siderophores and alpha haemolysin are important as survival factors for these invasive strains, which are responsible for colisepticaemia (Quinn *et al.*, 1994).

AEEC strains are involved in diarrhoea in 2 to 8-week old calves. The virulence factors of these bacteria include: the secretion of proteins involved in microvilli effacement, the production of the intimin, outer membrane protein involved in the intimate attachment of bacteria to epithelial cell and the production of shiga-like toxins (verotoxins), which destroy the microvilli by unknown means. 'Effacing' signifies the localized effacement of brush border microvilli (Quinn *et al.*, 1994; China *et al.*, 1998).

Salmonella species

Salmonella belong to the family enterobacteriaceae, rod-shaped, gram-negative straight rods, which are usually motile and produce gastroenteritis with nausea, vomiting, cramps and diarrhoea (Quinn *et al.*, 1994). *Salmonella* consists of 2501 serovars up to 2004 (Velge *et al.*, 2005). According to the Center for Disease control and prevention (CDC) system, the genus *Salmonella* contains two species, each of which contains multiple serotypes. The two species are *Salmonella enterica* (the type species) and *Salmonella bongori*, which was formerly the subspecies V (Brenner *et al.*; 2000). Some of the serovars like *S.* Typhi, *S.* Gallinarum, *S.* Dublin and *S.* Choleraesuis are host specific, the majority are non adapted and can cause infection in man and animals alike (Murugkar *et al.*, 2005).

Salmonellosis can occur in calves throughout the neonatal period and is often associated with a high mortality rate (Godden, 2007). *Salmonella* species may be particularly important in dairy calves (Marsolois *et al.*, 1978; de la Fuente *et al.*, 1998; Achá *et al.*, 2004). Moreover, *Salmonella* Typhimurium and *Salmonella* Dublin were isolated from calves with enteritis (Langoni *et al.*, 2004).

Salmonella infections are most frequent and of great concern to young animals. There are a whole range of *Salmonella* organisms, which can potentially cause scour in both calves and

humans. *Salmonella* Dublin can infect the gut causing scour and also cross the gut wall, to enter the blood stream causing systemic infections such as pneumonia, septicaemia and joint ill (Khan and Khan, 1991; Mason and Caldow, 2005).

Pathogenesis of *salmonella*

Salmonella pathogenesis is a complex and multifactorial phenomenon (Wallis and Galyov, 2000). *Salmonella* need to colonize the distal small intestine or colon to initiate enteric disease. Therefore, following oral ingestion, *Salmonella* colonize the intestines and invade the intestinal mucosa. But volatile organic acids produced by the indigenous normal anaerobic flora inhibit the growth of *Salmonellae* and the normal flora block the access to attachment sites required by the *Salmonella* species. The attachment of *Salmonellae* is by fimbrae. Some strains producing enteritis and diarrhoea are capable of forming enterotoxins and a cytotoxin (Quinn *et al*., 1994; Wallis and Galyov, 2000).

The invasive strains that produce septicaemia are able to escape destruction by the host and to multiply within the macrophages of the liver and spleen as well as intravascularly. Invasion of enterocytes results in the extrusion of infected epithelial cells into the intestinal lumen with consequent villus blunting and loss of absorptive surfaces. *Salmonella* also elicit a polymorphonuclear leukocyte (PMN) influx into infected mucosa and induce watery diarrhoea, which may contain blood (Quinn *et al*., 1994; Wallis and Galyov, 2000). *Salmonella* species interact with ileal mucosa and disrupt normal intestinal function, which results in an acute inflammatory influx, fluid secretion and enteritis (Wood *et al*., 1998; Wallis and Galyov, 2000). The severe fluid loss observed (E.g. during *S.* Typhimurium-induced enterocolitis) is in part due to an inflammatory mechanism, which causes liquid to flow from the blood to the intestinal lumen (Zhang *et al*., 2003).

The invasive abilities of some strains of *S.* Typhimurium are increased by the presence of genes carried on the plasmid. Destruction with in the blood stream is prevented by the O-repeat units of the lipopolysaccharide. It is thought that they may mask determinants on the bacterial cell surface that would normally bind complement and activate it by means of the alternate pathway. This would reduce the chances of chemotaxis, opsonization and phagosytosis. Any *Salmonellae*, in non-immune animals that are phagosytosed tend to survive with in the

phagocyte.Siderophores which remove iron from the iron binding proeins of the host, are secreted by these invasive *Salmonellae*. Multiplication of the organisms in the body leads to severe endotoxaemia. Some serotypes seem to be more commonly invasive than others. Invasive strains occur frequently in *S.* Typhi, *S.* Dublin and *S.* Typhimurium (Quinn *et al.*, 1994).

Protozoal causes

Cryptosporidium parvum

Cryptosporidia are well-recognized opportunistic protozoans mainly of the digestive or sometimes respiratory tract. The protozoan parasite, *Cryptosporidium parvum*, usually regarded as opportunist, causes gastrointestinal disease and diarrhoea in a variety of species including cattle, sheep, goat, pig, horses and humans worldwide. It is generally believed that the disease results in high morbidity and low mortality in calves. The diarrhoeic disorders are particularly serious in neonates and in immunosuppressed individuals (Naciri *et al.*, 1999; Lefay *et al.*, 2000; Castro-Hermida *et al.*, 2002; Langoni *et al.*, 2004).

The knowledge of *Cryptosporidium parvum* has increased considerably since the 1970s when it was first discovered as a cause of diarrhoea in humans and animals. The first report on bovine cryptosporidiosis was published in 1971, when *C. parvum* parasites were identified in the faeces of an 8-month-old heifer with chronic diarrhoea (Björkman *et al.*, 2003; Enemark *et al.*, 2003). It is a significant pathogen of livestock and humans and is increasingly being recognized as an important zoonotic pathogen (Bjo¨rkman and Mattsson, 2006). In immunocompromised humans, cryptosporidiosis may lead to life threatening chronic diarrhoea and because of the incidence of Acquired Immuno Deficiency Syndrome (AIDS) (Joachim *et al.*, 2003). The disease poses a significant public health problem in developing countries where AIDS is endemic (Singh *et al.*, 2006). Today *C. parvum* is recognized as an important infection in young calves. The severity of infection in both man and animals has been found to vary depending on the specific *C. parvum* isolate (Björkman *et al.*, 2003; Enemark *et al.*, 2003).

There are at least 11 *Cryptosporidium* species, and new species as well as genotypes are regularly described (Enemark *et al.*, 2003). Analysis of selected genetic loci by PCR-based methods or direct DNA sequencing can provide information on the genetic variability of parasite isolates. Application of such methods on oocysts of different origin revealed that there are two major genotypes of *C. parvum*: type 1 (or H), which seems to be exclusive to humans and primates (anthroponotic), and type 2 (or C), which has a broad host range and occurs in e.g. livestock, animals and humans (zoonotic). Furthermore, type 1 has known to be elevated to a new species, Cryptosporidium hominis (Bjo¨rkman and Mattsson, 2006).

Though calves 1–3 weeks old seem to be most, yet Cryptosporidium species has also been found in cattle over 2 years of age. The calf most often recovers spontaneously within 1–2 weeks even though there is a large variation between individuals in how they respond to and recover from infection. Concomitant infection with other enteric pathogens can aggravate the clinical signs and prolong the duration of disease. *Cryptosporidium parvum* is transmitted as microscopic oocysts that are excreted in the faeces from infected animals. The large numbers of oocysts that are excreted together with the strong resistance of the exogenous stages against disinfection can turn this parasite into a perpetual threat to animal health and productivity on affected farms. Finding oocysts in diarrhoeic faeces is indicative of *C. parvum* being the cause of the disease. During the first two weeks of infection a calf can shed millions of oocysts, which ensures efficient dissemination of the parasite (Naciri *et al.*, 1999; Björkman *et al.*, 2003; Enemark *et al.*, 2003; Joachim *et al.*, 2003; Singh *et al.*, 2006). Calves shedding *C. parvum* are three times more likely to present with clinical signs of diarrhoea (Godden, 2007).

Pathogenesis of *Cryptosporidium* species

The parasite generally affects calves between 4 days and three weeks of age. The damage caused by this infection is similar to that caused be rotavirus. The source of the parasite is thought to be either adult cows (which act as carriers without showing signs of the disease) or infected scouring calves passing the parasite in their faeces. 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 complete by 10 days (Mason and Caldow, 2005).

Table 1. Summary of pathogens associated with calf diarrhoea and their clinical sings.

Microbial Agent	Main Clinical signs	Reference (s)
Rotavirus	Neonatal diarrhoea in many mammalian and avian species	(Fukai *et al.*, 1999; Barreiros *et al.*, 2004)
Coronavirus	Enteric diseases of newborn calves and winter dysentery or chronic shedding in adult cattle	Ge´linas *et al.*, 2001; Mason and Caldow, 2005)
Esherchia coli	Diarrhoea in calves mainly during the first two weeks of life and even the highest frequency of *E. coli* occurs in calves younger than 3 days old. enterotoxic and septicemic colibacillosis in young calves enteritis	Quinn *et al.*, 1994; Achá *et al.*, 2004 (Langoni *et al.*, 2004).
Salmonella species	Gastroenteritis with nausea, vomiting, cramps and diarrhoea.	Quinn *et al.*, 1994
Cryptosporidium parvum	Gastrointestinal disease and diarrhoea (pasty to watery diarrhoea with dehydration), sometimes accompanied by depression, inappetence, fever, dehydration and/ or poor condition	Singh *et al.*, 2006; Godden, 2007

2.1.3. Diagnosis of calf diarrhoea

Diagnostic testing for the etiologic agent responsible for diarrhoea can only be performed in the laboratory because clinical signs do not allow differentiation of the causal microorganisms. The rapid detection of these pathogens will assist in treatment management (Nussbaum *et al.*, 1999). Diagnosis of the microbial pathogens associated with calf diarrhoea can be performed according to standard procedures, summarized in Table 2.

Table 2. Summary of techniques used for the diagnosis of pathogens causing diarrhoea

Causative agent	Diagnostic test and limitations for some methods	References
Rotavirus and Coronavirus	Electron microscopy, immunodiffussion, neutralization of indirect immunofluorescence, complement fixation, fluorescent antibody staining, agar gel precipitation immunoelectrophoresis or polyacrylamide gel electrophoresis (PAGE), hemagglutination, latex agglutination and Enzyme-Linked Immunosorbent Assay (ELISA). The demonstration of rotavirus double-stranded RNA in silver-stained polyacrylamide. Require expensive equipment and skilled operation.	Khan and Khan (1991); Nussbaum *et al.* (1999)
Cryptosporidium parvum	Identifying the oocyst in host feces. Detection is enhanced by the use of concentration procedures, or in histological sections taken during necropsy, by staining the faecal smears with methylene saffrainin blue stain and examination under microscope, antigenic ELISA	Khan and Khan (1991); de la Fuente *et al.* (1998); Naciri *et al.* (1999)
Escherchia coli	Bacterial culture, Demonstration fimbrial antigens (K88, K99, F41, 987P or F165) or the enterotoxin. Fimbrial adhesin can be detected using latex agglutination test or ELISA and the enterotoxins using ELISA The enterotoxigenic strains of *E.coli* are present in small intestine, but it is insufficient merely to isolate and identify the *E.coli*. Demonstration of the significant fimbrial antigens or the enterotoxin itself is necessary	Quinn *et al.* (1994); de la Fuente *et al.* (1998)
Salmonella	Bacteriological methods, which express the actual infection status and immunological methods identify previous exposure to *Salmonella*	ISO 6579, 2002/ FDAM 1: (2007); Forshell, and Wierup, (2006)

2.1.4 Predisposing factors for calf diarrhoea

Environmental factors

A pen area of between 8 to 12.6 m^2 has been found to be significantly associated with increased risk of diarrhoea. The association between pen area and diarrhoea might partly be due to the effect of housing system. On the other hand, pens with areas equal or greater than 12.6 m^2 have been associated with the low risk (Svensson *et al.*, 2006).

Factors related to birth

Several birth factors are associated with diarrhoeic hazard. Difficult calving causes stress for the newborn calf, which decreases resistance to pathogens owing to a combination of reduced calf vigour and delayed ingestion of colostrum. In addition, cows that require assistance during parturition give birth to calves that remain weak for long periods and thus become exposed to more faecal pathogens than calves that stand up shortly after birth. Hazards of diarrhoea have been reported in calvings that needed assistance (Lorino *et al.*, 2005). Dyspnoea may be associated with the hazard of diarrhoea (Lorino *et al.*, 2005; Trotz-Williams *et al.*, 2007).

Management and ambient conditions

The ammonia concentration could be associated with the hazard of gastroenteritis. This might be a consequence of bad ventilation, insufficient quantity of straw and inadequate drainage. Cleaning after each calving season probably leads to a reduction in the spread of the microorganisms that cause diarrhoea. Hazards are higher when calf pens are not clean leading to the build up of ammonia (Lorino *et al.*, 2005; Trotz-Williams *et al.*, 2007).

Prophylaxis

Hazards are high when cows are not vaccinated against a variety of specific causative agents of diarrhoea. Having dams vaccinated against agents *E. coli,* rotavirus and coronavirus decreases the hazards of diarrhoea (Lorino *et al.*, 2005).

Feed factors

Calves from herds with no concentrate feeding are at higher risk of diarrhoea. Feeding corn silage is associated with an increased risk of diarrhoea. This might be due to the fact that feeding corn silage could induce transient increases of triglyceride and urea nitrogen in the blood, which may contribute to the risk of diarrhoea. Minerals and vitamins offered to cows during the dry periods been associated with increased occurrence of diarrhoea (Lorino *et al.*, 2005).

2.1.5. Economic importance of calf diarrhoea

The neonatal calf diarrhoea (NCD) complex is one of the major causes of calf morbidity and mortality worldwide. It causes significant economic and production losses in the livestock industry (Radostits *et al.*, 1994; Khan and Khan, 1991; de la Fuente *et al.*, 1999; Naciri *et al.*, 1999; Nussbaum *et al.*, 1999; Garcia *et al.*, 2000; Langoni *et al.*, 2004; Trotz-Williams *et al.*, 2007; Godden, 2007). Neonatal calf mortality varies from 8.7 to 64 per cent throughout world. In addition the neonatal calf mortality in the first month of age accounts for 84 per cent of the total mortality and is particularly high in the third week of life (Khan and Khan, 1991). It is estimated that a calf mortality of 20 percent reduces dairy herd net profit by 38 percent. Moreover, the possible long-term effects of neonatal diarrhoea on the health, production and productivity performance of calves that survive diarrhoeic episodes might constitute even greater losses (de la Fuente *et al.*, 1998).

Diarrhoea of neonatal calves causes major economic loss directly through mortality and the need for treatment, in addition to professional expenses and indirectly from poor growth after clinical disease. It is usually high, affecting up to 90 to 100% of newborn calves and has been estimated that neonatal calf diarrhoea accounts for approximately 75% of the mortality of dairy calves under 3-weeks of age (Radostits *et al.*, 1994; de la Fuente *et al.*, 1999; Nussbaum *et al*, 1999 Garcia *et al.*, 2000; Langoni *et al.*, 2004; Trotz-Williams *et al.*, 2007). Moreover, the possible long-term effects of neonatal diarrhoea on the health and performance of calves that survive clinical episodes might constitute an even greater loss (de la Fuente *et al.*, 1998).

2.1.6. Management of calf diarrhoea

Generalized treatments

Neonatal diarrhoea is the major cause of illness and death for calves less than one month of age. Therefore, generalized treatments should be administered whatever the cause of scour and are aimed at correcting any dehydration and acidosis that may have occurred and minimizing intestinal damage. Guidelines on the treatment of scour should be included in the health plan. Some of the guidelines are given below (Mcclure, 2001; Mason and Caldow, 2005):

- If the calf is severely dehydrated (sunken eye), weak or collapsed with the absence of a suck reflex then veterinary attention should be sought and intra-venous fluids may be required.
- If the calf is mildly dehydrated, standing but scouring then oral rehydration fluids should be administered. The cornerstone of therapy for neonatal diarrhoea for calves is oral replacement fluid therapy (ORT) to correct numerous metabolic problems including dehydration, electrolyte imbalances and metabolic acidosis. Although modern ORT was introduced in the 1950s, the success of ORT therapy was not fully appreciated until the 1970s. Prior to this time intravenous fluid therapy was commonly utilized even for mild to moderate dehydration (Mcclure, 2001). Certainly calves should not be maintained on oral rehydration fluids alone for longer than 2 days as they are do not provide adequate nutrition for the calf.
- It was previously advised that calves were kept off milk while being given oral fluids; this is no longer considered to be the case. It may be best to ensure a gap of two hours after fluids are offered before sucking is allowed.
- If the calf is scouring but not dehydrated and is bright and alert then no specific oral rehydration treatment is indicated.
- In general antibiotics have no value in the treatment of the common causes of calf scour

Most of the guidelines recommended for the use of ORT in children have also been applied to ORT therapy in calves (Mcclure, 2001). These include:

- Sodium concentrations of ORT solutions should be between 60–90 mmol/L
- ORT can be used to treat mild, moderate and severe dehydration
- The glucose concentration in the ORT solution should be < 2:1 ratio with the sodium concentration
- Feeding should resume within 24 hours of starting ORT therapy. Access to water is also appropriate (Mcclure, 2001).

If the calf is able to drink an equivalent volume of fluid to that being lost in the faeces 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 eyes' and if severe can lead to collapse, circulatory failure and death. In addition, in some cases 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 diarrhoea are seen. Failure to recognize this state and seek veterinary attention will result in the death of the calf (Mason and Caldow, 2005).

Şentürk (2003) indicated that administration of low volumes of hypertonic sodium chloride and dextran 70 solution combinations with oral electrolyte solutions as a quicker, practical, economical and most importantly an effective way for the treatment of dehydrated diarrhoeic calves. In calves with diarrhoea and no systemic illness (normal appetite for milk or milk replacer, no fever), it is recommended that the health of the calf should be monitored and not administer parenteral antimicrobials (Constable, 2004).

Specific Treatments

A specific diagnosis of the causes of calf scour on the whole farm as specific control measures exist and can be administered (Mason and Caldow, 2005).

Rotavirus and Coronavirus

No specific therapy is available for these pathogens, as no anti-viral drugs are available. If the herd has an extended calving pattern, then it is suggested vaccination should be considered for cows greater than 1 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

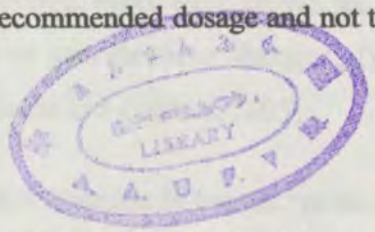
month of calving various immunological products are available in paste form. It should be emphasized that these will only provide short-term protection and these products should not be used as an alternative to vaccination (Mason and Caldow, 2005).

Salmonella and *E. coli* infections

Antibiotics should only be used in cases involving bacterial enteritis. Antibiotic sensitivity testing should be done to enable appropriate antibiotic selection (Mason and Caldow, 2005).

Cryptosporidia

Halofuginone is now available as an aid to treatment and prevention of cryptosporidium infection in calves and can be prescribed by your vet. The drug reduces the severity of disease in individual calves and suppresses the output of oocysts reducing the risk of disease spread. On a group basis, the drug works best when used to prevent further scour 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 (Mason and Caldow, 2005).



2.1.7. Prevention and control of calf diarrhoea

These different etiologies without specific symptomatology make the diagnosis and consequently the appropriate prevention or treatment difficult for veterinarians. In practice, a rapid diagnosis is *quasi* impossible and veterinarians prescribe usually an antibiotherapy and rehydration (Naciri *et al.*, 1999; Lorino *et al.*, 2005) but prevention should be centered on management factors (Lorino *et al.*, 2005).

Generalized Control Measures

Many husbandry measures will reduce the risk of scour and such measures carry little in the way of additional costs. Control focuses on reducing exposure to the infectious agents and optimizing the calves' resistance to them. Cleaning and disinfecting calf-rearing accommodation between calves is an essential means of disease control. For most circumstances general farm disinfectants will be satisfactory. However, cryptosporidium oocysts are highly resistant and persist in the

environment for long periods of time. Ammonia based disinfectants such as Oocide® (Antec), are the only effective agents, but because of the irritant fumes produced they can only be used after a building has been de-stocked (Mason and Caldow, 2005).

Colostrum Management

Because of the structure of the bovine placenta, calves are born without circulating protective antibodies against the common infectious organisms that cause scour and other diseases (Mason and Caldow, 2005; Godden, 2007). For protection against infectious pathogens during the first weeks of life, the calf is almost entirely dependent upon the absorption of maternal immunoglobulin (Ig) from colostrum. The colostrum at the first milking after calving is richest in antibody and will provide the calf with an excellent supply of antibody that is absorbed in the first 12 hours after calving. After this, the calf absorbs little or no antibody although antibodies can still work locally within the gut attaching to the infectious organisms such as rotavirus and coronavirus and inactivating them. This protection is enhanced if cows are vaccinated. Colostral protection for cryptosporidia is thought to be less effective. More over, calves are born without reserves of vitamin A or E so they rely on colostrum to provide these vitamins. These vitamins are essential for the calf's ability to fight infection. (Mason and Caldow, 2005).

