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PASSIVE IMMUNITY STATUS IN NEW BORN CALF UNDER PASTORAL PRODUCTION SYSTEM, ETHIOPIA

MSc Thesis

BY
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JUNE, 2018
BISHOFTU, ETHIOPIA

PASSIVE IMMUNITY STATUS IN NEW BORN CALF UNDER PASTORAL PRODUCTION SYSTEM, ETHIOPIA

A Thesis Submitted to the College of Veterinary Medicine and Agriculture of Addis Ababa University in partial fulfilment of the requirements for the degree of Master Science in Animal Production

By

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BISHOFTU, ETHIOPIA

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As members of the Examining Board of the final MSc open defence, we certify that we have read and evaluated the thesis prepared by Batiley Ambaw Tsegaye, entitled: **PASSIVE IMMUNITY STATUS IN NEW BORN CALF UNDER PASTORAL PRODUCTION SYSTEM, ETHIOPIA** and recommend that it be accepted as fulfilling the thesis requirement for the degree of Masters in Animal Production Science.

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DEDICATION

I dedicate this manuscript to my beloved wife Tseganesh Dessie, to my kind grandmother Wubalech Kassa and to my best friend Argeta Abddisa for their uninterrupted nursing and supporting me with deep affection and love, as well as for their dedicates partnership becomes mainstay in the success of my life.

STATEMENT OF AUTHOR

First, I declare that this thesis is my *bona fide* work and all sources of material used for this thesis have been duly acknowledged. This thesis has been submitted in partial fulfilment of the requirements for degree of Masters (MSc) in Animal Production Science at Addis Ababa University, College of Veterinary Medicine and Agriculture and is deposited at the University/College library to be made available to borrowers under rules of the Library. I solemnly declare that this thesis is not submitted to any other institution anywhere for the award of any academic degree, diploma, or certificate.

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LIST OF ABBREVIATIONS

AEA	Apparent Efficiency of Absorption
APT	Adequate Passive Transfer
BSTP	Blood Serum Total Protein
CFU	Colony Forming Units
CSA	Central Statistics Agency, Ethiopia
ELISA	Enzyme-Linked Immunosorbent Assay
FDRE	Federal Dimocratic Repeplic of Ethiopia
FPT	Failure of Passive Transfer
PPT	Partial Passive transfer
APT	Adequate Passive Transfer
GDP	Gross Domestic Product
GIT	Gastro Intestinal Tract
Ig	Immunoglobulins
IGAD	Intergovernmental Authority on Development
LMP	Livestock Master Plan
MC	Maternal Colostrum
MoARD	Ministry of Agriculture and Rural Development
MOFED	Ministry of Finance and Economic Development
RID	Radial Immunodiffusion
USDA	United States Department of Agriculter

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ABSTRACT

A longitudinal study was conducted from December 2017 to April 2018 in Amibara pastoral production system. The aims of this study were therefore, to determine passive immunity status in newborn calves and to identify possible managemental factors that have contribution for the failure of passive transfer of immunity in calves. Each selected calf was individually neck-tagged and monitored in weekly basis for clinical health problems up to an age of three months. Mean ± SD IgG concentration of serum samples determined with the bovine radial immunodiffusion assay was 1805.93 ± 718.68 mg/dL and ranges from 189.132 to 2783.39 mg/dL. The overall failure of passive transfer of immunity and adequate passive transfer of immunity status found in this study were 35.11% and 64.89%, respectively. Risk factors such as calve sex, dam parity, birth site, birth condition, mothering instinct and age at first colostrums ingestion were found to have statistically significant effect (P= 0.004 to 0.031). In the same way morbidity and mortality prevalence were significantly associated with serum IgG. Accordingly, 58.82%, 50% and 4.92 % of morbidity was found for complete failure, partial transfer and adequate transfer of passive immunity respectively. As well, 35.29% 12.5% and 1.64% morbidity was observed for complete failure, partial transfer and adequate transfer of passive immunity respectively. The incidence of morbidity and mortality was apparently higher in calves having complete failure and partial passive transfer of immunity than calves having adequate passive transfer. In this study, the highest proportion of failure of passive transfer of immunity might be due to improper calf and colostrum management practice and absence of regular care for pregnant cows. Hence improving knowledge of pastoralists concerning calf and colostrum management practice as well caring for pregnant cows and regular observation of pregnant cows would significantly reduce young stock mortality associated with failure of passive transfer of immunity.