Absorption of Ig from the intestine and into the calf's circulation is termed 'passive immunity'. Calves are defined as having failure of passive transfer if the calf serum IgG concentration is less than 10 mg/ml when sampled between 24 – 48 hours of age. Achieving early and adequate intake of high quality colostrum is the single most important management factor in determining calf health and survival. Additional benefits include improved growth rate and feed efficiency, reduced age at onset of puberty, reduced age at first calving, and improved first and second lactation milk production. Poor colostrum management is one of the key factors contributing to these excessive losses (Godden, 2007).

Specific Control Measures

Vaccination of the cow herd using one of the vaccines against rotavirus, coronavirus and *E coli* K99 is recommended in herds where calf scour is a recognized problem (Snodgras, 1986; Barreiros *et al.*, 2004; Mason and Caldow, 2005). Where herds suffer salmonellosis, vaccination

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. Vaccination of healthy calves is possible from 3 week of age however there is a delay in the development of immunity, which reduces the practicality of the vaccine for use in young calves (Barreiros *et al.*, 2004; Mason and Caldow, 2005).

2.2. Calf Pneumonia

2.2.1. The clinical picture and effects of calf pneumonia

Clinical respiratory-tract disease has been defined as either coughing or sneezing for more than two days, as labored breathing, severely abnormal respiratory noises at lung auscultation or as moderately increased respiratory sounds together with other signs such as coughing, sneezing or nasal discharge anorexia, moderate depression increased respiratory rate and pyrexia can also indicate Clinical respiratory-tract disease (Dowling *et al.*, 2002: Svensson *et al.*, 2006; Arcangioli *et al.*, 2007).

The occurrence of bovine respiratory disease (BRD) in recently weaned beef calves develops in a sudden and predictable fashion shortly after arrival at a feedlot. Respiratory disease in commingled beef calves is a complex disease syndrome caused by many bacterial and viral agents and is influenced by management and environment (DeRosa *et al.*, 2000).

Calf pneumonia may be broadly separated into two forms: acute and chronic. The chronic form is of insidious onset, with very few clinical signs apart from a dry cough and a slightly increased respiratory rate. The acute form often presents as an outbreak, with several animals succumbing to the disease within a 48-hour period. Common symptoms include fever, inappetance, dyspnoea and ocular/nasal discharge (Potter, 2007).

2.2.2. Microbial causes of Calf Pneumonia

There is a long list of bacterial pathogens that can be involved with calf respiratory disease, and one or more are usually isolated from most cases of disease (DeRosa *et al.*, 2000; Kedrak and Borkowska-Opacka, 2003; Potter, 2007). The cause of BRD is complex and involves the effect of stress and resultant depression of immune system functions, along with viral and bacterial

infections. Gram-negative organisms including *Manheimia haemolytica, Pasteurella multocida,* and *Histophilus somni* (formerly *Haemophilus somnus*) are the main bacterial components of the BRD complex (Norcia *et al.*, 2004).

Pasteurella and *Mannhaemia* species

To date, nine different genera (*Pasteurella, Actinobacillus, Haemophilus, Mannheimia, Lonepinella, Phocoenobacter, Histophilus, Gallibacterium,* and *Volucribacter*) have been described within the family Pasteurellaceae (Kuhnert *et al.*, 2004).

Pasteurella and *Mannhaemia* species are small (0.2-1.2μm), non-motile, gram-negative rods or coccobacilli that can colonize the mucosal surface of the respiratory and genital tracts. Important human and animal pathogens are found among this bacterial family. They are oxidase positive facultative anaerobes, and most species are catalase positive. Although non-enriched media supports their growth, these organisms grow best on media supplemented with blood or serum. They usually remain viable only a few days on culture plates. Some species, such as *Mannhaemia haemolytica, Pasteurella trehalosi* and *P. aerogenes* can tolerate the bile salts in MacConkey agar. In smears from infected tissues stained by Giemsa method, *Pasteurellae* exhibit bipolar staining (Jacques and Paradis, 1998; Quinn *et al.*, 2002).

Pasteurella haemolytica was classified into biotypes A and T according to its ability to ferment arabinose or trehalose, respectively, and the *P. haemolytica* complex negative to trehalose was reclassified into the new genus *Mannheimia,* which includes at least five species (Table 3). These changes were based on an extensive polyphasic investigation using quantitative evaluation of phenotypic data, ribotyping, multilocus enzyme electrophoresis, 16S rRNA sequencing, and DNA–DNA hybridization. *Pasteurella haemolytica* biotype T isolates is now used to denote *Pasteurella trehalosi* (Angen *et al.*, 2002; Quinn *et al.*, 2002; Jaramillo-Arango *et al.*, 2007).

Pasteurella multocida causes haemorrhagic septicaemia in cattle and buffalo, and plays a role in the respiratory disease complex of cattle. It has been extensively studied since it was first isolated in the late 1870s. It causes pasteurellosis, so-called Bollinger's disease (serotype B: 2), in older cattle, and it enters for polyethiological diseases of calf's respiratory tract (serotype A: 3) (Miflin and Blackall, 2001).

The previous species names, sites of isolation and the diseases caused by the *Mannhaemia* species are summarized in Table 3.

Table 3. The species present under the genus *Mannhaemia* and the diseases they cause

Species	Previous name (classification)	Site of isolation	Disease	Reference (s)
Mannheimia haemolytica	*P. haemolytica* biotype A	nasopharynx and tonsils of apparently healthy animals	respiratory diseases, particularly bovine pneumonic pasteurellosis or shipping fever pneumonia mainly in animals younger than one year of age recently transported or added to the herd	Ga°nheim *et al.*, 2003; Davies and Lee, 2006; Jaramillo-Arango *et al.*, 2007
M. glucosida	*P. haemolytica* biogroups 3A–H, and 9 and reference strains of serotype 11.	nasal cavity of ovines but can be isolated from healthy bovines	pneumonia or other diseases of both ovine and bovine	Angen *et al.*, 2002; Jaramillo-Arango *et al.*, 2007
M. ruminalis	*Actinobacillus lignieresii*, Bisgaard taxon 18 or *P. haemolytica* biogroup 8D	non-haemolytic strains isolated from rumen of cattle and sheep	have not been associated with disease conditions	Angen *et al.*, 2002
M. granulomatis	*P. granulomatis*, Bisgaard taxon 20 and *P. haemolytica* biogroup 3J	includes bovine strains as well as *P. haemolytica*-like strains isolated from rabbits and hares	pneumonia and purulent conjunctivitis in leprine species and with skin granulomas and other disease conditions in cattle	(Angen *et al.*, 2002).
M. varigena	*P. haemolytica* biogroup 6.	originate from both cattle and pigs	septicemia, pneumonia, enteritis and other disease conditions	(Angen *et al.*, 2002).

Table 3. Cont.

M. varigena	*P. haemolytica* biogroup 6.	originate from both cattle and pigs	septicemia, pneumonia, enteritis and other disease conditions affecting primarily cattle and pigs, other species of animals could also be affected	(Angen *et al.*, 2002).

Pathogenesis of *Pasteurella* and *Mannhaemia* species

Numerous infection factors such as viruses, mycoplasms, bacteria, and first of all *Pasteurella multocida*, *Pasteurella haemolytica* and *Haemophilus somnus* take part in enzootical pathogenesis of bronchopneumonia in calves (Kedrak and Borkowska-Opacka, 2003).

Many *P. multocida* infections are endogenous. The organisms, which are normally commensals of the upper respiratory tract, may invade the tissues of immuno-suppressed animals. Exogenous transmission can also occur either by direct contact or through aerosols. Factors of importance in the development of disease include; adhesion of pasteurellae to the mucosa and avoidance of phagocytosis. Fimbrae may enhance mucosal attachment and the capsule, particularly in type A strains has a major antiphagocytic role. In septicaemic pasteurellosis, severe endotoxaemia and disseminated intravascular coagulation cause serious illness, which can prove fatal (Quinn *et al.*, 2002).

Four main virulence factors have been identified in strains of *Mannhaemia haemolytica* and *P. trehalosi*; fimbrae may enhance colonization, a capsule that inhibits complement-mediated destruction of the organisms in serum, endotoxin which can alter bovine leukocyte functions and is directly toxic to bovine endothelial cells, leukotoxin, a pore forming cytolysin that affects leukocyte and platelate functions when present at low concentrations and causes cytolysis at high concentrations. The subsequent release from damaged cells of lysosomal enzymes and inflammatory mediators, such as tumour necrosis factor-α and eicosanoids, contributes to severe tissue damage in these infections (Quinn *et al.*, 2002).

Mannheimia haemolytica colonizes the mucosa of the upper respiratory tract and nasopharynx. By mechanisms poorly understood, *M. haemolytica* breeches the innate mucosal defense, including the mucociliary apparatus and antimicrobial factors to establish infection in the lung (Ackermann and Brogden, 2000).

Haemophilus somnus

Histophilus somni (*Haemophilus somnus*) is a gram-negative highly pleomorphic coccobacillus and member of the *Pasteurellaceae*. It is a host-specific opportunistic pathogen of cattle and sometimes sheep. *H. somni* is one of the pathogens responsible for bovine respiratory disease

complex. In addition to pneumonia, *H. somni* can cause meningoencephalitis, myocarditis, arthritis, septicemia, and other systemic infections. Although many pathogenic members of the family *Pasteurellaceae* are encapsulated, a capsule has not been identified on the surface of *H. somni*. However, there is a production of an exopolysaccharide, a common component of bacterial biofilms (Berghaus *et al.*, 2006; Sandal *et al.*, 2007).

Growth requirements include enriched media, such as brain heart infusion agar, supplemented with 5-10% bovine or sheep blood under 5- 20% carbon dioxide at 37^0 C. It does not require factors X (hemin) or V (NAD) for growth (Howard, 1998).

Haemophilus somnus, proposed new name, *Histophilus somni*, causes respiratory disease alone or in combination with other respiratory pathogens (Gershwin *et al.*, 2005). *Haemophilus somnus* infection in cattle was recognized first as an infectious disease affecting the central nervous system. However, several other syndromes in cattle (genital tract diseases, myocarditis, polyarthritis, septicaemia, weak calf syndrome) have been associated with infection by this organism. At the same time, *H. somnus* is also an important cause of bovine pneumonia. This pathogenic potential of *H. somnus* and the economic importance of the above diseases indicate that this organism is a significant bovine bacterial pathogen (Kwiecien and Little, 1991; Haziroglu *et al.*, 2000).

In general, the *Haemophilus somnus* complex is clinically manifested in the reproductive and urinary tract form, the respiratory form, the septicemic form, and a "catch-all" miscellaneous form (Richey, 2002). *Haemophilus somnus* is associated with a complex of diseases including thrombotic meningoencephalitis (TME), bronchopneumonia, myocardial abcessation, arthritis and mastitis (Kwiecien and Little, 1991).

Respiratory Form: The organism has the ability to attack both the upper and lower respiratory tract. In calves, one of the typical forms of the disease attacking the upper respiratory tract is "calf diphtheria." The surface tissue of the larynx (voice-box), affected by the interruption in blood supply, begins to die and slough. The calf exhibits difficulty in swallowing, bawling, and breathing. As the disease progresses, the wind pipe or trachea may also become infected. If the *H. somnus* organism reaches and attacks the lungs, severe pneumonia can result. The pneumonia caused by *H. somnus* can also result in rapid death of the animal before any clinical signs have

been detected. Quite often *H. somnus* is the primary cause of the pneumonia but can be quickly over-grown by opportunist. In either case, the pneumonia caused by *H. somnus* must be treated immediately or death usually occurs. If either the reproductive-urinary tract form or the respiratory form of *H. somnus* disease gains access to the blood stream, the organism can spread to all parts of the body. The diseases originating from the circulating infected blood are referred to as the "septicemic" form of *H. somnus* (Richey, 2002).

Reproductive and Urinary Tract Form: If *H. somnus* attacks the reproductive tracts of pregnant cows, the infection may result in the death of the fetus, at any age, with subsequent abortion and infection of the uterus. A long-term uterine infection will usually result in an extension of the infection into the vagina and a repeat-breeders syndrome. Because *H. somnus* has been found in the urinary tract and prepuce of bulls, it has been suggested that the organism is transmitted from the cow to the bull during mating. The organism is shed in uterine/vaginal discharges and urine of these infected animals. Susceptible cattle, in close proximity to the infected animals are routinely exposed to the organism by sniffing the discharges or being splattered by the urine. Subsequently, the exposed susceptible cattle become infected, usually resulting in the respiratory form of *H. somnus* disease (Richey, 2002).

Miscellaneous Form: *H. somnus* diseases have also occurred in the ears and eyes of cattle. The ear form is characterized by a large amount of thin, yellowish discharge flowing from the ear canal. The eye form is seen as an infection of the conjunctiva, the white part of the eye. As with any conjunctivitis, there is reddening of the eye, excessive tears that overflow and drain, and squinting (Richey, 2002).

Unfortunately, unless treated early, any form of *H. somnus* disease can cause another form of the disease (Figure 1). Regardless of how the disease was introduced into the herd, the infection has a tendency to reach the uterus. Because of this, the infected reproductive tract is thought to be the reservoir of the disease (Richey, 2002).

Pathogenesis of *Haemophilus*

It expresses a wide array of virulence factors, including phase variation of lipooligosaccharide epitopes, decoration of lipooligosaccharide with sialic acid and phosphorylcholine, expression of

high-molecular-weight immunoglobulin-binding proteins, intracellular survival in professional phagocytes, and induction of apoptosis. Although many pathogenic members of the family Pasteurellaceae are encapsulated, a capsule has not been identified on the surface of *H. somni*. However, researchers have described the production of an exopolysaccharide, which is a common component of bacterial biofilms (Sandal *et al.*, 2007).

When pathogenic strains of *H. somnus* enter the bovine bloodstream, they are able to evade complement mediated killing, while many commensal strains cannot. Binding of immunoglobulin by Fc receptors has been demonstrated in *H. somnus*, and may be one method by which pathogenic strains of this bacterium avoid complement mediated killing. *Haemophilus somnus* is able to adhere to a variety of host cell types, including bovine endothelial cells, turbinate cells, and vaginal epithelial cells. Cytotoxicity or interference with host cell function is another *H. somnus* virulence factor that enables it to avoid killing by phagocytic cells. Studies have shown that the function of bovine mononuclear cells and neutrophils, may become adversely affected by *H. somnus* and can even support replication of phagocytized *H. somnus*. This mechanism may also enable *H. somnus* to avoid humoral immunity, so long as phagocytized bacteria remain intracellular (Howard, 1998).

2.2.3. Diagnosis of calf pneumonia

When presented with an outbreak of pneumonia in a group of calves, visual inspection alone by looking for signs of coughing, tachypnoea, ocular or nasal discharge and depression may lead to under diagnosis of the problem, with animals in the early stage of the disease missing out on treatment. To this end rectal temperature is often employed as an effective method of assessing animals within a group, with temperatures in excess of 39.6°C being used as a threshold above which animals are affected probably by respiratory disease(Potter, 2007).

Bacterial pathogens are the most common agents but, currently, there is no efficient and simple test to determine the identity of the disease-causing pathogen ante mortem in the lungs (DeRosa *et al.*, 2000). The techniques available for microbiological examination of calf respiratory disease caused by viruses or bacteria:

Nasopharyngeal swabs: can be used for bacteriology and virus identification (Potter, 2007).

Bronchial alveolar lavage: can be used to examine for bacterial, viral, or mycoplasmal presence. The procedure involves passing a tube through the nose into the trachea; a second tube can then be passed through the first in order to prevent contamination with nasal commensals. The tube is advanced until it lodges in an end bronchus/ bronchiole. Once the tube is in place 60 mls of sterile isotonic saline is injected followed by 60 mls of air to flush the saline through the tube. Immediately afterwards as much fluid as possible is withdrawn through the tube; normally it is possible to collect 10-20 mls of frothy fluid (Potter, 2007).

Paired serology: two blood samples from affected animals, one at the beginning of an outbreak and a second 14-21 days later to look for serological conversion. At least six animals should be tested. A positive response is usually defined as a significant rise in titer in more than 30% of cases (Potter, 2007).

Post-mortem investigation: in severe outbreaks where there are mortalities, post-mortem examination can provide valuable diagnostic material, and visual inspection can give an idea of the potential pathogen involved. However, postmortem examination must be of cases that are representative of the problem being investigated. Post-mortem examinations of long-standing chronic cases are often unrewarding particularly if the on-farm problem is acute pneumonia (Potter, 2007).

2.2.4. Predisposing factors of calf pneumonia.

The cause of BRD is complex and involves the effect of stress and resultant depression of immune system functions, along with viral and bacterial infections (Härtel *et al.*, 2004; Norcia *et al.*, 2004). Stress is an important predisposing factor to BRD. Alteration in bronchoalveolar lavage fluid components in stressed animals may be due to a depressed efficiency of mucociliary system and/or decreased amount of alveolar spatial surfactant either or both of which may predispose affected livestock to show the presence of *P. multocida* in bronchoalveolar fluid (Mohammadi *et al.*, 2007). Management and environment play a key role in predisposing calves to pneumonia (DeRosa *et al.*, 2000).

The causation is multifactorial and the disease appears to be a result of the interaction of infectious micro-organisms and such predisposing factors as host defense, environment and stress (Härtel *et al.*, 2004).

2.2.5. Economic importance of calf pneumonia

Bovine respiratory disease (BRD, shipping fever) continues to be a primary cause of morbidity and mortality in feedlot production (Norcia *et al.*, 2004). It is the most economically important disease among beef cattle, and is second only to gastrointestinal disease as a cause of illness in dairy calves. Bovine respiratory disease is associated with stressful conditions coupled with viral and bacterial infection. Many bacterial species are associated with the pneumonia seen in BRD but *Mannheimia haemolytica* has been the most frequently isolated bacterium from fibrinous pleuropneumonia in cattle (Furrow *et al.*, 1986; Norcia *et al.*, 2004; Katsuda *et al.*, 2007).

Bovine respiratory disease (BRD) complex is a very important health problem for cattle industry world wide. It inflicts considerable financial losses in beef herds and is the most common cause of mortality in dairy cattle. It is also an important welfare problem of calves. BRD impairs animal welfare, causes excessive use of antibiotics and increases the rate of death or obligatory culling in calves as well as young stock. Hence, an important economical impact on cattle production is generated (Härtel *et al.*, 2004; Yeşilbağ and Güngör, 2008).

2.2.6. Management of Calf Pneumonia

Generalized Treatment

In situations where more than 30% of a group is affected antibiotic metaphylaxis is often advocated. There is considerable debate as to whether this is appropriate, and at what threshold it should be employed. On the positive side antibiotic metaphylaxis means 'subclinical' infections receive treatment, it helps reduce the environmental contamination with potentially pathogenic bacteria and it potentially enables a more convenient situation for farm staff, with animals requiring only one handling instead of repeated handling to monitor rectal temperatures during the initial stages of an outbreak. Treating early means a better response, reducing the knock-on costs described above. Possible disadvantages of metaphylaxis include cost, although this is often

reduced by opting for cheaper longer acting antimicrobial preparations. Other concerns over antibiotic metaphylaxis relate to the potential for antibiotic resistance and the fact that the true prevalence of respiratory disease will never be known, although active monitoring policies such as measuring rectal temperature can yield useful information on the disease progression within a group (Potter, 2007).

Specific Treatment

Treatment of *Pasteurella* and *Mannhaemia* species

Affected animals must be isolated and treated early in the course of the disease. Treatment with oxytetracycline, potentiated sulfonamides and ampicillin is usually effective (Quinn *et al.*, 2002).

Treatment of *Haemophilus somnus*

Early treatment is necessary to stop the disease progress. If clinical signs are missed and treatment is delayed, survival of the severely sick animals is questionable. The *H. somnus* organism is susceptible to several different antibacterial agents; primarily the new generation antibiotics such as enrofloxacin (Baytril®), ceftiofur hydrochloride (ExcenelTM), ceftiofur sodium (Naxel), tilmicosin (Micotil®) and florfenicol (Nuflor®) are effective. However, many of the old generation antibacterial agents such as tetracycline, penicillin, and sulfa groups are still used. The antibiotics halt the growth of the organism. It is the body defenses that actually kill it. Therefore, stopping treatment too early may allow the *H. somnus* organism to begin growing again and the disease relapses (Richey, 2002).

2.2.7. Prevention and Control of Calf Pneumonia

Generalized prevention and control measures

Calf pneumonia represents a complex and economically damaging disease process. Veterinary involvement should be aimed around the effective management in the face of an outbreak, coupled with investigations to enable long-term strategies to prevent future outbreaks. Changes, such as vaccination, housing conditions or alteration in calf management may be instituted to

reduce the impact of calf pneumonia on farm (Potter, 2007). In addition control of these Gram-negative pathogens generally resolves the illness (Norcia *et al.*, 2004).

Although treatment regimes are often implemented prior to definitive diagnosis of the causative agent, it is important to carry out diagnostics in order to refine treatment and develop long-term management strategies for the control of respiratory disease on a farm. Samples should be taken from typical cases in the early stages of an outbreak (Potter, 2007).

The investigation of bacterial virulence factors can result in improved methods for diagnoses and prevention of infection. Thus, an understanding of the bacterium, immunology, and the mechanisms involved in how each bacterium is able to avoid the host immune response is necessary before preventative measures can be optimized to combat infection (Howard, 1998).

Specific prevention and control measures

Stress factors must be kept to a minimum. Procedures such as castration, dehorning, branding and antihelimenthic therapy should be carried out several weeks before young cattle are transported. Vaccination regimes for respiratory pathogens should be completed at least 3 weeks before transportation. Vaccines for *Mannhaemia haemolytica* , which incorporate modified leukotoxin and surface antigens may induce protection (Quinn *et al.*, 2002).

Disease caused by *H. somnus* can be prevented by the appropriate use of vaccines. Appropriate use means: two doses administered at least 21 days apart, with the second dose given at least 30 days before the anticipated challenge. An annual booster is required to keep the resistance level high. In the case of an "out break," the challenge can be generally reduced by use of antibacterial drugs while the resistance is raised by vaccination. In this case we would use repeated doses of antibiotics or antibacterial drugs to reduce the challenge and allow time for the vaccinations (two doses) to raise the resistance. *Haemophilus somnus* is an organism that has the ability to cause many different diseases. Prevention is the best way to control the diseases (Richey, 2002).

2.3. The status of calf diarrhoea and pneumonia in Ethiopia

Limited works have been done about the incidence and causative agents of calf diarrhoea in some parts of Ethiopia (Abraham *et al.*, 1992; Simachew, 1998; Tadesse, 2004; Temesgen, 2004; Abebe, 2005; Demissie, 2007). They reported variable incidence rates and causative agents of diarrhoea in their respective study sites, and managemental conditions. But these researches were not able to apply effective control strategies to reduce the neonatal calf morbidity and mortality because: the areas of these researches do not represent the whole country and due to various reasons, most of the researches had focused on the bacterial and protozoan causes of calf diarrhoea. In addition, the other important cause of calf morbidity, calf pneumonia, has not been well studied in spite of its importance to the dairy and beef calves.

Baseline information concerning calf morbidity due to calf diarrhoea and pneumonia is lacking to apply effective control strategies in the country. Therefore it is essential to undertake this study in different agro climatic zones.

3. MATERIALS AND METHODS

3.1. Description of the study area and study population

3.1.1. Study Areas

Debrezeit

It is located 47 km south east of Addis Ababa at an altitude of about 1900 m a. s. l. The area experiences an annual mean rainfall of 1115.6mm with two rainy seasons. The short rainy season occurs between March and May, while the main rainy season is between June and September. The average minimum and maximum temperatures are 8.5^0C and 30.5^0C, respectively (NMSA, 2003).

Holeta

It is located 44 kms away from Addis Ababa in the central highlands of Ethiopia at 38^0 3'E and 9^0 3'N at an altitude of 2,400 m a.s.l. Holeta is characterized by mild subtropical weather with

minimum and maximum temperature ranges from 2^0C-9^0C and 20^0C-27^0C, respectively. The area experiences a bimodal rainfall pattern with a rainy season from March to April and also from June to September. The vegetation consists of annual legumes and perennial grass species. The major crops are wheat, barely, lentils, 'Teff' ('Eragrastis teff') and maize. The natural pastures in the area are predominantly composed of Andropgon, Hyperrhenia, Trifolium and some species of cyperaceae.

Muke Turi

It is a town and the seat of the administration. Its location is at 78 km North-West of Addis Ababa. The area has 3 agro-climatic zones: Temperate ('baddaa'), Subtropical ('baddaa-dare') and Tropical ('gamoojjii'), each sharing 87%, 11% and 2% of the total area. The altitude ranges from 1000-3000 m.a.s.l. (NSDAD, 2001). It receives an annual mean rainfall of 1028 mm. The mean minimum and maximum temperatures of North Shoa Zone are 11.23^0C and 20.86^0C, respectively. The climate of the area is favorable for crop and livestock production. It is the main milk shed for Addis Ababa.

3.1.2. Study Population

All calves under 6 months of age in the study areas formed the study population. Diarrhoeic and / or pneumonic calves in the study population during the study period were sampled for the detection of the causative agents.

3.2. Sample size determination

Cluster sampling was used and farms were purposively selected to get large number of calves and the sample size, for estimating disease problems when the number of clusters is fixed is used according to Thrusfield (2005):

$$Ts = \frac{1.96^2 \, g \, Pexp \, (1-Pexp)}{gd^2 - 1.96^2 Vc}$$

where, Ts = total number of sample

g = number of clusters to be sampled

d = Desired absolute precision

Vc = Between-cluster variance

Pexp = expected prevalence

As data derived from previous cluster samples, were not available, the between cluster-variance component (Vc) was obtained by guessing (Thrusfield, 1995). Thus, anticipated overall mean cluster prevalence of 0.336 (33.6 %) was used as reported by Demissie (2007). The average between this and the individual cluster prevalence was guessed to be 0.03 (3 %), and then the between-cluster variance component would be $0.03^2 = 0.0009$. Therefore this has been used to estimate the sample size. At a confidence level of 95% and required absolute precision of 5%, a total of 427 calves were included. Two additional calves were born and included. The calves have been closely monitored for the occurrence of diarrhoea and pneumonia during the study period.

3.3. Study design

3.3.1. Longitudinal study

A prospective observational study design was undertaken from November, 2007 to April, 2008 to determine the prevalence of major bacterial and viral causes of calf diarrhoea and bacterial causes of pneumonia in the study areas. Calves less than 6 months of age were used for the study. All calves under 3 months of age at the beginning of the follow up period and those borne in the subsequent 3 months were individually identified and monitored throughout the study period. The calves were excluded when they were 6 months of age. The calves were regularly visited every week. During each visit the main tasks performed were:

- Clinical examination of the calves for diarrhoea and pneumonia
- Observation and recording the calf management practices

- Collection of faecal and nasal swab samples from diarrhoeic and pneumonic calves, respectively.

3.3.2. Cross-sectional study

A pretested questionnaire was personally administered to the farm managers to gather basic information on the general managemental practices (Appendix 1). Visual observations were also recorded. All the informations obtained formed the basis for identifying the potential risk factors for the study diseases.

3.4. Sampling Design

Samples were selected from all intensive dairy farms with an average 61 calves. Based on this, two dairy farms were included in Holeta, one farm in Muke Turi and four farms in Debre Zeit. In total, 7 large scale dairy farms were included in the study. All calves less than 6 months of age in these farms were followed for the occurrence of diarrhoea and pneumonia. Calves in each farm were visited weekly. Those calves, which developed any or both diseases, were sampled during regular visit and on emergency calls.

3.5. Sample collection procedure

For the detection and isolation of pathogens associated with calf diarrhoea, faecal samples were collected from diarrhoeic calves immediately after the onset of diarrhoea. About 30 gm of faeces was collected aseptically, directly from the rectum of the diarrhoeic calves using sterile glove and universal bottles. These were kept in an icebox and transported to the Microbiology Laboratory of the Addis Ababa University, Faculty of Veterinary Medicine (AAU, FVM) in Debre Zeit. They were processed with in 48 hours. Collected samples were clearly labeled. The label contained information on the date of sampling, the age, breed, tag number of the calves and number of scouring calves < 6 months (Appendix 2). Only fecal samples obtained within 48 h of onset of clinical signs from untreated calves were included in this study.

The faecal samples were analyzed using ELISA for detecting the presence of rotavirus, coronavirus, *Cryptosporidium parvum* and *E. coli* K99 and bacterial culture for isolating and

identifying the presence of *E. coli* and *Salmonella* species. The faecal samples were stored at -20^{0}C according to Nussbaum *et al.* (1999). Similar authors describe the ELISA technique as the gold standard test although the ELISA sometimes gives false-positive results.

For the isolation of respiratory pathogens, nasal swabs (DeRosa *et al.*, 2000) were taken from the deep nasal cavity of calves showing pneumonic signs (n = 28) by rotating a sterile polysterene tipped swabs inside the cavities. Collected samples were preserved in Stuarts transport medium (DIFCO). The samples were kept under refrigeration for less than 48 h until processed at the Microbiology Laboratory of the AAU, FVM.

The nasal swabs were analyzed according to DeRosa *et al.* (2000) and Gershwin *et al.* (2005). Animals were considered as clinically affected by respiratory tract disease according to the definition given by Svensson *et al.* (2006), Jaramillo-Arango *et al.* (2007) and Arcangioli *et al.* (2007). Those animals showing clinical manifestations of an undifferentiated respiratory disease, such as: nasal discharge, coughing, hyperpnoea or dyspnoea, pyrexia, abnormal respiratory noises and retarded growth were sampled. Those not presenting these clinical manifestations were considered as clinically healthy so no samples were taken.

3.6. Identification of infectious agents associated with calf diarrhoea

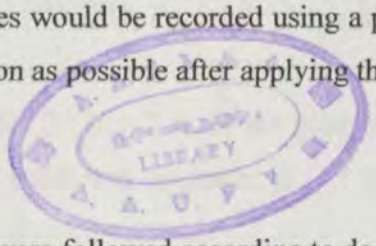
3.6.1. Antigenic ELISA

All the faecal samples were tested for the presence of rotavirus, coronavirus, *C. parvum* and *E. coli* F5 using a commercial ELISA kit (Bio-X Easy Digest®; Bio-X, Belgium). The sensitivities and specificities of the ELISA kit for detection of the pathogens were provided by the Manufacturer. The sensitivities for rotavirus, coronavirus, *E. coli* F 5 (K 99+) and *C. parvum* were given as 100 %, 90 %, 100 % and 97 %, respectively, while the specificities were 98 %, 95 %, 91 % and 91 %, respectively. The ELISA test was performed according to the manufacturer's instructions.

Procedures of the ELISA

Briefly, faeces were diluted in equal volumes with dilution buffer. This was a qualitative dilution, which allowed the pipetting of faecal suspensions. Any grud was discarded by decantation approach for about 10 minutes. The suspensions were not centrifuged. The diluted samples were pipetted into the micro-titration plate wells 100 µl per well. Pipettes were changed between different samples. The positive and negative controls were included in each plate. The plates were incubated at room temperature for 1 hour of incubation. They were then washed three times. After that, conjugates were added into the wells at the rate of 100 µl per well and incubated at room temperature for 1 hour. Following three washings, a 100 µl of the chromogen solution (substrate) was added to each well and incubated for 10 minutes at room temperature without covering the plates. The results were interpreted visually by checking for blue color formation.

If a plate reader was used for reading the optical density, 50 µl of the stop solution could have been added to each well. The optical densities would be recorded using a plate reader with a 450 nm filter. The readings would be made as soon as possible after applying the stop solution.



3.6.2. Isolation of *E. coli* Strains

For isolation of *E. coli*, standard procedures were followed according to de la Fuente *et al.* (1998) and Quinn *et al.* (1994). The faecal samples were plated on MacConkey agar. After overnight incubation, four colonies with typical characteristics of *E. coli* from each sample were chosen and subcultured onto nutrient agar. *Escherchia coli* strains were identified by biochemical tests, including Indole, Methyle Red, Voges Proskauer and Citrate (IMViC) test (Appendix 7 plate 10b), Triple Sgar Iron (TSI) (Appendix 7 plate 11b), Lysine decarboxylation (Appendix 7 plate 10a), urease and carbohydrate fermentation tests (Xylose and Sucrose). The isolates were also inoculated into Eosin Methylene blue (EMB) (Appendix 7 plate 6) to see the metallic sheen characteristics of the isolates. They were stored at + 4^0C in a nutrient agar (OXOID, England).

3.6.3. Isolation of *Salmonella* Species

Isolation and identification of *Salmonella* was carried out based on procedure made available by international organization for standardization (ISO 6579, 2002/ FDAM 1: 2007). In this procedure, the detection of *Salmonella* in animal faeces followed four stages:

Non-selective pre-enrichment in non-selective liquid medium

Buffered peptone water (BPW) (OXOID, England) was inoculated at ambient temperature with the test portion, then incubated at 37^0 C for 18 h $\pm$ 2 h. About 10 gram of faeces was inoculated into 100ml of buffered peptone water (BPW) in a ratio of 1 gram of sample to 9 ml of BPW.

Selective enrichment on selective semi-solid and liquid medium

A 0.1 ml of the culture obtained from the pre-enrichment was transferred to a 10 ml of Modified semi-solid Rappaport Vassiliadis (MSRV) agar (OXOID, England) plates (Appendix 7) and 1ml of the culture to Muller-Kauffmann tetrathionet/ novobiocin (MKTTn) broth (OXOID, England). The MSRV was incubated at 41.5^0C $\pm$ 1^0C for 24 h $\pm$ 3 h. If the plate was negative, it was incubated for a further 24 h $\pm$ 3 h. The MKTTn broth was incubated at 37^0C $\pm$ 1^0C for 24 h $\pm$ 3 h.

Selective plating out and identification

From the MSRV and MKTTn cultures, two selective solid media were inoculated, namely Xylose Lysine Deoxycholate (XLD) agar (OXOID, England) and *Salmonella–Shigella* agar (SS-agar) (OXOID, England) by means of loop (Appendix 7 plate 8). Both were incubated at 37 ^{0}C and examined after 24 h $\pm$ 3 h.

Confirmation of identity

Colonies presumptive of *Salmonella* were subcultured onto nutrient agar and confirmed by biochemical tests. Urea hydrolysis, TSI (Appendix 7 plate 11a), Lysine decarboxylation (Appendix 7 plate 10a), Voges Proskauer and Indole tests were done.

3.7. Identification of infectious agents associated with calf pneumonia

3.7.1. Isolation of *Pasteurella* and *Mannhaemia* Species

Isolation and identification of *Pasterella, Mannhaemia* and *Haemophilus* species was undertaken according to the method described by Quinn *et al.* (2002).

Nasal swab samples were directly inoculated into sheep blood agar (OXOID) and incubated for 24 - 48 hours at 37^0C. Individual colonies were sub-cultured in blood agar (5% sheep blood) at 37 °C for 24 h to obtain pure cultures of the bacteria. These colonies were transferred to nutrient agar. A preliminary identification of *Pasteurella* and *Mannhaemia* species was based on colony morphology on sheep blood agar, colour, and possible haemolysis on the plates incubated in a normal atmosphere, negative Gram staining and biochemical tests (positive oxidase and catalase reactions, carbohydrate fermentation, indole production, urease production, trehalose and aesculine fermentation were performed according to Tegtmeier *et al.* (1999) and Quinn *et al.* (2002).

3.7.2. Isolation of *Haemophilus somnus*

The procedure that was followed for the isolation of *Haemophilus somnus* was that recommended by Quinn *et al.* (1994), Howard (1998) and Ward *et al.* (2006). Briefly, the nasal swab sample was streaked directly to sheep blood agar and Chocolate agar. The media were incubated at 37°C in an environment having 10% of CO_2 and examined after 48 hrs. Individual colonies were identified and transferred to another chocolate agar. Gram staining, catalase and oxidase tests were done as described in Ward *et al.* (2006).

.8. Antimicrobial susceptibility test for *Salmonella, E. coli, Pasteurella* and *Mannhaemia* species

For antimicrobial susceptibility test, the National Committee for Clinical Laboratory Standards (NCCLS, 1997) guidelines was followed throughout. Briefly, the isolates were cultured on nutrient agar and then grown to 0.5 - 1 McFarland density in tryptone soya broth (the turbidity of the test brothes was adjusted with saline until the turbidity of the test suspension equated to that of the standard). The inoculated plates were allowed to stand for 3-5 minutes for any excess

moisture from the inoculum to be absorved by the agar before the antimicrobial discs were applied. The discs were then placed onto the agar surface using sterile forceps, gently pressed with the point of the forceps for ensuring complete contact with the agar surface. The discs were placed no greater than 24 mm (center to center). The plates were then inverted and placed in 35^0C incubator within 15 minutes of applying the discs and incubated aerobically for 16-18 hours.

The measurements taken included zone diameters to the nearest whole milli meter. The antimicrobials agents used, disc content, and the approximate Minimum Inhibitory Concentration (MIC) correlates are listed in table 4.

Table 4. Antimicrobial discs, symbols and zone diameters with the approximate minimum inhibitory concentration

Antimicrobial Reagents and symbols	Disc Content(µg)	Zone diameter, nearest whole mm				Approximate MIC Correlates (µg / ml)	
		R	I	MS	S	R	S
Ampicillin (AMP)	30	≤ 14	15-16	-	≥ 17	≥ 32	≤ 16
Erythromycin (E)	15	≤ 13	14-17	-	≥ 18	≥ 8	≤ 2
Gentamycin (CN)	10	≤ 12	13-14	-	≥ 15	≥ 8	≤ 4
Kanamycin (K)	30	≤ 13	14-17	-	≥ 18	≥ 25	≤ 6
Norfloxacin (NOR)	10	≤ 12	13-16	-	≥ 17	≥ 16	≤ 4
Streptomycin (S)	10	≤ 11	12-14	-	≥ 15	-	-
Trimethoprim-Sulfamethoxazole (SXT)	1.25/23.75	≤ 10	11-15	-	≥ 16	≥ 8/152	≤ 2/38
Amocacillin (AML)	2	≤ 13	-	14-17	≥ 18		
Clindamycin (DA)	2	≤ 14	15-20	-	≥ 21		
Tetracyclin (TE)	30	≤ 14	15-18	-	≥ 19	≥ 16	≤ 4
Neomycin (N)*	30	≤ 12	13-16	-	≥ 17		
Chloramphenicol (C)*	30	≤ 12	13-17		≥ 18		

Keys: R= Resistant, I= Intermediate, MS= Moderately Susceptible, S= Susceptible (Source: NCCLS (1997), (*) Disc diameter for these drugs was obtained from the drug manufacturers leaflet, Becton Dickinson Microbiology systems.

The zone diameters generated by the test are meaningless without reference to the MIC correlates and interpretive guidelines published in NCCLS (1997).

3.9. Identification of the predisposing factors for calf diarrhoea and pneumonia

According to Langoni *et al.* (2004), Svensson *et al.* (2006) and Lorino *et al.* (2005), various factors predispose calves to diarrhoea. Therefore a total of 25 potential risk factors (explanatory variables) categorized as calf factors, management factors farm factors and prophylaxis and miscellaneous categories associated with diarrhoea and pneumonia were considered (Table 5). All variables were dichotomized taking care of the categorization.

Table 5. Categories of potential risk factors for calf diarrhoea and pneumonia

Risk Calf factors	
Variables	Description of categories
Sex	• Male • Female
Age	• 0-30 days • 31-60 days • 61-90 days • > 90 days
Management factors	
Time of first colostrum feeding	• ≤ 6 hours • > 6 hours
Method of colostrum feeding	• Suckling from its dam • Hand feeding
Frequency of feeding on the first day of life	• Sufficient ≥3 times • Insufficient < 3 times

Table 5. Cont.

Feeding concentrate	• Yes • No
Amount of milk fed daily	• < 4 liters • ≥ 4 liters
Weaning age	• < 3 months of age • ≥ 3 months of age
Housing condition	• Separate calf pen • In the same barn with cows
House cleanness	• Clean • Unclean
Farm factors	
Age of the farm	• ≤ 5 years • > 5 years
Farm as a source of income	• Primary source of income • Secondary source of income
Gender of calf caretaker	• Males only • Females only • Both
Experience of calf caretaker	• ≤ 5 years experience • > 5 years experience
Knowledge about the importance of colostrum	• Yes • No
Knowledge on the optimum age to feed colostrum	• Yes • No
Prophylaxis and miscellaneous categories associated with diarrhoea	
Disinfecting calf area (frequently)	• Yes • No
Cleaning before calving season in calf barn	• Yes • No

Table 5. Cont.

Cleaning after calving season in calf barn	• Yes • No
Cleaning after each diarrhoea episode in calf barn	• Yes • No
Dam vaccinated against other agents	• Yes • No
Additional vitamins and minerals to cows	• Yes • No
Additional vitamins and minerals to calves	• Yes • No

3.10. Data Management and Statistical Analysis

3.10.1. Describing cumulative incidences of calf diarrhea, pneumonia and mortality

Animals in the present study were followed for different periods of time for the two diseases. The incidence rate (True rate) and cumulative incidences (CI) of calf diarrhoea and pneumonia were calculated according to Thrusfield (2005),

- True rate (IR) = Number of new cases of disease that occur in a population during a particular period of time

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  The sum, over all individuals, of the length of time at risk of developing disease (animal time at risk)

- Cumulative Incidence (CI) = $1\text{-}e^{-I}$,

  Where, e = the base of the natural logarithms, 2.718

  I = The Incidence rate (True rate)

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. Cumulative incidence was also calculated for mortality.

3.10.2. Data Analysis

Data entry and validation were done using Microsoft excel, and processed using the appropriate statistical analysis. Description of the variables was done first. The database file was transferred to SPSS followed by statistical analysis of the associations between risk factors (explanatory variables) and outcome variables (disease status). Kalpan-Meier time-to-event analysis for the association of potential risk factors with the occurrence of diarrhoea and pneumonia was then performed. The association of the risk factors was also further analyzed using multivariable Cox regression (Cox' proportional hazard model) (SPSS, 2006).