Keywords:*Pastoral System, Calves, Passive Transfer, Immunity, Radial Immunodiffusion*

1. INTRODUCTION

Ethiopia, the agricultural sector is a corner stone of the economic and social life of the people (Asresie and Zemedu, 2015). Livestock production, as one component of agriculture, covers 40 percent of agricultural output and it also plays an important role in the national economy (Yami and Sileshi, 2001). Ministry of Finance and Economic Development's (MOFED) estimates placed livestock's contribution at about 25% of total agricultural GDP (Behnke and Metaferia, 2013). Livestock is characterized by high mortality. Annual direct losses from ruminant mortality are generally estimated at about 8–10% of the cattle herd, 14–16% of the sheep flock, and 11–13% of the goat flock (Tsegaw *et al.,* 2016). The development of Ethiopian Livestock Masterplan (LMP) indicated the presence of very high young stock mortality in the country and planned to reduce the challenge, by implementing health intervention, vaccinations and parasite control programs through private and veterinary service (LMP, 2015).

Pre-weaning mortality of young stock appeared to be one of the major constraints of livestock production in all major production systems of Ethiopia, hampering the development of replacement stock. Regardless of species and production system, a high loss of young stock was reported during the first one month of life extending up to the third month of age (Tsegaw *et al.,* 2016). The greatest factor contributing to mortality of pre-weaned calves is the failure of passive transfer (FPT), associated with 39 to 50% of pre-weaned calve mortality (Margerison and Downey, 2005).

This high morbidity and mortality occur in claves because calves are born with no immunity against disease, so must depend on their dam to provide passive immunity through colostrum (Alberghina *et al.,* 2010). This has to do with one significant aspect of placentation and fetal growth in bovine. The syndesmochorial placenta of bovine forms a syncytium between the maternal endometrium and the fetal trophectoderm separating the maternal and fetal blood supplies and preventing the transmission of immunoglobulin in to utero Salazar (2008), consequently, calves are born agammaglobulinemic, rendering ingestion and absorption of adequate amount of colostral immunoglobulins essential for establishing passive immunity (Weaver *et al.*, 2000).

Bovine colostrum is concentrated in essential proteins called immunoglobulins (Ig) (Arthington, 2001). The primary immunoglobulin in bovine colostrum is IgG1, which is derived from maternal serum IgG (Sasaki *et al.*, 1977). This bovine IgG provides the major component of passive immunity to the new-born calve (Lascelles, 1963). Passive immunity is that which is received passively from an outside source; conversely, active immunity is derived from the calve's own body (Arthington, 2001).

Inadequate ingestion or absorption of colostral Ig leads to a secondary immunodeficiency condition termed FPT that predisposes ruminant neonates to the development of bacterial septicemia and common neonatal diseases (Weaver *et al.*, 2000; Barrington and Parish, 2002). It has been estimated that as many as 35% to 40 % of dairy calves in developed countries suffered from FPT, which indicates that many calves have inadequate immunity and are more likely to get sick (Lang, 2008). To ensure adequate passive transfer of immunity a calf must ingest 2.5 litre of colostrum with in the first 6 hours of life and four litres within the first 12 hours (Margerison and Downey, 2005). Active uptake of large molecules, such as IgG, ceases after 24–36 hours in neonatal calves Staley and Bush (1985), and for this reason, timely provision of an adequate mass of absorbable Ig from maternal colostrum (MC), particularly IgG, is essential to minimize the risk of failure of passive transfer of immunity in new-born ungulates (Godden, 2008).

High serum IgG concentrations after colostrum ingestion are associated with decreased morbidity and mortality from most calve hood infectious diseases (Fetcher *et al.*, 1983). If the serum IgG concentration exceeds some critical level, then the calve is thought to be relatively well protected against pathogens. In different production system, FPT has long been defined as an IgG of <1,000 mg/dL and/or < 800 mg/dL (McGuire *et al.*, 1976; Weaver *et al.*, 2000; Godden, 2008). Although some researchers have used other threshold serum IgG concentrations (Quigley, 2002). The United States Department of Agriculture recommends passive transfer status is excellent if its serum IgG level is 1,500 mg/dL or more and adequate if its serum IgG level is 1,000 to 1,499 mg/dL. And calve has a failure of passive transfer if its serum IgG level is below 1,000 mg/dL (USDA, 2010).

Several assays are available for evaluating the adequacy of passive transfer of immunity in neonatal calves (Tyler *et al.*, 1998). Of these methods that have been assessed for their

effectiveness: the radial immunodiffusion (RID) and enzyme-linked immunosorbent assay (ELISA) are tests that directly determine the serum-plasma IgG concentration. The sodium sulphate turbidity test, zinc sulfate turbidity test, serum total protein concentration and digital or optical refractometer are used to estimate IgG concentration indirectly (Dawes, *et al*., 2002; Godden, 2008; Morrill *et al*., 2013). Currently, RID is considered the gold standard for determining FPT in calves (Dawes *et al.*, 2002). It has been used extensively for quantitation of immunoglobulins (Ig) in blood serum and also to evaluate Ig in colostrums (Quigley, 2008).

Failure of passive transfer of immunity prevalence varies with management condition and type of animal. In Ethiopia, 32 %, 30.2% and 65.2% failure of passive transfer was reported in market-oriented dairy farm Ibrahim and Lemma (2009), Amoki (2001), in Holeta and Yeshwas (2015), in Bahr Dar Milk-shed respectively. Previously, studies conducted in our country have not focused specifically on FPT prevalence, but they have studied as one factor for calf morbidity and mortality. Also, thus studies are focused on peri-urban and market-oriented dairy farms. Regarding prvevalence of FPT, there has been no research done in pastoralist areas, whereas in the present study, an attempt has been made to determine condition of passive immunity transfer and assess management practice accountable to occurrence of FTP in calves, it is hypothesized that colostrum feeding and calve management practices in the pastoral system have an influence on passive immunity transfer in newborn calves; therefore, the objective of this study is:

- To determine the prevalence of failure of passive transfer of immunity in calves under pastoral production system using radial immunodiffusion test
- To assess the role of colostrum feeding and cow and calve management practices under pastoral production system for the occurrence of FPT.

2. LITERATURE REVIEW

2.1. Livestock Production in Ethiopia

According to the report of MacDonald and Simon (2011), Ethiopia is home to Africa's largest livestock population and it is the continent's top livestock producer and exporter. There are 52.13 million cattle, 24.2 million sheep, 22.6 million goat, 2.5 million camels, 44.89 million poultry, 1.96 million horses, 0.37 million mules and 6.4 million donkeys (CSA, 2012; FAO, 2012). The Livestock production system is categorized as pastoral, agro-pastoral, mixed crop-livestock farming, urban and peri-urban farming and specialized intensive farming systems (Eshetu and Abraham, 2016).

In Ethiopia, agriculture is the main economic activity and more than 80 % of Ethiopian population is dependent on agriculture of which livestock plays a very important role (Duguma *et al.*, 2012). It is an integral part of agriculture and the contribution of live animals and their products to the agricultural economy accounts for 40 %. Over 85% and 90% of the farm and pastoral incomes, respectively, are generated by or from livestock (LMP, 2003). Also in the majority of the rural areas of Ethiopia, livestock production plays an important role in the provision of draft power, food, cash income, transportation, fuel and, especially in pastoral areas, social prestige (Birara and Zemen, 2016). On the other hand, domestic demand for animal products in Ethiopia is increasingly driven by the urban middle and upper classes; the export potential is the key force encouraging expansion and intensification of livestock production (NMSA, 2001).

2.2. Pastoralist Cattle Production System

Pastoralism is an economic activity and land use system with its own distinct characteristics and it is a way of life for people who derive most of their income or sustenance from keeping domestic livestock reared in conditions where most of the feed is natural rather than cultivated or closely managed (Sandford, 1983). In Ethiopia, pastoralism is one of the oldest socio-economic systems, in which livestock husbandry in open grazing areas represents the major means of subsistence (Mussa, 2004). On average, the pastoral livestock population accounts for an estimated 40% of the total livestock population of the country, according to IGAD estimated in 2010 that pastoralist livestock makes up 30% of the nation's cattle, 70% of the

goats and sheep and all camels in the country (Shitarek, 2012). And pastoralist livestock contributed 35 billion Ethiopian Birr (ETB) O'Lakes (2010), out of the total national livestock value of 86.5 billion ETB to the national economy for 2008/09 (Morton, 2013).