4.1. Description of the farms

A total of 25 potential risk factors (explanatory variables) were considered to analyze their association with calf diarrhoea and pneumonia. Due to the similarity of the farm management, no statistics were computed for most of the farm factors (Appendix 4). But some of the potential risk factors (age, breed, etc) were different among the farms and were included in the study.

Generally, 100 % of the farms in this study were intensively managed with an average number of 61 calves per herd. Majority (71.4 %) of the farms had crossbreed calves, but there were also farms having Boran breeds and Holstein Friesians. The Boran breed and Holstein Friesian calves were 22 (5.1 %) and 126 (29.4 %), respectively and the rest 281 (65.5 %) were cross breed calves. All of the farms had the knowledge of colostrum feeding.

Calves were housed in separate pens with bedding of 'Teff' straw and the pens were cleaned twice a day. During cleaning, the calves were kept in group pens except in two farms where there was no mixing up of calves. In the group pens, they were supplied with water, concentrates and / or straw. In the majority of the farms, the water troughs were well constructed so that the calves would not enter into them, except in one farm (Pictures 1 and 2). In addition, disinfection of the calf pens was not practiced after diarrhoeal episodes in the majority (85.7 %) of the farms and foot and truck bathes were not used in 71.43 % of the farms.

Picture 1. Watering trough in the group pens used in the majority of the farms

Picture 2. A calf inside a watering trough at Farm 1

4.2. Cumulative Incidence of Calf Diarrhoea and Pneumonia

A total of 429 calves less than 6 months of age were followed up for six months. Calves were considered lost in the follow up period if they were sold, dead or culled from the herd when they were affected with some diseases. Usually male calves from the commercial private dairy farms remained in the farms for not more than two weeks due to economic reasons, but the female calves were kept for replacement in all the farms.

The result of the present study revealed that the CIs of NCD in Debre Zeit, Holeta and Muke Turi dairy farms were 0.37, 0.41 and 0.63, respectively. Similarly, the CIs of calf pneumonia were 0.07, 0.12 and 0.09, respectively (Figure 1 and appendix 5A).

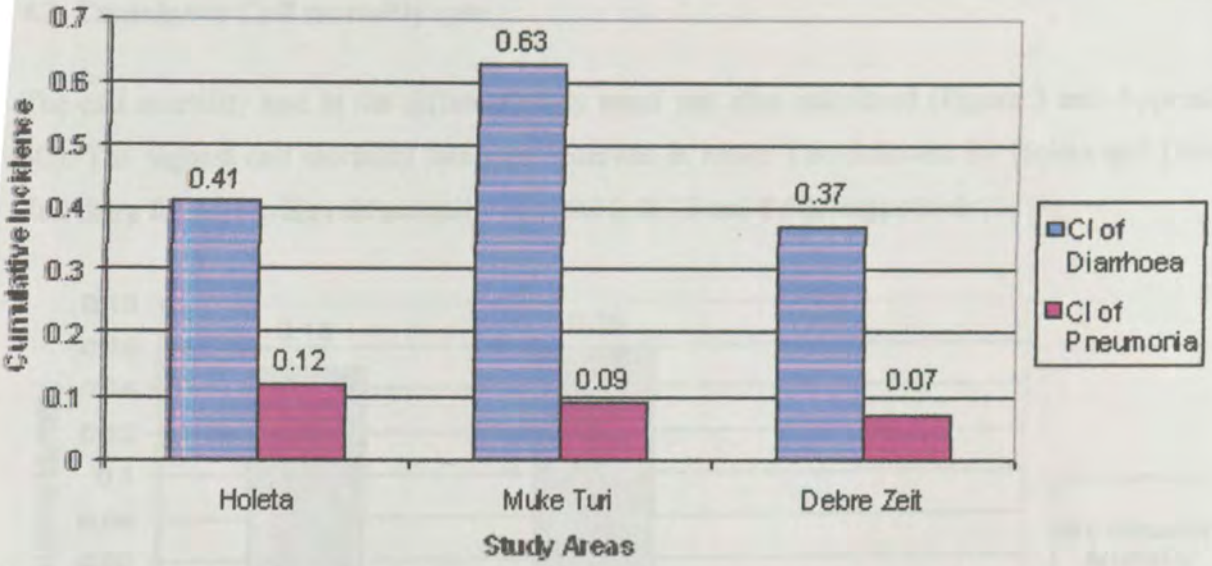

Figure 1. Cumulative incidences of calf diarrhoea and Pneumonia in different study areas

The CI for the entire follow-up period for diarrhoea and pneumonia were also calculated at a farm level and the highest one for diarrhoea was recorded at farm 1 (0.65) followed by farm 3 (0.63), farm 5 (0.55), farm 4 (0.36), farm 7 (0.34), farm 6 (0.22) and farm 2 (0.11). In the case of calf pneumonia, highest CI was seen in farm 6 and the least in farm 5 (Figure 2 and appendix 5B).

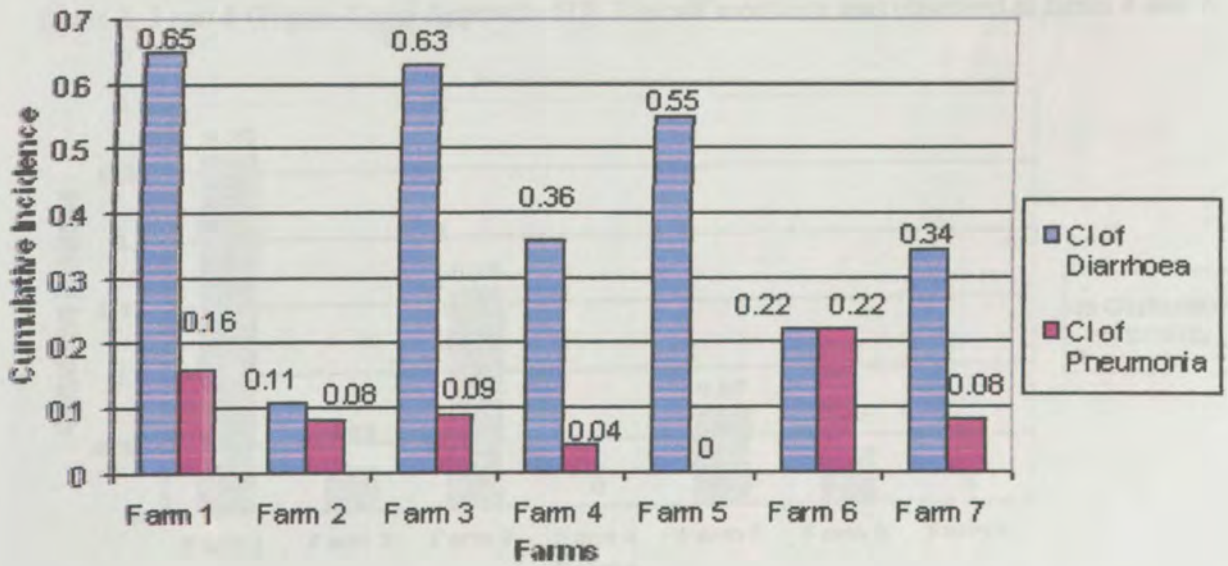

Figure 2. Cumulative incidence of calf diarrhoea and Pneumonia by different farms

4.3. Cumulative Calf mortality rate

The calf mortality rate in the different study areas was also calculated (Figure 3 and Appendix 5C). The highest calf mortality rate was observed in Muke Turi followed by Holeta and Debre Zeit dairy farms, having calf mortality rates 0.16, 0. 15 and 0.02, respectively.

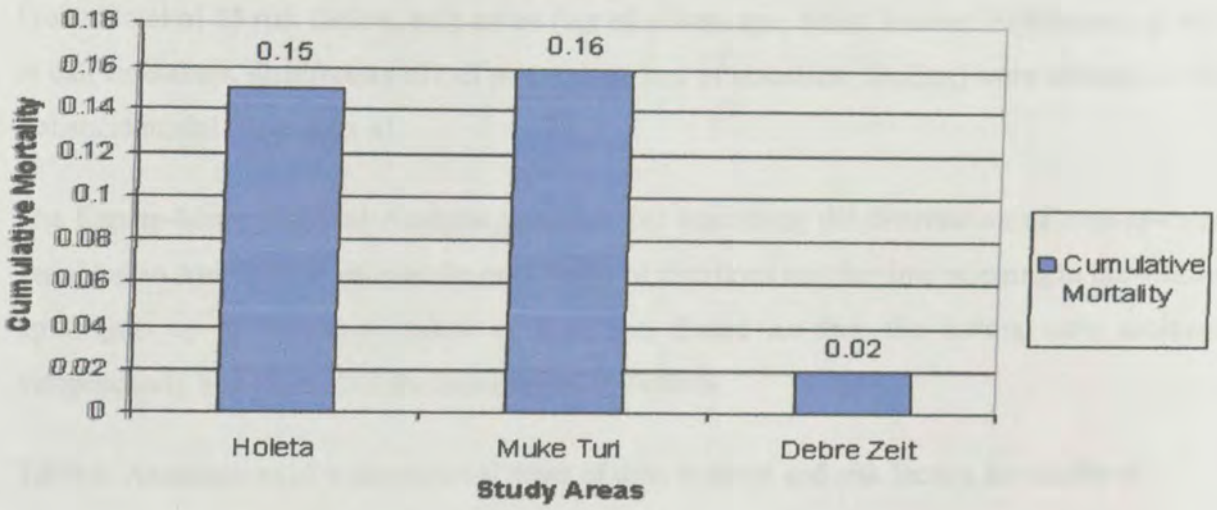

Figure 3. Calf mortality rate in different study areas

When calf mortality was calculated at a farm level, highest was found at farm 1 followed by farms 3, 5 and 6 (Figure 4 and Appendix 5D). No calf mortality was observed at farms 4 and 7.

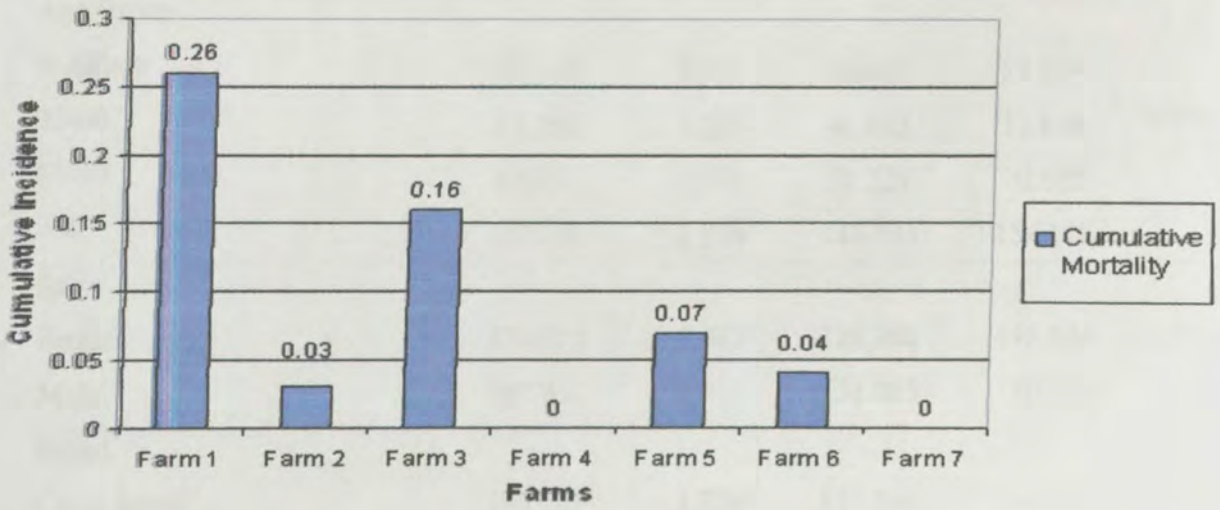

Figure 4. Cumulative calf mortality rate by different farms

The calf mortalities observed in the age-groups 0- 30 day old calves was, 5 (14.7%), 31-60 day old calves was 14 (41.2 %), 61-90 days old 7 (20.6 %), greater than 90 days 8 (23.5 %). Highest mortality was observed in the age group 31-60 day old calves

4.4. Relationships between risk factors and survival times for calf diarrhoea

From a total of 25 risk factors, only seven (sex of calves, age, breed, vitamin supplement, gender of calf caretakers, disinfection of calf pen and method of colostrum feeding) were included in the statistical model (Appendix 4).

The Kaplan-Meier Survival Analysis was used for examining the distribution of time-to-event variables, in which the event was the occurrence of diarrhoea and the time referring to the follow-up lengths up to the development of diarrhoea. Based on this, the factors were analyzed independently and the results are summarized in Table 6.

Table 6. Associations of mean survival times of time to event and risk factors for diarrhoea

			95% Confidence Interval		
Risk Factor	Mean Survival Time	Std. Error	Lower Bound	Upper Bound	P-value
Age Group					
0-30days	22.212	2.955	16.421	28.004	
31-60	47.350	3.223	41.033	53.666	0.000
61-90	60.953	4.965	51.221	70.685	
>90	147.581	3.336	141.043	154.120	
Sex					
Female	136.915	4.443	128.206	145.624	0.570
Male	140.911	5.014	131.085	150.738	
Breed					
Cross Breed	146.707	3.734	139.389	154.026	0.000
Holstein Friesian	113.435	7.092	99.533	127.336	

vitamins and Minerals supplements					
No	137.367	3.742	130.033	144.702	0.354
Yes	138.426	6.650	125.391	151.460	
Gender of calf caretaker					
Females only	130.120	7.158	116.091	144.150	
Males only	118.466	6.358	106.004	130.928	0.000
Both	157.431	4.080	149.433	165.428	
Disinfection of calf pens					
No	137.367	3.742	130.033	144.702	0.354
Yes	138.426	6.650	125.391	151.460	
Method of colostrum feeding					
Suckling	143.125	5.298	132.740	153.510	0.596
Bucket feeding	137.046	4.165	128.883	145.208	

From the seven analyzed variables, the result of the Kaplan-Meier Survival Analysis showed that only the three variables; age of the calves, breed of the calves and gender of the calf caretakers were significantly ($P < 0.05$) associated with the occurrence of calf diarrhoea. The other factors were not significantly ($P > 0.05$) associated.

Age of calves was categorized according to Lorino *et al.* (2005) and Lee *et al.* (2007) to facilitate the analysis. When the occurrence of diarrhoea was compared among the different age-groups, the confidence intervals for the calves within age-group 31-60 and 61-90 days overlapped indicating the absence of significant difference in the occurrence of diarrhoea in those two age-groups. But the confidence intervals for the rest of the age-groups did not overlap indicating the presence of statistically significant ($P = 0.000$) differences among them. The hazard rates of diarrhoea in calves within the age-group 0-30 days was the highest followed by 61-90, 31-60 and > 90 days old calves (Figure 5).

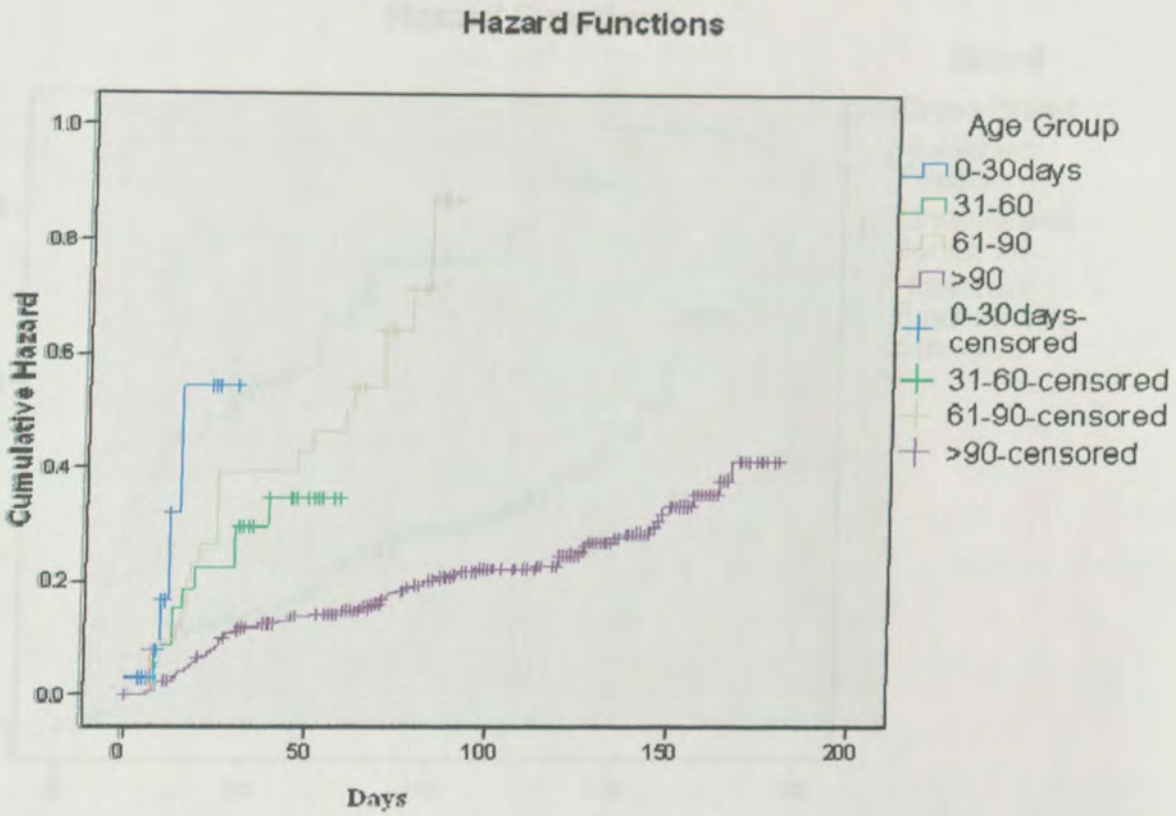

Figure 5. The hazard functions of diarrhoea compared by the different age-groups using Kaplan-Meier Survival Analysis

In the case of breed, the Boran breeds were not included in the analysis because there was no event (diarrhoea) which occurred in these breeds. Only crossbreeds and Holstein Friesians were compared. According to the results, the confidence intervals for the mean survival times for the two breeds did not overlap indicating that there was statistically significant ($P < 0.05$) difference in the occurrence of diarrhoea between these breeds. The hazard function plot indicates that the hazard rate of diarrhoea is more in Holstein Friesians than the cross breeds (Figure 6).

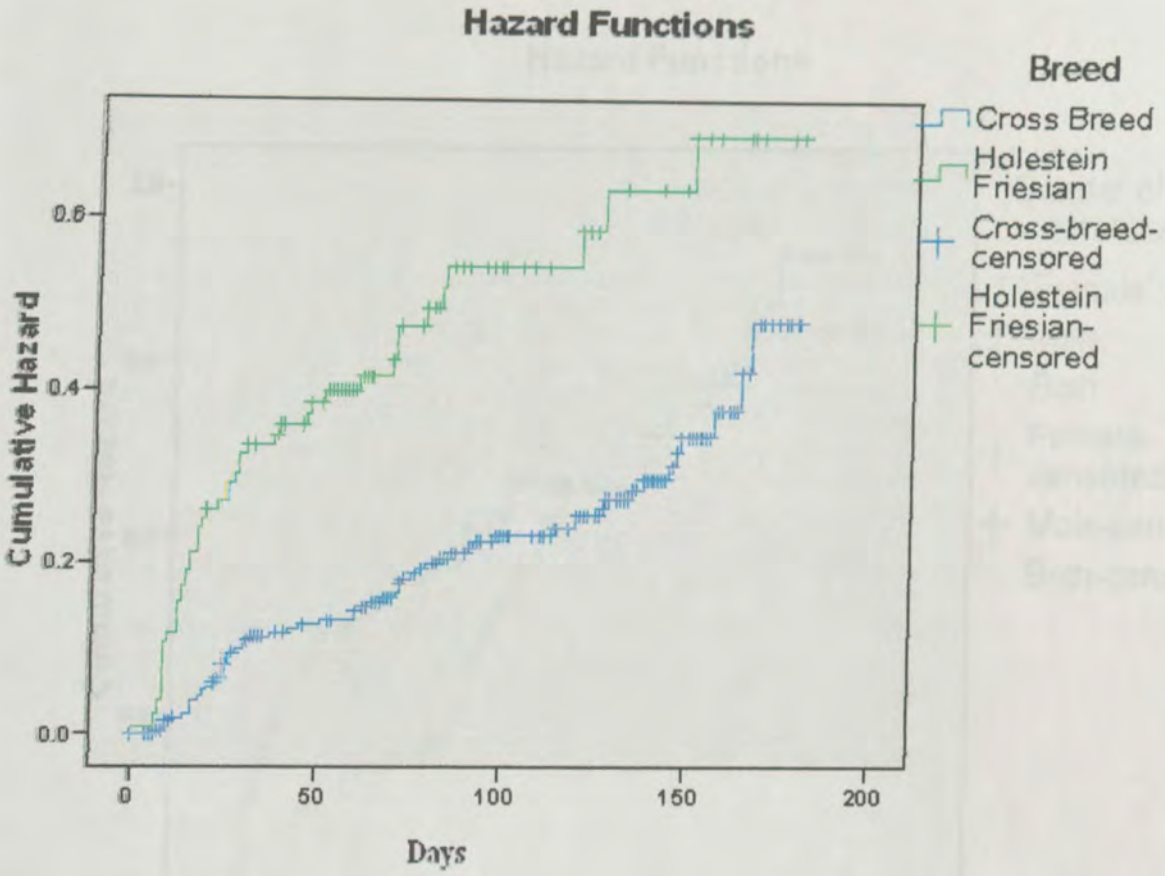

Figure 6. The hazard functions of diarrhoea compared by the different breeds using Kaplan-Meier Survival Analysis

The difference in the gender of calf caretakers, when analyzed to see the association with the occurrence of diarrhoea, the specific confidence intervals for the only male or only female calf caretakers overlapped indicating the absence significant difference in the occurrence of diarrhoea between calves managed by male or female calf caretakers. But the confidence intervals for farms having both male and female calf takers did not overlap with the other two categories. This implied that there was statistically significant difference in the occurrence of diarrhoea among calves managed by both male and female calf caretakers compared to the ones with specifically only male or female calf caretakers (Figure 7).

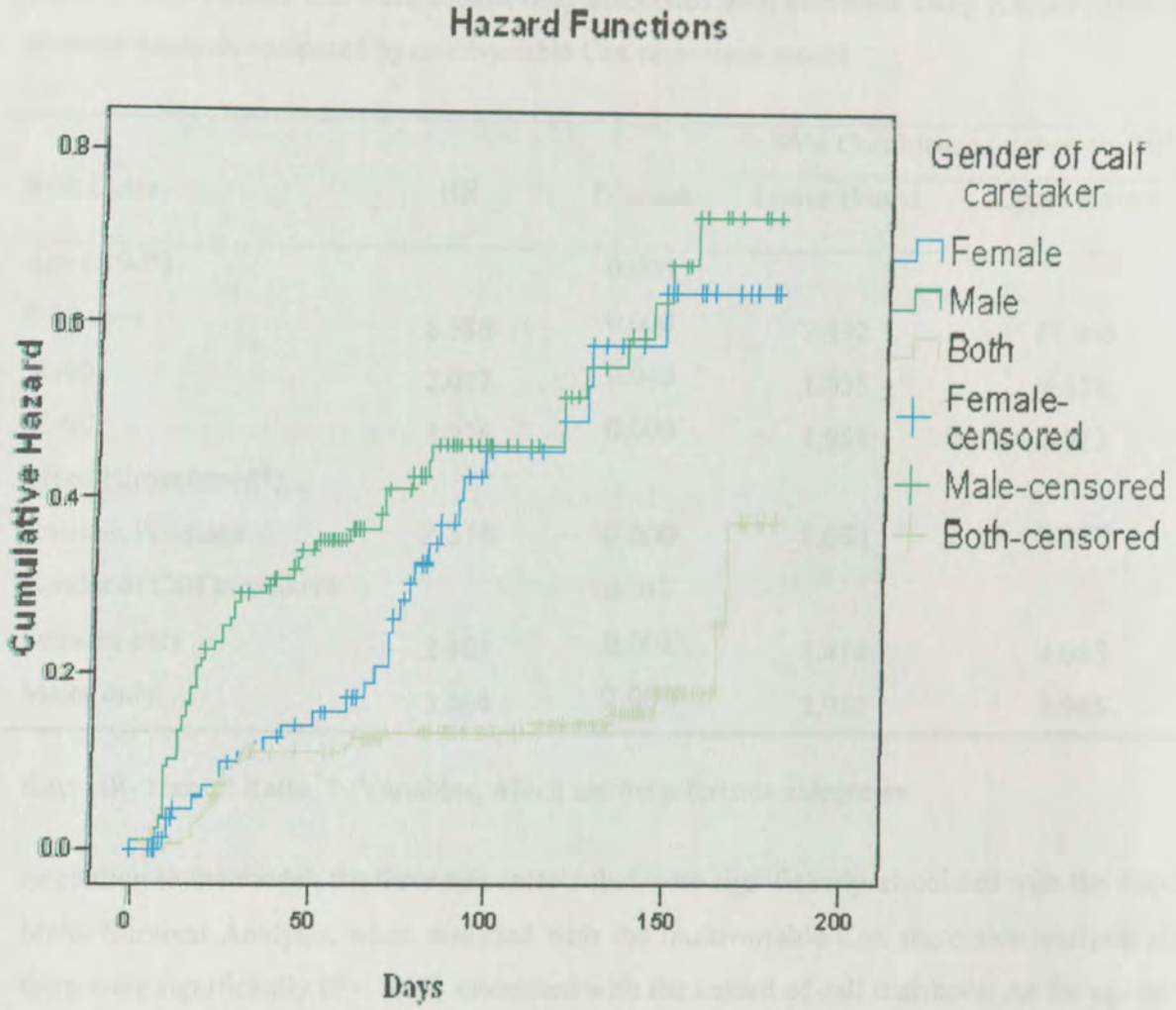

Figure 7. The hazard functions of diarrhoea by different gender groups of calf caretakers using Kaplan-Meier Survival Analysis

Risk factors that were significantly associated with the occurrence of calf diarrhoea using the Kaplan-Meier time-to-event analysis were further analyzed with multivariable Cox regression proportional hazards model, to see the degree of associations of calf diarrhoea and the risk factors (Table 7).

Table 7. Risk Factors that were significantly associated with diarrhoea using Kaplan-Meier time to event Analysis compared by multivariable Cox regression model

Risk factor	HR	P- value	95% Confidence Interval for HR	
			Lower Bound	Upper Bound
Age (> 90*)		0.000		
0-30 days	6.586	0.000	2.492	17.405
31-60	2.077	0.040	1.035	4.170
61-90	3.225	0.000	1.958	5.313
Breed (Cross breed*)				
Holstein Friesians	2.316	0.000	1.591	3.370
Gender of Calf caretakers		0.017		
Females only	2.403	0.001	1.414	4.085
Males only	3.104	0.000	1.933	4.985

Key: HR- Hazard Ratio, *- Variables, which are the reference categories

According to the model, the three risk factors that were significantly associated with the Kaplan-Meier Survival Analysis, when analyzed with the multivariable Cox regression analysis all of them were significantly ($P < 0.05$) associated with the hazard of calf diarrhoea. As for age-group of calves, different values of HRs were obtained. Significant ($P < 0.05$) difference of diarrhoeal hazard was seen in the age groups 0-30, 31-60 and 61-90 days old calves when compared with calves greater than 90 days old. The HR for the age-group 0-30 was 6.586. This indicates that the hazard of diarrhoea in 0-30 days old calves is 6.586 times higher than the calves greater than 90 days old. This was statistically ($P= 0.000$) significant. Similarly, calves in the age-group 31-60 days old were 2.077 times more at risk of diarrhoea than the older age groups ($P= 0.040$). The hazard of diarrhoea in the age group 61-90 days old calves was 3.225 times more than calves greater than 90 days old and was statistically significant ($P= 0.000$).

Similarly breed had significant effect on the hazard of diarrhoea. The Boran breeds were also not included in the analysis because of the absence of diarrhoea in these breeds. According to the

above analysis Holstein Friesian calves were 2.316 times more at risk of developing diarrhoea than the cross-breed calves (P = 0.000).

In the case of the gender of calf caretakers, the calves managed with female calf caretakers were 2.403 times at risk of developing diarrhoea than calves managed by both sexes and it is statistically significant (P = 0.001) and those managed with male calf caretakers were 3.104 times at risk of developing diarrhoea than calves managed by both sexes and was statistically significant (P = 0.000).

In addition to the above, the occurrence of calf diarrhoea was compared by the different farms to see if there were significant difference0s among farms and occurrence of diarrhoea (Table 8).

Table 8. Associations of mean survival times for diarrhoea in different farms and risk factors

Farm	Mean Survival time	Standard Error	95% Confidence Interval		P- value
			Lower Bound	Upper Bound	
Farm 1	113.435	7.092	99.533	127.336	
Farm 2	170.675	3.471	163.871	177.479	
Farm 3	108.981	12.176	85.116	132.847	
Farm 4	138.426	6.650	125.391	151.460	0.000
Farm 5	124.455	10.212	104.439	144.470	
Farm 6	159.213	9.258	141.067	177.358	
Farm 7	154.067	8.740	136.937	171.196	

There was no significant difference in the mean survival times of calves to diarrhea among Farm 1 compared with Farm 3, Farm 4 and Farm 5; Farm 2 compared with Farm 6 and Farm 7; Farm 3 compared with Farm 4, Farm 5 and Farm 7; Farm 4 compared with Farm 5, Farm 6, and Farm 7; Farm 5 compared with Farm 6 and Farm 7; and Farm 6 compared with Farm 7. Statistically significant (P = 0.000) difference in the occurrence of calf diarrhoea was observed between Farm 1 and Farm 2, Farm 1 and Farm 6, Farm 1 and Farm 7, Farm 2 and Farm 3, Farm 2 and Farm 4, Farm 2 and Farm 5, Farm 3 and Farm 6 and Farm 3 and Farm 7.

When the hazard functions plot was made for different farms, the Hazard of diarrhoea in farm 1 and farm 3 were higher than the others, while the least was observed at Farm 2 (Figure 8).

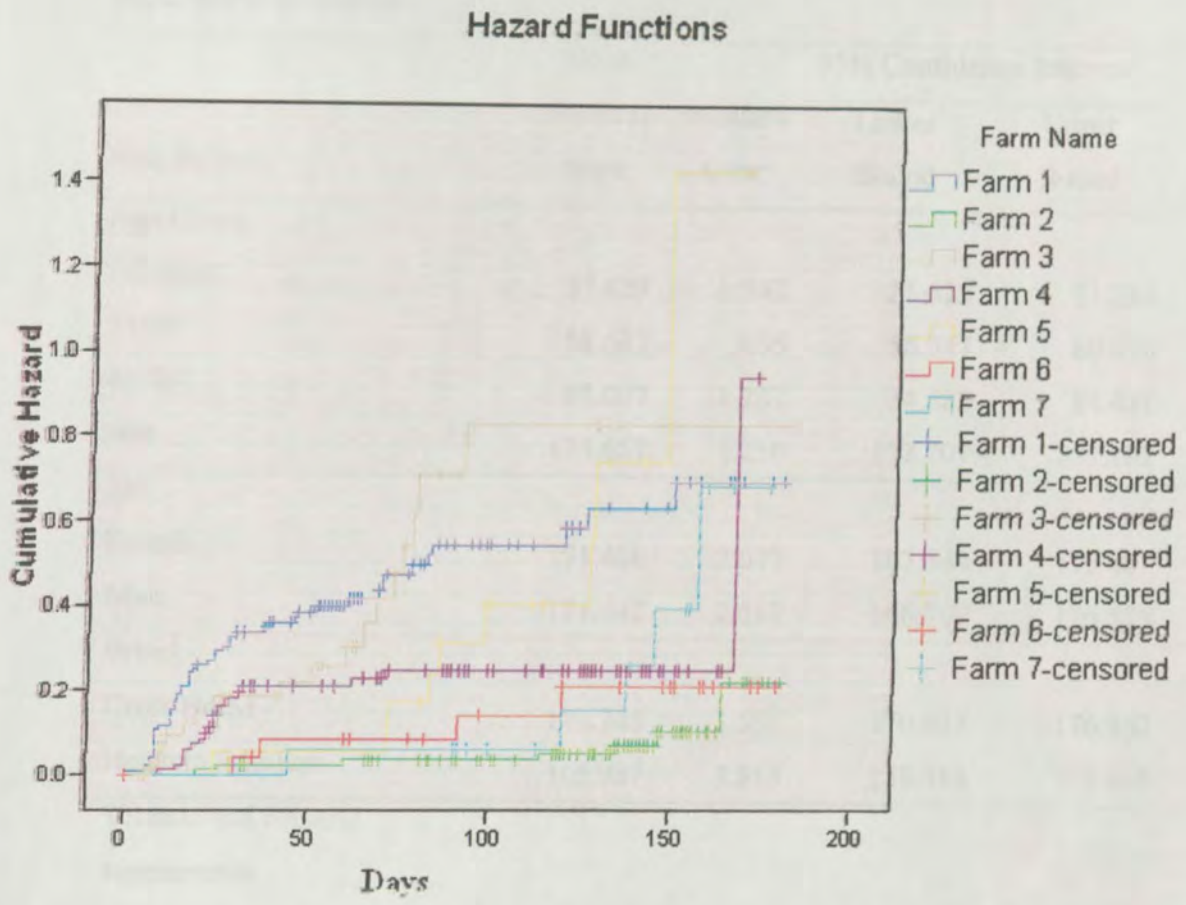

Figure 8. The hazard functions of diarrhoea in different farms compared by the Kaplan-Meier Survival Analysis

4.5. Association of potential risk factors with the incidence of calf pneumonia

The risk factors that were analyzed as a predisposing factor for the occurrence of calf diarrhoea were also analyzed with the Kaplan-Meier Survival Analysis to see if they can predispose calves to pneumonia. The results are summarized in Table 9.

Table 9. Association of potential risk factors to the occurrence of pneumonia using the Kaplan-Meier Survival Analysis

Risk factor	Mean Survival time	Standard Error	95% Confidence Interval		P- value
			Lower Bound	Upper Bound	
Age Group					
0-30days	27.429	1.942	23.623	31.234	.000
31-60	58.682	.885	56.947	60.416	
61-90	87.007	1.237	84.582	89.431	
>90	174.657	1.250	172.207	177.107	
Sex					
Female	171.416	2.077	167.344	175.487	0.939
Male	171.642	2.517	166.709	176.575	
Breed					
Cross Breed	173.745	1.591	170.627	176.862	0.052
Holstein Friesian	165.987	3.913	158.318	173.455	
Vitamin and Mineral supplements					
No	170.382	1.880	166.698	174.067	0.121
Yes	172.707	2.258	168.281	177.132	
Gender of calf caretaker					
Female	170.733	3.561	163.752	177.713	0.080
Male	167.150	3.434	160.419	173.881	
Both	175.634	1.582	172.534	178.734	
Disinfection of calf pens					
No	170.382	1.880	166.698	174.067	0.121
Yes	172.707	2.258	168.281	177.132	
Method of colostrum feeding					
Suckling	173.406	2.390	168.721	178.090	0.503
Bucket feeding	170.808	2.014	166.861	174.755	

The results showed that only age-group of calves was significantly associated (P= 0.000) with the occurrence of pneumonia. The mean survival time for the occurrence of pneumonia in the different age-groups was significantly (P = 0.000) different. The hazard functions of all the age-groups are given in Figure 9.

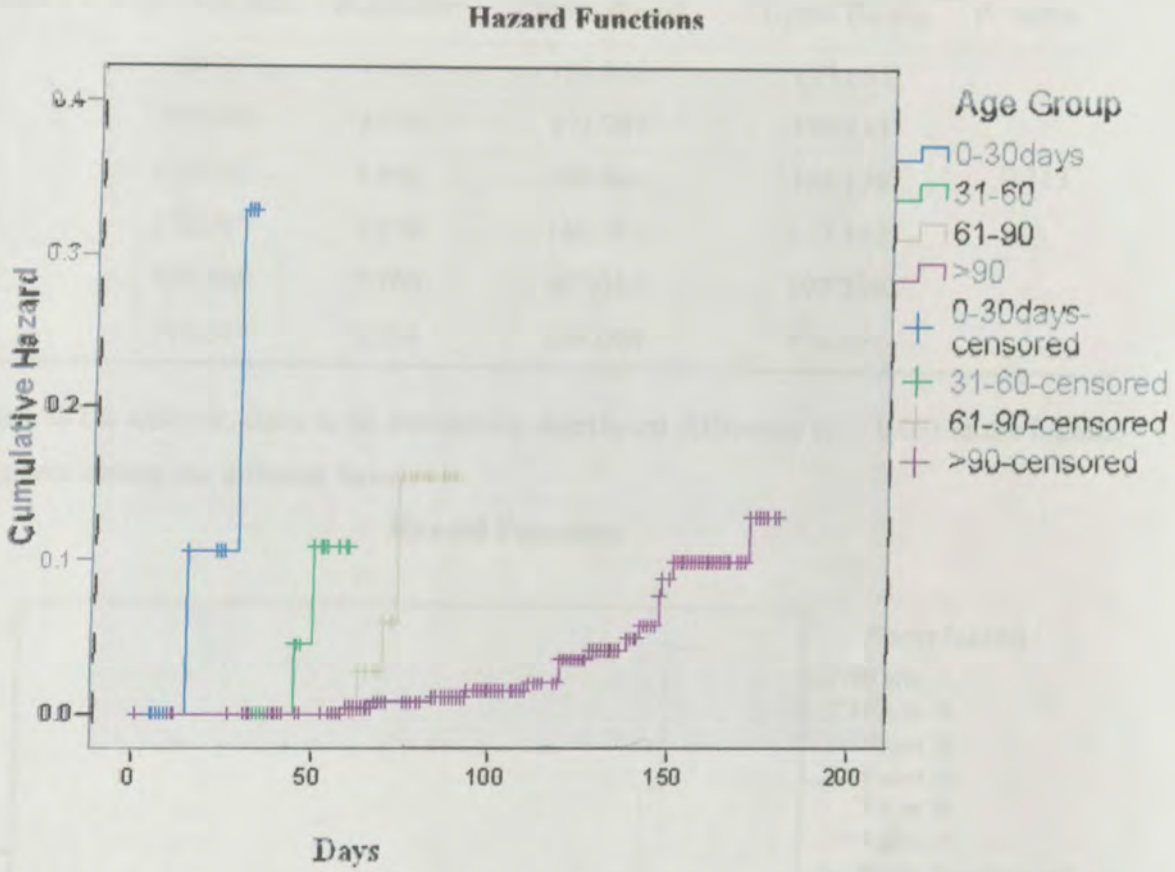

Figure 9. The hazard functions of pneumonia by the different age-groups of calves using Kaplan-Meier Survival Analysis

The risk factor (age-group) was further analyzed using a univariable Cox proportional hazards model to see the association well. But the result was found to be insignificant (P> 0.05).

The hazard of pneumonia was also compared among farms using Kaplan-Meier Survival Analysis. The result is summarized in Table 10. Farm 5 was excluded from the analysis because no case of pneumonia occurred. Statistically, there were no significant differences among the farms in the hazard rates of developing pneumonia and the hazard functions are depicted in Figure 10.

Table 10. The occurrence of pneumonia among the different farms analyzed using the Kaplan-Meier Survival Analysis

Farm Name	Mean Survival time	Std. Error	95% Confidence Interval		P- value
			Lower Bound	Upper Bound	
Farm 1	165.987	3.913	158.318	173.655	
Farm 2	175.192	2.036	171.201	179.183	
Farm 3	172.271	5.055	162.364	182.178	0.113
Farm 4	172.707	2.258	168.281	177.132	
Farm 6	162.108	7.700	147.016	177.200	
Farm 7	172.545	3.294	166.090	179.001	

According to the analysis, there is no statistically significant difference ($P > 0.05$) in the hazard of pneumonia among the different farms.