Pastoralists constitute a minority in Ethiopia, with an estimated 12–15 million people (14% to 18%) out of the total population of 83 million people (Catley, 2007). And the pastoral population occupies a disproportionately large area and produces much more than its share of national livestock output. The Ministry of Agriculture estimates that pastoralists use 60% of the country's land area, though exact figures of the pastoral livestock population in Ethiopia are unknown (MoARD, 2015). Smallholders, pastoralists and their animals often live in harsh environments which may be hot and dry, hot and humid, or high in altitude and cold. Moreover, these environments can be characterized by scarce feed and water resources and high disease pressure with large seasonal and annual variation Mirkena *et al.* (2010), and their cattle are dependent upon communal grazing systems on rangelands as feed resources (Hassen *et al.*, 2017). According to Shitarek (2012) the livestock numbers in such systems change mostly in response to annual rainfall variation, which has a direct effect on the availability of vegetation and water for livestock which is causing herd mortality, resulting in food insecurity and poverty (Mussa, 2004).

2.3. New-Born Calf Physiology

Calves have some special features in their body system that have relevance in disease occurrence and accordingly require special attention in management. Those that have particular importance are the poorly developed defence mechanism and a dynamic digestive system that has to evolve from milk digestion to a solid feed digestion (Wudu, 2004). As soon as birth, a calve's gastrointestinal tract is designed to temporarily allow the absorption of large molecules including antibodies ("Immunoglobulins") from the small intestine (Yeshwas, 2015).

Pre-weaned calves have physiologically monogastric type stomach. For the newborn calves, the presence of milk in the rumen and reticulum is considered to be abnormal and is undesirable from a physiological and nutritional standpoint (Blowey, 1999; Costello *et al.*, 2010). There are also certain alterations in the digestive system of newborn calves. There is a delay in acid secretion from the stomach and in the development of pancreatic function; thus

acid and trypsin digestion of protein is not started. Calves less than one month of age lack sufficient post ruminal digestive enzymes to break down most sugars and are limited in their ability to utilize starch, maltose, sucrose, or dextran (Heinrichs *et al.*, 2007).

Because of calves are born as functional monogastric digesters the reticulorumen is initially morphologically underdeveloped and free of any microbes Baldwin *et al.* (2004). Over the first few hours of life, the rumen will be colonized by bacteria from the environment (Fonty *et al.*, 1989). The eventual establishment of a healthy anaerobic microbial population is critical to becoming a healthy ruminating animal capable of digesting plant matter (Jami *et al.*, 2013). Although evidence of fermentation can be observed within two weeks and the entire process of becoming a functional ruminant is long and complex, with the expression of hundreds of genes altered Connor *et al.* (2013), that lead to developmental changes throughout the gastrointestinal tract (Baldwin *et al.*, 2004).

2.4. Passive Immunity

Passive immunity refers to components of the immune system that were externally received, such as a neonatal mammal will obtain maternally through the placenta or colostrum until its own immune system is fully functioning (Jainudeen and Hafez, 2000). A basic aspect of animal husbandry is the absolute requirement for newborn animals to take in maternal colostrum during a narrow window of opportunity immediately after parturition. This transfer of maternal immunoglobulin (together with other proteins, lymphocytes, and cytokines) confers temporary immune protection upon the newborn animal until it is capable of activating its own endogenous immunity (Day and Schultz, 2014). This transfer of immunoglobulins from the dam to neonate is of paramount importance Godden (2008), because calves less than five weeks of age do not have active immunity, and colostral antibodies are the only source of immunoglobulins to protect calves from infectious disease immediately after birth (Weaver *et al.*, 2000). As a consequence of the placenta morphology in cattle, large molecules such as immune proteins are unable to cross the placental barrier from maternal to fetal blood circulation and calves will not receive this passive transfer of immunity while in-utero. This means that calves are born agammaglobulinemic effectively immunocompromised and will be entirely dependent on colostrum to provide maternal protection from disease insults for the first few weeks of life (Godden *et al*., 2009; Beam *et al.*, 2009).

Passive immunity is particularly relevant in the control of infection disease. An important consideration in materno-fetal-neonate interactions is the influence of maternally derived immunoglobulins and other maternally derived factors on the immunological status of the neonate (Meyer *et al.*, 1976). Partial or complete failure of passive transfer of colostral immunoglobulins is a primary cause of disease and mortality in neonatal calves (McGuirk and Collins, 2004).

Successful transfer of passive immunity has been determined by measuring the concentration of IgG in calve serum at 24 to 48 hours after birth. If serum IgG concentration exceeds the critical level, then calve is thought to be relatively well protected against pathogens. The greater the concentration of IgG in the circulation of calves at 24 to 48 hours after birth, the greater is the protection against the array of pathogens to which the calve might be exposed (Quigley, 2004).

2.5. Failure of Passive Transfer of Immunity

Failure of passive transfer of immunity (FPT) is not a disease, but a condition that predisposes the neonate to the development of disease (Beam *et al.*, 2009). While failure of passive transfer antibody is a major determinant of neonatal disease, it is not the sole determinate (Radostits *et al.*, 2000). FPT occurs when a calf fails to absorb an adequate quantity of Ig (Beam *et al.*, 2009), it is related both to characteristics of the mother (deficient production or low quality of the colostrum) and of the newborn calves (inadequate intake due to insufficient ingestion or intestinal absorption) Besser *et al.* (1991), poor colostrum feeding methods (Arnold, 2014). The prevalence of failure of passive transfer varies according to management system and types of animal but, whenIgG level in calve blood is less than 10 mg/mL at 24 hours after birth is indicator of failure of passive immunity Godden (2008), Jone and Heinrichs (2011), or failure of passive transfer of immunity exists if the IgG concentration is below 800 mg/dL Beam *et al.* (2009), and < 1,000 mg/dL Garry *et al.* (1993), reported that the concept of FPT is sound and has proven useful, but the diagnosis and its subsequent recommendation have been confusing. He reported that it is incorrect to assume that the published guidelines are applicable under all management situation and environmental condition.

2.6. Consequence of Failure of Passive Transfer of Immunity

Calves with inadequate colostral immunoglobulin concentration within 24 hours of birth were at greater risk of neonatal morbidity and mortality (Poulsen *et al.*, 2010). Failure of passive transfer in a dairy herd puts calves at a higher risk for septicemia, diarrhoea, respiratory disease, navel infections or other illness before weaning. Even without illness, individual calves with FPT shed more pathogens and contaminate the calve housing environment (McGuirk, 2010). In addition, FPT could be due to bacterial contamination of the fed colostrum (Poulsen *et al.*, 2010). Generally, the chances of calves surviving the first few weeks of life are greatly reduced if they do not ingest timely and absorb these antibodies into their bloodstream (Moran, 2002).

2.7. Colostrogenesis

Colostrogenesis is defined as the prepartum transfer of immunoglobulins from maternal circulation into mammary secretions and it is a finite stage (Barrington *et al*., 2001). During the period of colostrogenesis, approximately 3 to 4 weeks prior to parturition, the bovine mammary gland begins to selectively transfer IgG from blood circulation into the forming colostrum (Baumrucker *et al.*, 2010). These immunoglobulin transported from the blood into the mammary glands through receptors on the alveolar epithelial cells Singh and Shivahre (2015), and ceases abruptly at parturition (Foley and Otterby, 1978). As previously mentioned the syndesmochorial placenta of the cow separates the maternal and fetal blood supplies Arthur *et al.* (1996), thus, preventing the transmission of immunoglobulins in utero and categorizing the neonatal calve as agammaglobulinemic (Weaver *et al.*, 2000). This is produced during a distinct physiological and functional stage of mammary gland (MG) development that is markedly different from the gland's primary role in milk production (Barrington and Parish, 2001).

2.8. Colostrum

The period following parturition, when the mammary secretion is considered as colostrum, colostrum is the mammary secretion, the production of which starts closely before parturition and is released as long as the total protein and whey protein content decrease and the content of fat and lactose increase with unchanged levels of total solids (Klobasa *et al.* 1987; Marnila

and Korhonen 2011). The term colostrum is generally used to describe all the milk produced by cows up to five days after calving (Moran, 2002). This secretion is fundamental for the survival of mammal offspring, especially for ungulates (Kehoe *et al.* 2007; Langer 2009). It contains less lactose and more fat, protein, peptides, non-protein nitrogen, ash, vitamins and minerals, hormones, growth factors, cytokines and nucleotides than mature milk; except in the case of lactose, the levels of these compounds decrease rapidly during the first 3 days of lactation Blum and Hammon (2000), and immunoreactive cells Concha *et al.* (1980), needed by the neonate to sustain life. Colostrum provides nutrition of new-borns, enhances protection against pathogens, promotes the development of immune system and ensures the growth, maturation and repair of several tissues (McGuirk and Collins 2004; Shen *et al.*, 2015). Provision of adequate good-quality colostrum results in improved daily weight gain and feed conversion efficiency in both pre-weaning and post-weaning, although the effect of this on joining dates.