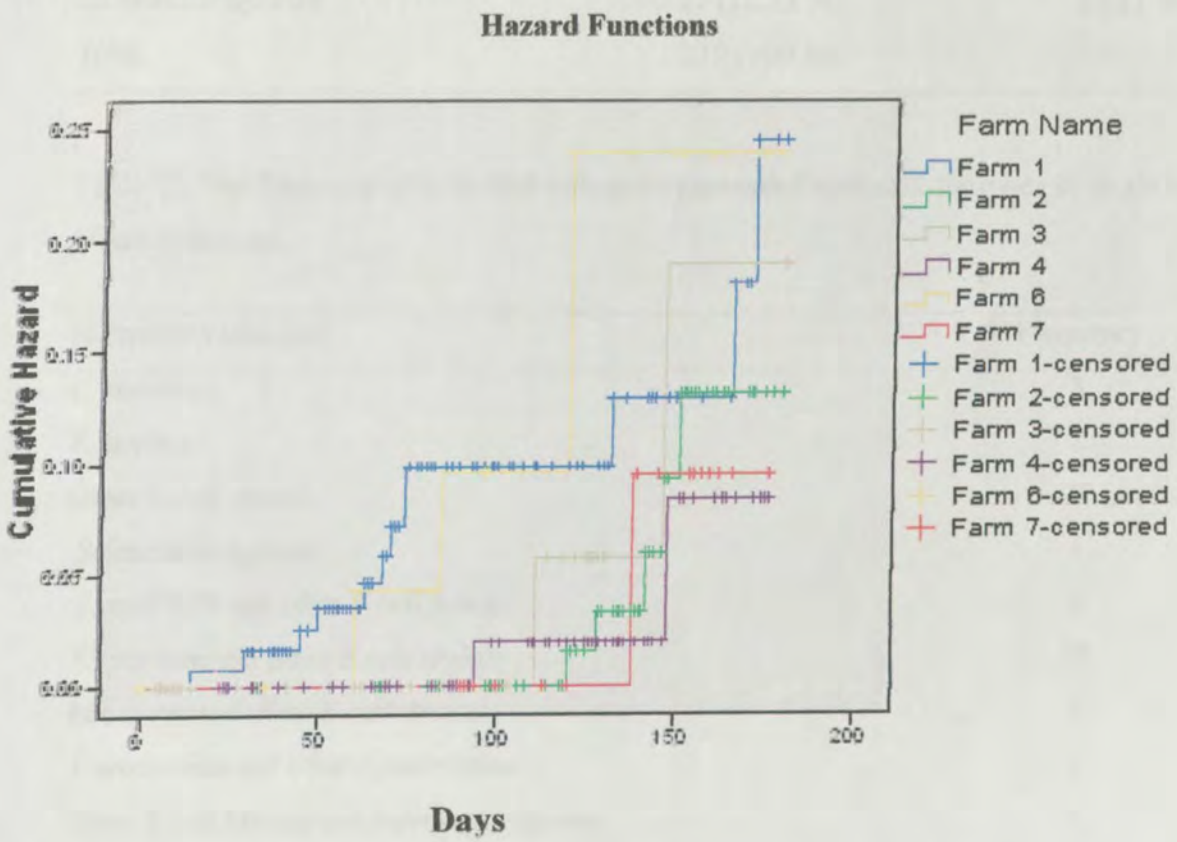

Figure 10. The hazard functions of pneumonia among the different farms compared by the Kaplan-Meier Survival Analysis

4.6. Major bacterial and Viral Pathogens Associated with calf diarrhoea

ELISA test and standard bacterial culture were performed for the identification of microbial pathogens associated with calf diarrhoea. The results are summarized in Tables 11, 12, and 13.

Table 11. Number of antigen ELISA positive faecal samples and prevalence of the pathogens identified in 112 samples

Bacterial and viral pathogens	Frequency of isolation	Prevalence
E. coli K99	3 (1.37 %)	2.68 %
C. parvum	58 (26.48 %)	51.79 %
Rotavirus	22 (10.05 %)	19.64 %
Coronavirus	6 (2.74 %)	5.36 %
Other *E.coli* Strains	103 (47.03 %)	91.96 %
Salmonella Species	27 (12.33 %)	24.11 %
Total	219 (100 %)	

Table 12. The frequency of microbial pathogens associated with calf diarrhoea in single and mixed infections

Microbial Pathogens	Frequency
C. parvum	5
Rotavirus	2
Other *E.coli* strains	27
Salmonella species	1
E. coli K99 and other *E.coli* strains	2
C. parvum and other *E.coli* strains	28
Rotavirus and other *E.coli* strains	7
Coronavirus and other *E.coli* strains	2
Other *E.coli* Strains and *Salmonella* species	9
C. parvum, *Salmonella* and other *E.coli* strains	11
C. parvum, rotavirus and other *E.coli* strains	7

C. parvum, coronavirus and Other *E.coli* strains	4
C. parvum, rotavirus, *Salmonella* and other *E.coli* strains	3
Rotavirus, *Salmonella* species and other *E.coli* strains	2
E. coli K99 + Other strains of *E.coli*, rotavirus and *Salmonella*	1

From all the cases of diarrhoea, the pathogens were found singly and in combination with each other, except *E.coli* K 99 and coronaviruses, which were not detected singly. *Escherichia coli* strains other than those with the K 99 strain had the highest frequency (103), followed by *C. parvum*, *Salmonella* species, rotavirus, coronavirus and *E.coli* K 99. *Escherchia coli* K 99 was detected from 3 cases only. The frequency of diarrhoeal pathogens with a single infection detected by the ELISA test was 5 and 2 for *C. parvum* and rotavirus, respectively. On the other hand, the single infection of calves with *Salmonella* species and *E.coli* strains other than K 99 were 1 and 27, respectively (Table 12).

Table 13. Microbial pathogens detected and isolated from diarrhoeic calves in different farms

	Microbial pathogens					
Farm (N)	*E.coli* K99	*C. parvum*	Rotavirus	Coronavirus	Other strains of *E.coli*	*Salmonella* species
Farm 1 (51)	1	28	12	5	46	8
Farm 2 (7)	0	3	0	0	7	1
Farm 3 (17)	1	8	7	0	15	7
Farm 4 (19)	0	11	0	1	18	5
Farm 5 (9)	1	3	2	0	9	2
Farm 6 (4)	0	2	0	0	4	2
Farm 7 (5)	0	3	1	0	4	2
Total (112)	3	58	22	6	103	27

Key: (N): number of cases

4.7. Bacterial Pathogens Associated with Calf pneumonia

Nasal swab samples were taken from calves with clinical respiratory tract disease for the purpose of isolating *Pasteurella, Mannhaemia* and *Haemophilus* species. The results are shown in Tables 14 and 15. *Haemophilus somnus* was not isolated from any of the cases.

Table 14. Frequency of bacterial isolates from pneumonic calves (n = 26)

Farm	Peumonia Cases (N)	*Pasteurella* spp.	*Mannhaemia* spp.	*Pasteurella* spp.	*Mannhaemia spp.*
Farm 1	12	4	1	*P.trehalosi, P.aerogens* and *P. lymphangitidis*	*M. haemolytica*
Farm 2	5	3	2	*P.trehalosi* and *P.aerogens*	*M. haemolytica*
Farm 3	2	1	0	*P.trehalosi*	
Farm 4	2	0	0	-	
Farm 6	4	1	0	*P.trehalosi*	
Farm 7	1	0	0		
Total	26	9	3		

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Table 15. Frequency of *Pasteurella* and *Mannhaemia* species isolated from pneumonic calves by different farms

Farm Name	Bacterial pathogens	
	Pasteurella and *Mannhaeimia* Species	*Haemophilus* species
Farm 1 (12)	5	0
Farm 2 (5)	5	0
Farm 3 (2)	1	0
Farm 4 (2)	0	0
Farm 5 (0)	0	0
Farm 6 (4)	1	0
Farm 7 (1)	0	0
Total (26)	12	0

4.8. Antimicrobial Susceptibility test for *Salmonella*, *E.coli*, *Pasteurella* and *Mannhaemia* isolates

Antimicrobial susceptibility test was performed for the bacterial isolates. Ten antimicrobial impregnated discs (OXOID) were used for *Salmonella* and *E.coli* isolates and eight for *Pasteurella* and *Mannhaemia* species (Tables 16, 17, and 18 and appendices 6A, 6B and 6C). Multi drug resistance was seen in all of the bacterial isolates.

Table 16. Antimicrobial susceptibility patterns of *E. coli* isolated from diarrhoiec calves

Antimicrobial	Resistant	Intemediate	Moderately Susceptible	Susceptible	Total
Amoxicillin	86 (83.5 %)	0 (0 %)	13 (12.6 %)	4 (3.9 %)	103 (100%)
Streptomycin	70 (68.0 %)	13 (12.6 %)	0 (0 %)	20 (19.4 %)	103 (100%)
Norfloxacin	0 (0 %)	0 (0 %)	0 (0 %)	103(100 %)	103 (100%)
Gentamycin	2 (1.9 %)	3 (2.9 %)	0 (0 %)	98 (95.1 %)	103 (100%)
Trimethoprin Sulphamethoxazole	12 (11.7 %)	1 (1.0 %)	0 (0 %%)	90 (87.4 %)	103 (100%)
Erythromycin	65 (63.1 %)	27 (26.2 %)	0 (0 %)	11 (10.7 %)	103 (100%)
Tetracyclin	77 (74.8 %)	5 (4.9 %)	1 (1.0 %)	20 (19.4 %)	103 (100%)
Ampicillin	36 (35.0 %)	6 (5.8 %)	0 (0 %)	61 (59.2 %)	103 (100%)
Clindamycin	101 (98.1%)	1 (1.0 %)	0 (0 %)	1 (1.0 %)	103 (100%)
Kanamycin	13 (12.6 %)	1 (1.0 %)	0 (0 %)	89 (86.4 %)	103 (100%)

The proportion shows that 98.1% of *E. coli* isolates were resistant to clindamycin, 1 % intermediate and 1 % susceptible. Similarly, 83.5 % of the isolates were resistant to amoxicillin, 12.6 % moderately susceptible and 3.9 % susceptible. In the case of tetracyclin 74.8% were resistant, 4.9 % intermediate, 1 % moderately susceptible and 19.4 % of them were susceptible, while 68 % and 63.1 % of the isolates were resistant to streptomycin and erythromycin, respectively.

Table 17. Antimicrobial susceptibility patterns of *Salmonella* isolated from diarrhoeic calves

Antimicrobial	Resistant	Intermediate	Moderately Susceptible	Susceptible	Total
Amoxicillin	24 (88.90%)	0 (0%)	2 (7.40%)	1 (3.70%)	27 (100%)
Streptomycin	10 (37.00%)	7 (26.00%)	0 (0%)	10 (37.00%)	27 (100%)
Norfloxacin	0 (0%)	0 (0%)	0 (0%)	27 (100.00%)	27 (100%)
Gentamycin	0 (0%)	0 (0%)	0 (0%)	27 (100.00%)	27 (100%)
Trimethoprin Sulphamethoxazole	5 (18.50%)	3 (11.1%)	0 (0%)	19 (70.40%)	27 (100%)
Erythromycin	24 (88.90%)	2 (7.40%)	0 (0%)	1 (3.70%)	27 (100%)
Tetracyclin	10 (37.00%)	25.90%	0 (0%)	10 (37.00%)	27 (100%)
Ampicillin	15 (55.60%)	2 (7.40%)	0 (0%)	10 (37.00%)	27 (100%)
Clindamycin	27 (100.0%)	0 (0%)	0 (0%)	0 (0%)	27 (100%)
Kanamycin	6 (22.22%)	0 (0%)	0 (0%)	21 (77.78%)	27 (100%)

All (100 %) of *Salmonella* isolates showed resistance to clindamycin and 88.9 % of the isolates showed resistance to amoxicillin and erythromycin, which had 3.7 % of them susceptible to both antimicrobials. Streptomycin and tetracycline, on the other hand were resisted by 37 % of the isolates having the same proportion of bacterial isolates being susceptible.

Table 18. Antimicrobial susceptibility patterns of *Pasteurella and Mannhaemia* isolated from pneumonic calves

Antimicrobial	Resistant	Intemediate	Moderately Susceptible	Susceptible	Total
Streptomycin	6 (50.0 %)	2 (16.7 %)	-	4 (33.3 %)	12 (100 %)
Norfloxacin	1 (8.3 %)	-	-	11 (91.7 %)	12 (100 %)
Gentamycin	-	-	-	12 (100.0 %)	12 (100 %)
Erythromycin	3 (25.0 %)	2 (16.7 %)	-	7 (58.3 %)	12 (100 %)

Tetracyclin	2 (16.7 %)	2 (16.7 %)	-	8 (66.7 %)	12 (100 %)
Ampicillin	1 (8.3 %)	1 (8.3 %)	-	10 (83.3 %)	12 (100 %)
Neomycin	-	-	-	12 (100 %)	12 (100%)
Chloramphenicole	-	-	-	12 (100 %)	12 (100 %)

Pasteurella and *Mannhaemia* species isolated were all (100 %) susceptible to gentamycin, neomycin and chloramphenicole and 91.7 % were susceptible to norfloxacillin The highest resistance of antimicrobial agent by ***Pasteurella and*** *Mannhaemia* isolates was observed in streptomycin, in which 50 % of the isolates developed resistance.

The antimicrobial susceptibility pattern in ***Pasteurella and*** *Mannhaemia* species is summarized in table 19.

Table 19. Antimicrobial susceptibility pattern of *Pasteurella* and *Mannhaemia* species

	TE		S			AMP		N	CH	E				
Bacterial species	R	S	R	I	S	R	S	S	S	R	I	S	R	S
P. trehalosi (6)	1	5	2	0	4	1	5	6	6	0	1	5	6	0	6
P. aerogenes (1)	0	1	0	0	1	0	1	1	1	1	0	0	1	0	1
P. lymphangitidis (2)	0	2	2	0	0	0	2	2	2	1	0	1	2	0	2
M. haemolytica (3)	1	2	0	1	2	0	3	3	3	0	0	3	3	1	2

Keys: R- Resistant, I- Intermediate, S- Susceptible, numbers in bracket are the total number of the isolates.

All of the isolates were susceptible to neomycin, chloramphenicole and gentamycin. ***Mannhaemia*** *haemolytica* isolates were susceptible to ampicillin, neomycin, gentamycin, erythromycin and chloramphenicole. *Pasteurella lymphangitidis* isolates on the other hand were resistant to streptomycin.

5. DISCUSSION

The present study attempted to identify the incidence of calf diarrhoea and pneumonia. Investigations of the potential risk factors which could influence the occurrence of calf diarrhoea and pneumonia, detection and isolation of the etiological factors were also done. Further, determination of the antimicrobial susceptibility patterns of the bacterial isolates from the clinical cases was carried out. In addition, the calf mortality rates mostly due to diarrhoea and pneumonia were performed. The study mainly focused on intensively managed dairy farms in some selected parts of the country, where there was large number of calves and major complaints about calf diarrhoea and pneumonia. Temesgen (2004) reported that there were small numbers of calves (average 2 calves per farm) in small and medium scale market-oriented dairy farms, which make the number of calves for the study to be minimal. Therefore, in order to solve the problem of limited number of calves for prospective study, farms with a large number of calves were considered.

Unlike other studies in the country, which focus mainly on the identification of bacterial or parasitic causes of calf diarrhoea, this research was designed to investigate bacterial, viral and protozoal causes of diarrhoea and pneumonia and antimicrobial susceptibility of the bacterial isolates from diarrhoeic and pneumonic calves.

5.1 Description of farms

The farms that were included in this study had almost similar managmental practices. Due to this the analysis was merely based on limited number of risk factors. Since all the farms had at least one professionally experienced manager, risk factors like time of first colostrum feeding, frequency of feeding on the first day of life, pen hygiene were easily gathered using a pre-tested questionnaire.

The interview with the farm managers revealed that the major health problems of calves were mainly calf diarrhoea and pneumonia similar to the report of Temesgen (2004). Due to these diseases, there were high mortality rates recorded in some of the farms particularly in one of the farms at Holeta. But, no investigations on the causes had been done previously in this and other farms.

There were significant variations among and within farms in some management practices for example in vitamin supplementation, colostral feeding and disinfection of the calf pens. Two methods of colostrum feeding were practiced, namely suckling and bucket feeding. Disinfection of calf pens was practiced only in one of the farms. The calf caretakers were only females in 42.8%, males 28.6 % and both males and females in 28.6 % of the farms.

5.2. Cumulative incidences of Calf diarrhoea and Pneumonia and calf mortality rate

The CI of calf diarrhoea in Holeta, Muke turi and Debre Zeit dairy farms were 0.41, 0.63 and 0.37, respectively. The incidence that was recorded in Holeta was comparable to that reported by Temesgen (2004) but higher than that reported in Demissie (2007), while the CI of calf diarrhoea in Debre Zeit was lower than previous reported (Temesgen, 2004) and slightly lower than reported by Demissie (2007). On the other hand, CI of calf diarrhoea at Muke Turi dairy farm was much higher than in the other study farms. In a study conducted by Svensson *et al.* (2006) in Sweden, the CI of diarrhoea was 0.63, which was similar to the one in this study at Muke Turi. The higher CI of calf diarrhoea obtained in the present study may be due to the other factors not included in the analysis like the absence of vaccination against enteropathogens and other related managemental practices (Lorino *et al.*, 2005).

The calculated CI of calf pneumonia was higher than at reported by Temesgen (2004) who reported only 0.049. Poor management in colostrum feeding of calves may predispose them to gut, respiratory, systemic and other infections. Such infections result in the development of diarrhoea, pneumonia, pleurisy, septicaemia and other conditions due to weak immunity (Mellor and Stafford, 2004). In addition, large herd size might have contributed to the high incidence of calf pneumonia, because as the number of calves in the pen increases, the amount of ammonia produced increases too, which predisposes calves to pneumonia (Bogale, 1999).

The calf mortality rate recorded in the present study was higher than the economically tolerable 5 % rate. This finding is in agreement with Temesgen (2004), Demissie (2007) and Gulima (2008), who also reported a calf mortality rate higher than the economically tolerable limit. In the mentioned studies, they have reported higher incidence of calf diarrhoea followed by pneumonia except in the report of Gulima (2008), who reported the reverse. They have indicated that these diseases contributed much to the high mortality. The high mortality rate observed in the present

study might also be due to the high cumulative incidences of these diseases. The highest calf mortality was observed in calves between the age-group of 31-60 days, which is comparable to the report by Gulima (2008), who reported high mortality rate in calves aged between 31-90 days.

5.3. Potential risk factors associated with calf diarrhoea and pneumonia

Risk factors postulated to positively influence the occurrence of diarrhoea and pneumonia were investigated. Based on the results, some factors were found to be significantly associated but others were not, although biologically they were hypothesized to be associated with the occurrence of these diseases. One of the factors that significantly was associated both diarrhoea and pneumonia was the ages of calves. According to the result of the present study, young calves were at a significantly high risk of being affected with diarrhoea (HR = 6.586 for 0-30 days, 2.077 for 31-60 days and 3.225 for 61-90age group of calves) compared to calves greater than 90 days old. This finding compared well with the findings of Temesgen (2004). Trotz-Williams *et al.* (2007) and Lorino *et al.* (2005) also reported that calves aged between 0 – 30 days were at great risk of diarrhoea, particularly during the first week of life, and this risk decreases with age. This is well explained by Godden (2007) and Mellor and Stafford (2004) who reported that the structure of the bovine placenta impedes easy acquisition of immunoglobulins by unborn calves during pregnancy and therefore calves are borne without circulating protective antibodies. So they are more susceptible to different pathogens. In addition, Gulliksen *et al.* (2007) explained that calves are agammaglobulinemic at birth, which makes them more susceptible to diseases. The age-dependent difference in pathogenicity of bacteria as mentioned by Lee *et al.* (2007) might be due to age-related differences in the development of rumen function, where a combination of a high concentration of volatile fatty acids and a low pH inhibits the growth of the organism as well as the immune response.

Breed in the present study was found to be significantly associated with the hazard rate of developing diarrhoea. Similar to this finding, Weaver *et al.* (2000) described in their review that Holstein Friesians have lower immunoglobulin secretion as compared to other breeds, which makes these breeds susceptible to different diseases.

The gender of calf caretakers when analyzed with the multivariable Cox regression, showed significant association between the hazard rates of diarrhoeal development in only female and

only male calf takers as compared to calves managed with male and female calfcaretakers. There is no previous report to compare the present result with. But previous study by Ibrahim (2007) indicated that the presence of attendant during birth of calves had significant interaction with calf morbidity. The reason for the lower incidence of disease in farms having female and male calf caretakers in the present study might have also been due to labor division particularly in critical times of birth, which require special attention of calves and their dams.

Vitamins, particularly Vitamin E influences both the cellular and the humoral immune function. Vitamin E positively influences cellular immune system by increasing production of the T-helper cells leading to an increased function of the cellular immunity. The effect on the humoral immune system is by increasing production of antibody producing B-cells, apparently in collaboration with the T-helper cells in the initial phase of the immune response. Due to this fact, vitamin supplementation was included in this study analysis. However, it was found to be insignificant. This finding contrasted the finding of Jensen *et al.* (2007), who has reported that calves have higher disease resistance if they get sufficient amounts of vitamin E. The difference could have arised from the amount and the quality of the vitamin E supplemented in the calf feeds and also the small number of study calves, 86 (20 %) given this supplement.

In the present study, method of colostrum feeding was not significantly associated with the incidence of diarrhoea which differs from the work by Selman *et al.* (1971), who reported significantly higher average serum immunoglobulin concentrations from calves that were left for long with their dams. However, a review by Weaver *et al.* (2000) points to the fact that all calves that receive sufficient quantities (2 L) of colostrum may have effective passive immunity. However no difference in the rates of IgG absorption nor the final serum IgG concentration in bottle-fed calves was significantly different from that of the naturally suckled calves. It is also indicated that the risk of failure of passive immune transfer is greater in naturally suckled calves because of intake of inadequate colostrum volume and IgG and the mothering effect does not provide suitable gain to advocate leaving calves with the dam.