As the principle, suckling should always be supervised, and assisted where necessary, to achieve colostrum intake levels of 2.5 L within the first 6 houres of life and 4 L within the first 12 hours of life. Where the calve is not able to suckle successfully, it should be offered milked colostrum from a nursing bottle (Moran, 2012) (Table. 1).

Table 1: Recommended colostrum allowance for newborn calves

Age (day)	Period	Allowance (Liter)
1	< 6hours	2.5
1	<12 hours	4
2-3	Daily	3-4
Continued	Continued	5% total milk offered

2.9. Immunoglobulin

Immunoglobulins (Ig) present in the body are produced by plasma cells that are originally derived from bone marrow cells. These plasma cells are present in various locations in the body and secrete immunoglobulins that collect in the blood and then can be utilized by the calf for the required immune response (Singh *et al.*, 2011). It is transferred from the cow's

blood to cow colostrum by means of (colostrogenesis) at a rate of 500 g/week in the udder during the late dry period (4-6 weeks pre-partum) (Barrington *et al.*, 2001).

2.10. Class of Immunoglobulins

Immunoglobulins are divided into several classes including IgA, IgG, IgD, IgE and IgM that prove to be effective in defending the body against bacteria, virus, parasites and fungi (Mehra *et al.*, 2006). Each of these classes is then further divided into subclasses. Of these five classes, IgG, IgM and IgA are the major immunoglobulin classes in mammary secretions. There are two subclass of immunoglobulin G: G1 and G2, the most dominated immunoglobulin are G1 and it makes 80% of total IgG concentration (Musayeva *et al.*, 2015). It accumulates selectively from the blood circulation into the colostrum by an active receptor mediated transfer across the mammary gland secretory epithelium (Besser and Gay, 1994).

2.11. Colostral Immunoglobulins

Colostrum is characterized by its very high concentration of immunoglobulin G (IgG), which is of particular importance to the neonate, whose gut, immediately following parturition, allows the passage of large immunoglobulins, thereby conferring passive immunity (Stelwagen *et al.* 2009). IgG present in the body is produced by plasma cells that are originally derived from bone marrow cells. These plasma cells are present in various locations in the body and secrete immunoglobulins that collect in the blood and then can be utilized by the calve for a required immune response (Sasaki *et al.*, 1976).

The IgG concentration blood serum may vary due to various factors including animal's disease history, lactation number, volume colostrum, season, breed, health and nutritional status. The survival of the neonate is ultimately dependent on the maternal, the passive/humoral and later the active/cellular immunity and colostrum is the only source of sufficient circulatory Ig for newborn ruminant farm animals where it provides the neonates with passive immunity for the first 30-90 days of life and direct protection of the intestinal tract against infection (Guy *et al.*, 1994). Colostrum from second, and later, lactation healthy cows generally have a greater Ig concentration as these cows have been exposed to more disease, antigens and/or vaccines in their lifetime. As they have had the opportunity to develop antibodies against such disease organisms it is preferred to feed newborn calves

colostrum from such cows (Devery and Larson, 1983). Immunoglobulins, or antibodies, found in colostrum or milk are the same as those found in the blood or mucosal secretions. Ig's concentrations in colostrum are very high and provide passive immunity to new-borns during the development of their own immune systems but can also be useful for providing adults with protection against infection (Lilius and Marnila, 2001; Singh and Shivahre, 2015).

2.12. Absorption of Immunoglobulins

Absorption of intact macromolecules across the intestinal epithelium into the neonatal circulation is possible for approximately 24 hours after calve is born. The absorption of Ig occurs by an active process called pinocytosis, which moves Ig (and other molecules) across the intestinal epithelium. After leaving the epithelium, Ig molecules move into the lymph and then to the circulation. Maturation of the small intestine begins shortly after birth and the ability of the intestine to absorb macromolecules without digestion is lost by about 24 hours after birth (Quigley, 2002).

At six hours post-partum, the gut wall's ability to absorb Ig is already reduced by 33 % and at 24 hours post-partum this absorption is decreased by around 90 % (Heinrichs and Jones, 2003). This loss of absorptive ability appears related to the development of the digestive apparatus in intestinal epithelial cells and turnover of cell populations. After about 24 hours of age, the chance to provide calve with antibodies is gone. However, it is important to continue to feed colostrum for 2 to 3 days after birth. The Ig in colostrum will bathe calve's digestive tract and make it difficult for bacteria to attach to the intestinal wall. This "local effect" can reduce the incidence of scours during the first several weeks of life (Quigley, 2002).