The risk factors, which were analyzed for their association with diarrhoea, were also analyzed for calf pneumonia. But they were not associated with the occurrence of pneumonia. The difference could be due to the small number of pneumonic cases which could affect the results of the

analysis as a result of few degrees of freedom and poor power of the statistical test. The mean survival times for the occurrence of pneumonia is different among different age-groups. But in the Cox regression analysis, age turned out to be not significantly associated with the occurrence of pneumonia. Previous study by Hägglund (2005) however indicated the relationship between calf pneumonia and age of calves. It is suggested as, due to the fact that many of the disease-causing pathogens are ubiquitous and adult animals are often immune, bovine respiratory disease occurs most frequently in young calves, during the period between passive and active immunity (Hägglund, 2005). The results of a study by Svensson *et al.* (2006) underlined the importance of diarrhoea as a risk factor for CRTD in older calves. They have indicated that the association may be linked to an immunosuppressive role of diarrhoea or to common predisposing factors, such as inadequate colostrum management. Previous studies reported higher incidence of pneumonia in older calves than young ones. But a study conducted in Sweden as well as in the USA indicated that, young calves are most often housed individually while older calves are generally kept in groups. It is reasonable to believe that it is more difficult for the caretaker to recognize disease in animals kept in groups and this may contribute at least to some extent to the lower incidence reported in older calves (Svensson *et al.*, 2006).

5.4. Microbial Pathogens associated with calf diarrhoea and Pneumonia

In this study, the major pathogens associated with calf diarrhoea were detected and isolated. Similar to the result obtained by de la Fuente *et al.* (1998), mixed infections were much more commonly detected in diarrhoeic calves. These authors suggested that the presence of more than one enteropathogen may be one of the factors determining whether an infection results in a clinical or subclinical disease. On the other hand, mixed infections may be associated with more severe disease (de la Fuente *et al.*, 1998). In contrast to Demissie (2007), who reported *C. parvum* as the only pathogen detected concurrently with other pathogens, in the present study, all of the pathogens occurred concurrently.

According to the present finding, the antigenic ELISA test, *C. parvum* was the leading cause of calf diarrhoea having a frequency of isolation of 58 (detected in 51.79 % of the diarrhoeal cases) followed by rotavirus 22 (19.64 %). This finding is comparable to that reported by Geurden *et al.* (2007) with 63 % and Demissie (2007) with 63.9%. But the present finding is much higher than

that reported by Abraham *et al.* (1992), Huetink *et al.* (2001) and (2002) and Temesgen (2004) with 0 % in central highlands of Ethiopia, 7.2 % in and around Debre Zeit and 22.2 % in the Netherlands, respectively. The differences could be due to the managemental practices which increase the opportunity for exposure to these organisms, sampling procedure analysis, the sample sizes used and disease predisposing factors among different study areas.

Rotavirus was the second most abundant viral pathogen detected by ELISA, which is contrary to the finding of Demissie (2007) who reported that rotavirus had the least frequency of occurrence. However, this study finding was similar to that reported by Marsolais *et al.* (1978), Abraham *et al.* (1992) and de la Fuente *et al.* (1998) who found it to be 14%, 16.7 % and 17.9%, respectively. Finding of coronavirus of 5.36 % was much lower than reported by Marsolais *et al.* (1978) of 53 % and Abraham *et al.* (1992) of 38.9 % but lower than reported by Demissie (2007), which was 2.8 %. The difference could have arised from the method of sample analysis (some used electron microscopy, FAT) used by the researchers.

A 24.11 % of *Salmonella* species greatly differs from Abraham *et al.* (1992), who did not isolate *Salmonella* species from all diarrhoeic calves. It was higher than the results by and Temesgen (2004) and Demissie (2007) who reported an isolation rate of 2.6 % and 16.7 %, respectively. *Salmonella* infection of a farm is maintained by transmission of the agent from the faeces of infected animals to susceptible animals, which is fecal-oral route. The epidemiology of salmonellosis is primarily the epidemiology of fecal pollution (Gay, 1999). Therefore, in the present study, since the calves had access to contaminate the water (Picture 2), it may be one reason for the high prevalence. Bischoff *et al.* (2004) reported a higher proportion of *Salmonella* species from calves. But in this case, only one farm was sampled and the results might have represented an extreme situation.

Previous studies (Abraham *et al.*, 1992; Demissie, 2007) in the country focused only on the *E. coli* species with the K 99 antigen although no toxigenic genes were detected, *E. coli* strains other than the K 99 were also isolated to perform serotypic identification and toxigenic gene detection. Determination of the serotype may help in confirming the hypothesis that the toxins could be aggravating factors (China *et al.*, 1998). But due to the shortage of time in carrying out laboratory analysis, this was not done. Despite the evidence for a bovine reservoir of VTEC O157 (Porter *et*

al., 1997; Manna *et al.*, 2006), little is understood about the ecology of the organism in farms in our country. Similar authors reported a higher faecal load of *E. coli* O157. In addition, STEC strains besides producing certain life threatening diseases in humans are also capable of causing diarrhoea in calves (Wani *et al.*, 2003; Wani *et al.*, 2005; Manna *et al.*, 2006). Based on that, investigation aimed at generally identifying *E. coli* was performed. The present finding of *E. coli* species with a proportion of 91.96 %, which do not posses the K 99 antigen, was the highest of all the microbial pathogens detected and isolated. Nevertheless, it was comparable to that reported in buffalo by Ribeiro *et al.* (2000), in Brazil. The objective of screening of different bovine breeds in different farms (dairy or meat) was to establish precisely the identity and prevalence of virulence factors associated with colibacillosis (NCD), which could assist in revealing the actual magnitude of the problem caused by different strains of *E.coli* (e.g. VTEC, ETEC, etc). This would provide an important epidemiological data about this disease and also give an early warning regarding any outbreaks in future (Arya, 2005).

Pasteurella and *Mannhaemia* species were identified from calves with clinical signs of respiratory disease. The *Pasteurella* and *Mannhaemia* species were isolated from 12 (42.86 %) pneumonic calves in the present study. Even though these bacteria are normal flora of the respiratory tract, according to DeRosa *et al.* (2000), a nasal culture of a clinically ill animal can be predictive of the pathogen in the lungs and is genetically identical in most of the time. Moreover these bacteria are reported to be the most pathogenic in different investigations (Miflin and Blackall, 2001; Norcia *et al.*, 2004). The results of this study suggest that a nasal swab culture can be a useful diagnostic tool that can be applied in guiding the specific bacterial pneumonic therapy and especially with regard to antibiotic susceptibility (DeRosa *et al.*, 2000). The negative result for nasal bacterial culture in the pneumonic calves in the present study may not be a true representative of the healthy status of the calves, in that other pathogens, viral and / or other bacterial species might be involved (E. g. BRSV, Tuberclosis) (Bogale, 1999; Berghaus *et al.*, 2006).

The current study did not find *Haemophilus somnus* in the calves with clinical respiratory disease. Since there is no previous study on this bacterium in the country, comparison could not be made. The reason for the failure in the isolation of this bacterium might be due to the absence of selective media as recommended by Slee and Stephens (1985) and Ward *et al.* (2006), which

involves the incorporation of vancomycin (5 micrograms/ml), neomycin (5 micrograms/ml), sodium azide (50 micrograms/ml), nystatin (100 iu/ml) and cyclohexamide (100 micrograms/ml) into 5 per cent horse blood agar, which results in a selective medium for the primary isolation of *Haemophilus somnus* from cattle and sheep. Due to the absence of these supplements, the bacterium could not be isolated. In addition, the transport media and the time of processing the samples, which was 48 hrs might have destroyed the bacterium and result in the failure to detect it. A research undertaken on the nasopharyngeal bacterial flora of Rhesus Macaques by Bowers *et al.* (2002) indicated that *Haemophilus* species were very prevalent from pharyngeal swabs but not present in nasal swabs. Since the samples in the present study were nasal swab samples, this may be another reason for not detecting the bacterium in the calves.

5.5. Antimicrobial sensitivity test

Antimicrobial susceptibility test was performed to all of the bacterial isolates. Almost all of the bacterial isolates were susceptible to Norfloxacillin, Gentamycin and Kanamycin and resistant to clindamycin. On the other hand, most of the *E. coli* isolates were resistant to amoxicillin, Tetracyclin, streptomycin and erythromycin. These results are similar to those of Werckenthin *et al.* (2002) and Aksoy *et al.* (2007), who reported high resistance rates of *E. coli* to many antimicrobial agents. This bacterium is part of the normal faecal flora and hence used as a potential indicator for resistance trends in men and animals Werckenthin *et al.* (2002). *E. coli* isolates (98.1 %) were resistant to Clindamycin. Antibiotic use leads to resistance in pathogenic bacteria as well as the development of resistant strains in flora bacteria.

The resistance that develops in flora bacteria may be transferred to other bacteria and infect humans through direct or indirect routes (meat and meat-by-products). In particular, resistant strains form associations with the antibiotics used in veterinary medicine (tetracycline, streptomycin, ampicillin, trimethoprim-sulfamethoxazole, enrofloxacin, etc.) (Aksoy *et al.*, 2007). Information about the prevalence of antibiotic resistance in commensal enteric bacteria is of interest because these bacteria are potential indicators of selection pressure on enteric bacteria and represent reservoirs of resistance genes in potentially pathogenic bacteria (O'Connor *et al.*, 2002)

The antimicrobial sensitivity patterns of the *Salmonella* isolates in this study is in agreement with van Duijkeren *et al.* (2003), Murugkar *et al.* (2005), Sibhat (2006) and Demissie (2007), who reported resistance to different antimicrobial agents like erythromycin, streptomycin, tetracycline, etc. Least resistance by these isolates was observed in norfloxacin and gentamycin. The finding compares well with the observations of other researchers (Murugkar *et al.*, 2005; Sibhat, 2006). Murugkar *et al.* (2005) indicated that Fluoroquinolone group (E.g. Norfloxacillin) of antibiotics have rapid and prompt bactericidal action at a very low minimum inhibitory concentration against *Salmonellae*. However, caution is warranted against their indiscriminate usage as evidenced from the resistance problems faced by many developed countries, where more than a ten-fold increased resistance has been observed against the quinolones in a two decade study reference. Excessive or inappropriate use of antibiotics in the rearing of farm animals represents a major factor in the emergence, persistence and spread of resistant *Salmonellae* even in the humans who are the *cul-desac* of the food chain. Hence, it is imperative that judicious use of antibiotics in the treatment and prophylaxis, after *in vitro* testing, be practiced to sustain the usefulness of the antibiotics in controlling salmonellosis on long-term basis (Murugkar *et al.*, 2005). The high prevalence *Salmonella* resistant to many commonly used therapeutic drugs on that farm of is exemplary of the animal health problems faced by producers.

In the antimicrobial sensitivity test for *Pasteurella* and *Mannhaemia* species, there was development of drug resistance against different antimicrobials used except for gentamicin and norfloxacillin. Malik *et al.* (2005) reported little or no resistance in a poultry isolate of *Mannhaemia haemolytica* to gentamycin and ampicillin but high resistance to penicillin and tetracycline similar to the present finding, while their finding on the absolute resistance of erythromycin is contrary to the present finding of the calf isolate.

6. CONCLUSIONS AND RECOMMENDATIONS

High CI of diarrhoea was found in the different study areas. Diarrhoea represented an increasing and recurrent problem in young calves. It remains an important cause of morbidity and mortality in dairy calves. The CI of calf pneumonia was also high particularly in one of the study areas contributing to the increased morbidity and mortality of calves in the area.

In the investigation of potential risk factors for the occurrence of NCD and RDC, age of calves was found to be significantly associated with the hazard of diarrhoea and pneumonia. Young calves were found to be more susceptible to the occurrence of diarrhoea and pneumonia. But sex of calves, breed, vitamin supplement, etc were not significantly associated with the occurrence of diarrhoea.

Five enteropathogens, rotavirus, coronavirus, *C. parvum, E. coli* and *Salmonella* species involved in causing diarrhoea were detected and isolated. Therefore, it is concluded that the viral, bacterial and protozoal causes of calf diarrhoea are also contributing to the occurrence of NCD in the present study areas. *Cryptosporidium parvum* was detected at a higher rate than others. In most of the cases, mixed infections were common. Similarly, *Pasteurella* and *Mannhaemia* species were also isolated from calves with pneumonia.

The antimicrobial susceptibility test result indicated an increased antimicrobial resistance to different antimicrobial agents tested. But from all the drugs, gentamycin and norfloxacillin were effective in all tested bacterial species. Majority of the isolates were resistant to clindamycin.

Therefore based on these conclusions, the following recommendations were reached:

- The high CI of diarrhoea highlights the need for a long-term longitudinal epidemiological and microbiological investigations of calf diarrhoea that would result in designing and implementing cost-effective and appropriate prevention and control strategies
- Because of the large number of etiological agents, the prevention of neonatal diarrhoea is difficult but should be centered around improving calf management practices like optimizing colostral transfer of passive immunity and improved treatment protocols are required for calf diarrhoea and pneumonia

- The bacterial strains identified revealed high resistance rates to antibiotics, which indicates the need for greater scrutiny in the usage of antibiotics
- The feeding and watering troughs should be constructed in such a way that calves should not have access to enter into the troughs so as to reduce the faeco-oral route of transmission of infectious agents
- The development and application of effective vaccines offers a potential means of reducing losses due to calf diarrhoea, pneumonia, in addition to public health risks from zoonoses by enhancing pathogen specific immunity
- ORT should be used instead of antibiotics for calves especially with NCD for effective response and reduction of antimicrobial resistance. To solve the problem of bacterial resistance tot antibiotics, use of many antibiotics should be limited. Where bacterial transmissibility is high, the importance of simple infection control measures (e.g. adequate management practices) should be practiced.

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8. APPENDICES

Appendix 1. Questionnaire format

1. Farm description

1.1. 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

1.2. Herd size: Cows ....., Bulls ....., Heifers .....

Male calves ....., Female calves .....

1.3. Breed and age of animals kept

1.4. Age of the farm

1.5. The farm as a source of income: a) Primary income

b) Secondary income

2. Management data

2.1 Calf caretakers (attendants)

2.1.1. Sex: a) Male

b) female

2.1.2. Experience: a) ≤ 5 years

b) > 5 years

2.2. Periparturient care

2.2.1. Calving facilities: a) Calving pen

b) The same barn

2.2.2. Navel treatment: a) Practiced

b) Not practiced

2.2.3. Awareness of importance of colostrums 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

2.3. Feeding

2.3.1. Type of feeding: a) Milk

b) Milk replacer

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

2.3.3. Frequency: a) Once a day

b) Twice a day

c) Three times a day

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

a) Grazing

b) Concentrates

c) Hay

2.3.5. Weaning age

3. Housing

3.3. 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. Bedding: a) Present

b) Absent

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

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

4.3. Major health problems for the farm

4.4. Number of calves the farm lost during the last one year

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

4.6. Measures taken to treat sick calves

4.7. Measures taken to prevent disease problems

Appendix 2. Sample of calf card format

1. Genealogy and periparturient care

1.1. Date of birth: Day ……..……Month……..Year…….

1.2. Site of birth:
a) The same cow barn
b) Calving pen

1.3. Condition of birth:
a) Normal delivery
b) Dystocia

1.4. Sex:
a) Male
b) Female

1.5. Exotic blood level:
a) < 50%
b) 50 – 75%
c) > 75%

1.6. Navel disinfection:
a) Yes
b) No

1.7. Chemical used in navel disinfection

1.8. Time of colostrums ingestion:
a) ≤ 6 hrs
b) > 6 hours

1.9. Method of colostrums feeding:
a) Bucket feeding
b) Suckling

1.10. 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

Appendix 3. Result recording format

A. Data recording format for_____ dairy Farm

No.	Calf ID	Birth date	Date Of sample collection	Age (Days)	Sex	Sample Type	ELISA				*E.coli*	*Salmon*	*Past.*	*Haem*	Death (Date)	General remark
							E.coli K_{99}	*C.parvum*	Rota	Corona						
1																
2																
3																
4																
5																
6																
7																
8																
9																
10																
11																
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18																
19																
20																
21																
22																

B. Isolation and identification of *E.coli*

No.	Farm	Calf ID	Age	Sex	MacConkey (Pink/Straw)	EMB Agar (MS/ NMS)	IMViC				Xylose	Sucrose	Urease	LIA			TSI				General remark
							Indole	MR	VP	Citrate				Slant	Butt	H_2S	Slant	Butt	H_2S	Gas	
1																					
2																					
3																					
4																					
5																					
6																					
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23																					

C. Isolation and identification of *Salmonella* Species

No.	Farm	Calf ID	Age (Days)	Sex	XLD Agar	SS Agar	SIM Medium		Urease	Voges Proskauer	TSI				LIA			Remark
							H_2S	Indole			Slant	Butt	H_2S	Gas	Slant	Butt	H_2S	
1																		
2																		
3																		
4																		
5																		
6																		
7																		
8																		
9																		
10																		

D. Isolation and identification of *Pasteurella* Species

No.	Farm Name	Calf ID	Haemolysis	Chocolate Agar	Catalase	Oxidase	MacConkey			Urease	Glucose	Maltose	Sucrose	Lactose	D-xylose	Mannitol	Ornith. Decarb.	Trehalose	L-arabin	Sorbitol	Remark
								Indole	S I M												
								H_2S													
1																					
2																					
3																					
4																					
5																					
6																					
7																					
8																					
9																					
10																					

Appendix 4. Questionnaire results of the farms

Risk Factors		Farms						
		Farm 1	Farm 2	Farm 3	Farm 4	Farm 5	Farm 6	Farm 7
Sex of calves	FC	57 (45.2)	46 (52.3)	39 (60.9)	59 (68.6)	9 (47.4)	17 (58.6)	8 (47.1)
	MC	69 (54.8)	42 (47.7)	25 (39.1)	27 (31.4)	10 (52.6)	12 (41.4)	9 (52.9)
Breed	Boran	0 (.0)	17 (19.3)	0 (.0)	5 (5.8)	0 (.0)	0 (.0)	0 (.0)
	CB	0 (.0)	71 (80.7)	64 (100.0)	81 (94.2)	19 (100.0)	29(100.0)	17 (100.0)
	HF	126 (100.0)	0 (.0)	0 (.0)	0 (.0)	0 (.0)	0 (.0)	0 (.0)
Vaccination[a]	No	126 (100.0)	88 (100.0)	64 (100.0)	86 (100.0)	19 (100.0)	29 (100.0)	17 (100.0)
Vitamins and Minerals	No	126 (100.0)	88 (100.0)	64 (100.0)	0 (.0)	19 (100.0)	29 (100.0)	17 (100.0)
	Yes	0 (.0)	0 (.0)	0 (.0)	86 (100.0)	0 (.0)	0 (.0)	0 (.0)
Caretaker	F	0 (.0)	0 (.0)	64 (100.0)	0 (.0)	19 (100.0)	29 (100.0)	0 (.0)
	M	126 (100.0)	0 (.0)	0 (.0)	0 (.0)	0 (.0)	0 (.0)	17 (100.0)
	Both	0 (.0)	88 (100.0)	0 (.0)	86 (100.0)	0 (.0)	0 (.0)	0 (.0)
Disinfection[b]	No	126 (100.0)	88 (100.0)	64 (100.0)	0 (.0)	19 (100.0)	29 (100.0)	17 (100.0)
	Yes	0 (.0)	0 (.0)	0 (.0)	86 (100.0)	0 (.0)	0 (.0)	0 (.0)
Colostrum feeding	Suckling	0 (.0)	17 (19.3)	64 (100.0)	5 (5.8)	19 (100.0)	29 (100.0)	17 (100.0)
	Bucket	126 (100)	71 (80.7)	0 (.0)	81 (94.2)	0 (.0)	0 (.0)	0 (.0)
Time of 1st feeding colostrum	≤ 6 hrs	126 (100.0)	88 (100.0)	64 (100.0)	86 (100.0)	19 (100.0)	29 (100.0)	17 (100.0)
	> 6 hours	0 (.0)	0 (.0)	0 (.0)	0 (.0)	0 (.0)	0 (.0)	0 (.0)
Frequency on the first day	Sufficient ≥3	126 (100.0)	88 (100.0)	64 (100.0)	86 (100.0)	19 (100.0)	29 (100.0)	17 (100.0)
	Insufficient >3	0 (.0)	0 (.0)	0 (.0)	0 (.0)	0 (.0)	0 (.0)	0 (.0)
Feeding concentrate	Yes	126 (100.0)	88 (100.0)	64 (100.0)	86 (100.0)	19 (100.0)	29 (100.0)	17 (100.0)
	No	0 (.0)	0 (.0)	0 (.0)	0 (.0)	0 (.0)	0 (.0)	0 (.0)
Amount of milk fed daily	< 4 liters	0 (.0)	0 (.0)	0 (.0)	0 (.0)	0 (.0)	0 (.0)	0 (.0)
	≥ 4 liters	126 (100.0)	88 (100.0)	64 (100.0)	86 (100.0)	19 (100.0)	29 (100.0)	17 (100.0)
Weaning age	<3 months	0 (.0)	0 (.0)	0 (.0)	0 (.0)	0 (.0)	0 (.0)	0 (.0)
	≥3 months							

Housing condition	Separate pen	126 (100.0)	88 (100.0)	64 (100.0)	86 (100.0)	19 (100.0)	29 (100.0)	17 (100.0)
	Same barn with cows	0 (.0)	0 (.0)	0 (.0)	0 (.0)	0 (.0)	0 (.0)	0 (.0)
House cleanness	Clean	126 (100.0)	88 (100.0)	64 (100.0)	86 (100.0)	19 (100.0)	29 (100.0)	17 (100.0)
	Unclean	0 (.0)	0 (.0)	0 (.0)	0 (.0)	0 (.0)	0 (.0)	0 (.0)
Age of the farm	≤ 5 years	0 (.0)	0 (.0)	0 (.0)	0 (.0)	0 (.0)	0 (.0)	0 (.0)
	> 5 years	126 (100.0)	88 (100.0)	64 (100.0)	86 (100.0)	19 (100.0)	29 (100.0)	17 (100.0)
Farm as a source of income	Primary source	126 (100.0)	88 (100.0)	64 (100.0)	86 (100.0)	19 (100.0)	29 (100.0)	17 (100.0)
	Secondary source	0 (.0)	0 (.0)	0 (.0)	0 (.0)	0 (.0)	0 (.0)	0 (.0)
Experience of calf caretaker	≤ 5 years	0 (.0)	0 (.0)	0 (.0)	0 (.0)	0 (.0)	0 (.0)	0 (.0)
	> 5 years	126 (100.0)	88 (100.0)	64 (100.0)	86 (100.0)	19 (100.0)	29 (100.0)	17 (100.0)
Knowledge on the importance of colostrum	Yes	126 (100.0)	88 (100.0)	64 (100.0)	86 (100.0)	19 (100.0)	29 (100.0)	17 (100.0)
	No	0 (.0)	0 (.0)	0 (.0)	0 (.0)	0 (.0)	0 (.0)	0 (.0)
Knowledge on the optimum age to feed colostrum	Yes	126 (100.0)	88 (100.0)	64 (100.0)	86 (100.0)	19 (100.0)	29 (100.0)	17 (100.0)
	No	0 (.0)	0 (.0)	0 (.0)	0 (.0)	0 (.0)	0 (.0)	0 (.0)
Disinfecting calf area (frequently)	Yes	0 (.0)	0 (.0)	0 (.0)	0 (.0)	0 (.0)	0 (.0)	0 (.0)
	No	126 (100.0)	88 (100.0)	64 (100.0)	86 (100.0)	19 (100.0)	29 (100.0)	17 (100.0)
Cleaning before calving	Yes	126 (100.0)	88 (100.0)	64 (100.0)	86 (100.0)	19 (100.0)	29 (100.0)	17 (100.0)
	No	0 (.0)	0 (.0)	0 (.0)	0 (.0)	0 (.0)	0 (.0)	0 (.0)
Cleaning after calving season	Yes	126 (100.0)	88 (100.0)	64 (100.0)	86 (100.0)	19 (100.0)	29 (100.0)	17 (100.0)
	No	0 (.0)	0 (.0)	0 (.0)	0 (.0)	0 (.0)	0 (.0)	0 (.0)
Dam vaccinated against other agents	Yes	126 (100.0)	88 (100.0)	64 (100.0)	86 (100.0)	19 (100.0)	29 (100.0)	17 (100.0)
	No	0 (.0)	0 (.0)	0 (.0)	0 (.0)	0 (.0)	0 (.0)	0 (.0)

Keys: a- Disinfection of calf pens after diarrhoea, b- vaccination against enteropathogens, FC- Female calves, MC- Male calves, CB- Cross breed, HF- Holstein Friesian, F- Female, M- male (Numbers in Parenthesis are the percentages).