The diagram below illustrates the temporal relationship between closure, IgG absorption, colostral IgG, and calve serum IgG. The process of macromolecular absorption is initially high at the first suckling and then declines gradually. Intestinal closure to the uptake of macromolecules has occurred when no more intact macromolecules can be absorbed. An intestinal closure is a continual, gradual process that starts immediately after birth and proceeds until there is no longer transport of macromolecules. Time of closure is the time after birth when macromolecules (including immunoglobulins) can no longer pass from the intestinal lumen, through the intestinal cell and into the neonate's vascular system. Closure is complete in the calve by about 24 hr after birth (Hurley, 2010) (Fig. 1).

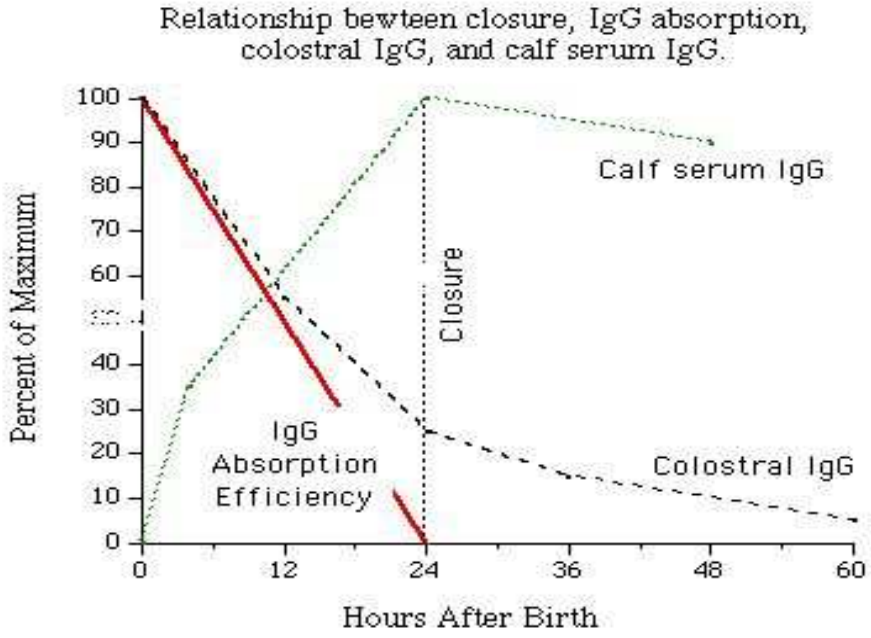

Figure: 1. The relationship between closure and IgG absorption

2.13. Colostrum Cleanliness

Colostrum quality is typically expressed in terms of IgG, but contaminants also influence quality. Obviously, fewer contaminants mean higher quality (Chester-jones, 2009). Common contaminants include blood, mastitis, and bacteria. Even good colostrum can be damaged if a cow's udder and teats are not well cleaned, sanitized, and dried before the initial milking or nursing (Chester-Jones, 2009). Quality colostrum refers to the absence of bacteria in colostrum; unfortunately, a large percentage of colostrum has high levels of bacteria, greater than 100,000 colony forming units (cfu)/mL. Bacteria in colostrum have a negative impact on IgG absorption by the calve because they may bind IgG in the calve’s small intestine or they may directly block the uptake of IgG by the intestinal cells (Godden, 2008). Also, the presence of bacteria in colostrum may also be pathogenic and cause diseases such as diarrhoea. There are several management practices that should be followed to minimize bacterial contamination of colostrums (Doepel and Bartier, 2014). Avoiding feeding excessively bloody or mastitic colostrum and regularly maintaining and cleaning milking equipment's, especially waste milk cans and their lids are major means to reduce bacterial contamination (Chester-jones, 2009).

2.14. Factors Influencing Immunoglobulin Transfer

Factors that often are cited as having an effect on passive transfer in the calve are; the timing of colostrum ingestion, the method and volume of colostrum administration (Weaver *et al*., 2000). Also factors like age, parity, breed, nutritional status, length of dry season, premature milking, dystocia, birth time, presence or absence of attendant and udder health status have considerable effect on IgG concentration in colostrum and amount of colostrum produced by cow and passive transfer (Amoki, 2001; Ibrahim and Lemma, 2009; Yeshwas, 2015).