Appendix 5. Cumulative incidences of calf diarrhoea and pneumonia

A. Cumulative incidence of calf diarrhoea and pneumonia in different study areas

Study Area	No. of cases		Calf days at risk		True rate per 6 calf months at risk		Cumulative incidence	
	D	P	D	P	D	P	D	P
Holeta	58	17	20072	23552	0.520	0.130	0.41	0.12
Muke Turi	17	2	3073	4017	0.996	0.090	0.63	0.09
Debre Zeit	37	7	14633	16777	0.455	0.075	0.37	0.07
Total	112	26	37778	44366	0.534	0.105	0.41	0.10

*Key: D - Diarrhoea, P – Pneumonia

B. Cumulative incidence of calf diarrhoea and Pneumonia by different farms

Farm	No. of cases		Calf days at risk		True rate per 6 calf months at risk		Cumulative incidence	
	D	P	D	P	D	P	D	P
Farm 1	51	12	8730	12070	1.052	0.179	0.65	0.16
Farm 2	7	5	11342	11482	0.111	0.078	0.11	0.08
Farm 3	17	2	3073	4017	0.996	0.090	0.63	0.09
Farm 4	19	2	7571	9265	0.452	0.039	0.36	0.04
Farm 5	9	0	2004	2255	.808	0	0.55	0
Farm 6	4	4	2862	2944	0.252	0.245	0.22	0.22
Farm 7	5	1	2196	2313	0.410	0.078	0.34	0.08

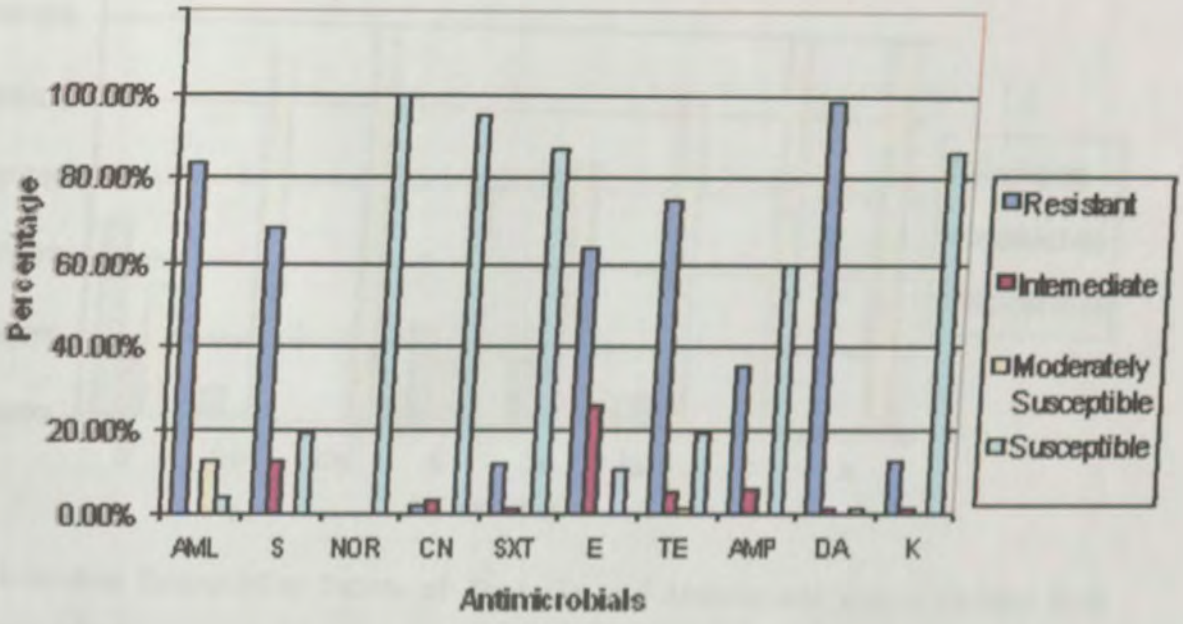
C. Calf mortality rate in different study areas

Study Area	Number of calves died	Calf-days-at-risk	True mortality rate per 6 calf months at risk	Cumulative Mortality
Holeta	27	29210	0.166	0.15
Muke Turi	5	5329	0.169	0.16
Debre Zeit	2	20370	0.018	0.02

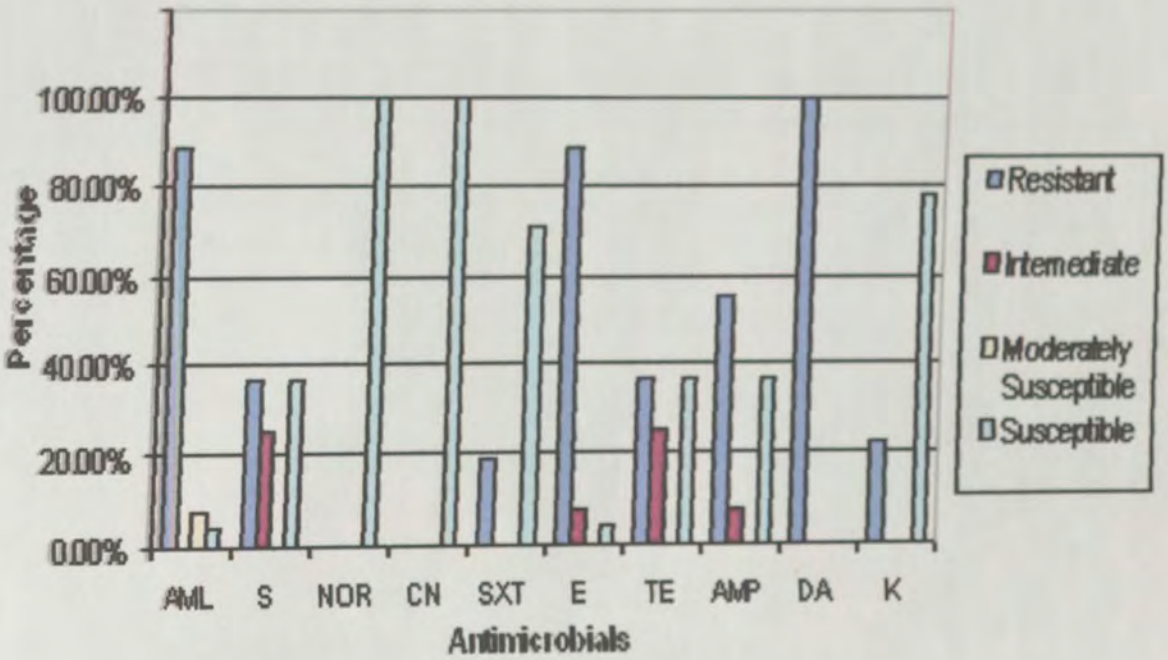
D. Calf mortality rate by different farms

Farms	Location	Mortality	Calf days at risk	True rate per 6calf months at risk	Cumulative incidence
Farm 1	Holeta	26	15820	0.296	0.26
Farm 2	Holeta	1	13390	0.027	0.03
Farm 3	Muke Turi	5	5329	0.169	0.16
Farm 4	Debre Zeit	0	11481	0	0
Farm 5	Debre Zeit	1	2453	0.073	0.07
Farm 6	Debre Zeit	1	4077	0.044	0.04
Farm 7	Debre Zeit	0	2359	0	0

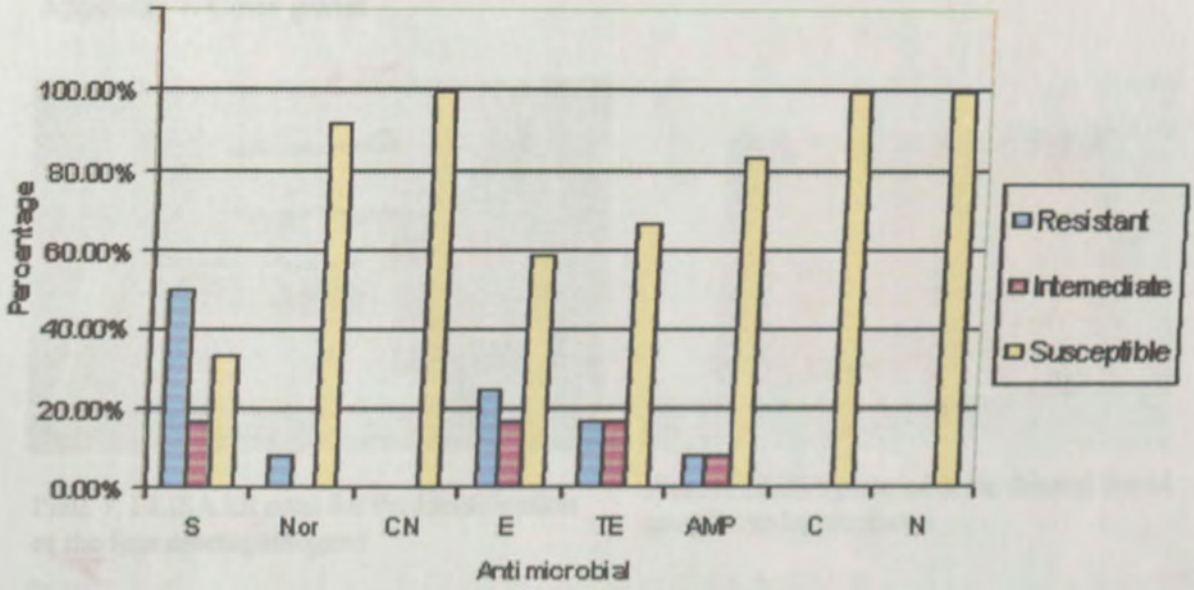
Appendix 6. Antimicrobial susceptibility Patterns of different bacterial pathogens



A. Antimicrobial Susceptibility Pattern of *E. coli* isolated from diarrhoiec calves



B. Antimicrobial Susceptibility Pattern of *Salmonella* isolated from diarrhoiec calves



C. Antimicrobial Susceptibility Pattern of *Pasterella and Mannhaemia* species isolated from pneumonic calves

Appendix 7. Color plates

Plate 1. ELISA kit used for the identification of the four enteropathogens

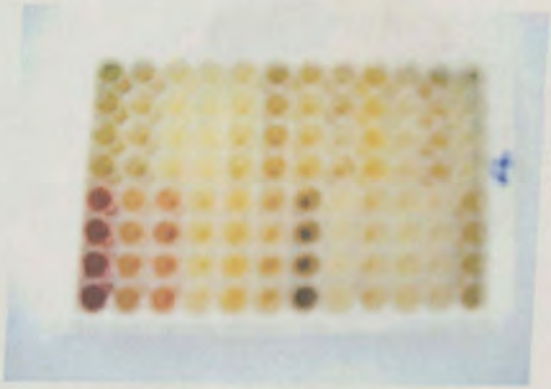


Plate 2. ELISA plate with the diluted faecal samples to be incubated

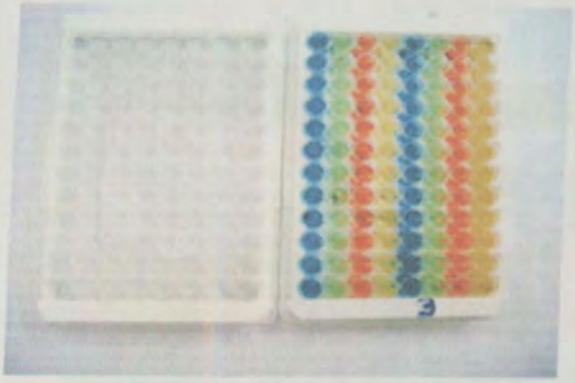


Plate 3. ELISA plate uninoculated (left) and a plate with conjugates (right)(from right to left, anti- *E.coli*, anti-Crypto., anti-rota and anti corona)

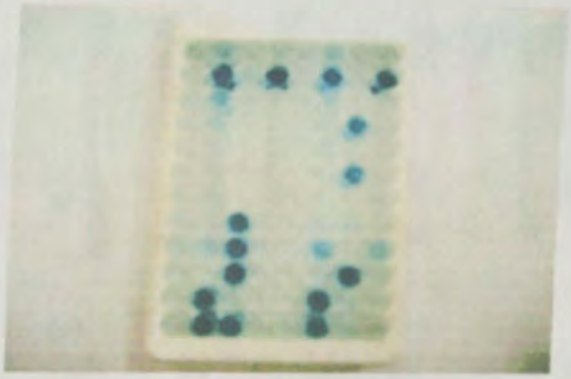


Plate 4. Blue plates indicating positive samples

Plate5. Pink *E. coli* colonies on MacConkey agar

Plate 6. Characteristic Metallic sheen appearance of *E. coli* on EMB agar

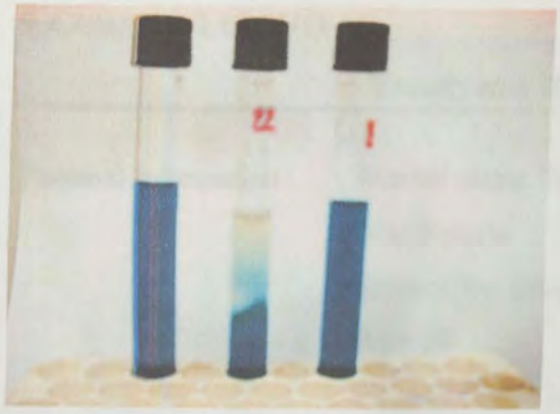


Plate7. Left un inoculated, middle, a possible *Salmonella* growth in MSRV broth after overnight incubation and left no growth

Plate 8. *Salmonella* suspected isolates on SS agar (transparent colonies with black center)

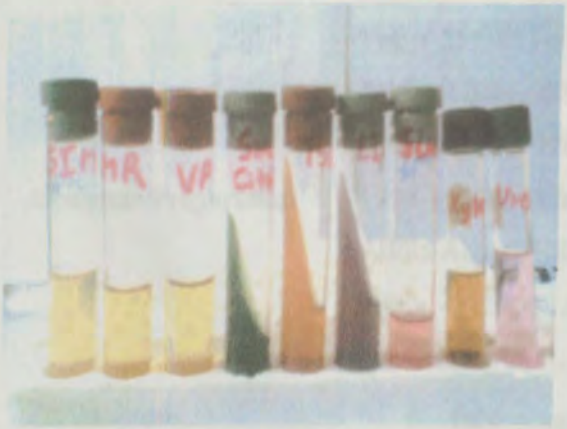
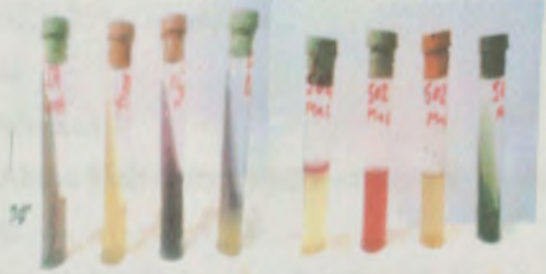


Plate 9. Biochemical tests performed for isolation of *E. coli* and *Salmonella* species



(a) b)

Plate 10: (a) Plate. LIA agar from left to right: uninoculated, Negative, Positive, positive

(b) IMViC Test result typical of *E. coli* species (+, +, -, -) from left to right

(a) (b)

Plate 11. TSI agar inoculated with *Salmonella* (a) and *E. coli* (b)

Plate 12. Antimicrobial susceptibility test for the bacterial isolates

9. CURRICULUM VITAE

Yenehiwot Berhanu Welde Aregay

Personal Information

Marital status: Single

Sex: Female

Nationality: Ethiopian

Age: 28

Place of birth: Gondar, Ethiopia

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Academic Preparation

1998 -2000: Addis Ababa University Faculty of Natural Science, Addis Ababa, Ethiopia

2000 – 2004 Addis Ababa University Faculty of Veterinary Medicine (AAU, FVM), Debre Zeit, Ethiopia; **DVM**

Professional memberships

- Ethiopian Veterinary Association
- Society of Animal Welfare- Ethiopia

Professional experience

2003 – 2005:

- Abattoir Experience in Addis Ababa Abattoirs Enterprise (Addis Ababa, Ethiopia)
- Laboratory and clinical work experience at the AAU, FVM (Debre Zeit, Ethiopia)

2005 – 2007

- Instructor at Alagae ATVET College, Department of Animal Health (Alagae, Ethiopia)

Additional Professional Activities

Practical Coordinator at Alagae ATVET College

Research and Seminar

- *Improved Animal Heallth and Production for Poverty reduction* (Seminar presented for the course concepts and problems in livestock development)
- *Prevalence of small ruminant mastitis in Debre Zeit area* (Research thesis defended for the partial fulfillment of Degree of Doctor of Veterinary Medicine)
- *Development of Biofilm based vaccine against Salmonellosis in poultry* (Seminar presented for the course, Seminar on the current Microbiology topics)

Trainings

Certified:

- Computer skill (Application software, Data com. Computer training center)
- Veterinary Public Health (At AAU, FVM)
- Continuing Professional Development on the health and welfare of working donkeys, organized by the Donkey Sanctuary and AAU
- HIV/ AIDS and reproductive health peer education training

Teaching Methodology (At Kotebe Teacher's Training College)

FVM (At AAU, FVM)

Patents and Publications

Prevalence and predisposing Factors of Small Ruminant Mastitis in Debre Zeit area, published in *Ethiopian Veterinary Journal* (Volume **10** N0. 2, 2006)

Languages

- Amharic - Speak, Read and write
- English - Speak, Read and write
- French - Read and write

Interests and activities

I have a huge interest to research works in the field of veterinary medicine, particularly Microbiology and in clinical works.

Volunteer experience

Participation in counseling students in different socioeconomic and academic issues while I was working at Alagae ATVET College

Professional memberships

- Ethiopian Veterinary Association
- Society of Animal Welfare - Ethiopia

References

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  Post Graduate Associate Dean of AAU, FVM
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  Debre Zeit, Ethiopia

10. 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 Yenehiwot Berhanu Welde Aregay

Signature _____

Date of submission June 20, 2008

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

Dr. Moses Kyule (BVM, MSc, MVPM, PhD, Associate Professor) _____

Dr. Kelay Belihu (DVM, PhD, Assistant Professor) _____