2.14.1. **Timing of ingestion**

The timing of colostrum intake by calve can have a role in the transfer of passive immunity. Passing of antibodies from the dam to calve via the colostrum (or first milk after calving) transfer only occurs during the first 24 hours following birth (Mendonsa, 2011). Beam *et al.* (2009), have reported that calves fed with colostrum more than 4 hours after birth were 2.7 times more likely to be susceptible to FPT than calves fed with the first colostrum within 4 hours. As optimal absorption occurs within 4 hours of birth and declines rapidly after 6 hours, calves should ideally be fed within the first 4 hours of birth, with the first feeding not later than 6 hours after calving (Godden, 2008). Although, the volume of colostrum produced is one factor that affects the concentration of IgG. Conneely *et al.* (2013), reported that for every liter of colostrum produced, IgG concentration decreases of 3.7%, suggesting that colostrum should be harvested as soon as possible after calving.

Calves fed earlier will have significantly higher serum IgG concentrations than those fed later when similar concentrations and volumes of colostrum are fed (Weaver *et al*., 2000). Suckling within 6 h of birth attained higher levels of all sub-classes of Ig than calves failing to suckle. However, most calves which we1l assisted to suckle soon after this period still attained levels of Ig well in excess of those considered adequate to provide protection against neonatal disease. It thus appears that assisting calves to suckle at any time during the first 7 h postpartum will ensure that they attain a good immunity status. Calves which failed to suckle within 6 h of birth and were not subsequently assisted to suckle had much lower levels of IgG 1, the predominant Ig in colostrum, but levels of the other sub-classes of Ig were no lower. It may be that, although the ability to absorb Ig does decrease from birth, the first 6 hours does

not constitute such a critical period as previously suggested provided that ample colostrum is obtained relatively soon after this time (Waldhalm *et al.*, 1971).

2.14.2. Amount of colostrum to be feed

Because of concern about colostrum quality that varies a lot, it is now recommended that calve should be given four litres of colostrum in the first feeding (Besser *et al.*, 1991). Calves that ingest ≥ 3 L at the first feeding will need to ingest 1 L of colostrum within 12 hours to have an optimum colostral intake (Chigerwe *et al.*, 2009). Because, passive immunity is determined by the quantity of serum gamma immunoglobulins (IgG) absorbed via intestinal enterocytes by 24 h after birth (Stott *et al.*, 1979). The relationship between serum IgG and colostral IgG intake (colostrum IgG concentration × amount of colostrum fed) is linear in experiments (McEwan *et al.*, 1970). It is recommended that four quarts of colostrum should be fed between birth and 4 hours after calve is born to ensure high absorption rates (Kirk, 2011). Calves that receive sufficient immunoglobulin are known to perform better than calves with insufficient level (Robison *et al.*, 1988).

2.14.3. Parity

IgG is the predominant colostral immunoglobulin. The concentration of IgG may vary considerably among cows (Weaver *et al.*, 2000). Cows that have had multiple lactations tend to have a higher quality of colostrum in regards to antigen level because they have been on the farm longer and had a greater period of exposure to various pathogens and antigens (Godden, 2008). Primiparous animals have not had the time to accumulate and build up resistance to the various herd-specific pathogens. Older cows build up a larger amount of antibodies because of a greater exposure time to pathogens. This exposure provides the calve with higher amounts of antibodies from the maternal colostrum. Immunoglobulin concentrations in primiparous cows significantly differed from older cows, with multiparous cows secreting 1.3-1.6 times higher antibody concentration (Liu *et al.*, 2009). However, if older cows are not exposed to many pathogens, the colostrum produced may not have high levels of Ig. This is also why a good dry cow vaccination program can improve the quality of colostrum. Moreover, cattle raised on a farm will produce colostrum with antibodies specific for the organisms on that farm which is an added benefit. Finally, prepartum milking or

leaking of milk from the udder prior to calving can reduce the concentration of Ig in colostrum (Quigley, 2002).

2.14.4. Sex of calve

The gender of the neonatal calve has been shown to affect colostrum production and later lactation milk production (Hinde *et al.*, 2014). The total Ig concentration was also affected by the sex of calve and was higher for males than for females. According to Angulo *et al.* (2015), both total immunoglobulin concentration and colostral volume were affected by the sex of the neonate. Total immunoglobulin concentration was higher in dams carrying males than for those carrying females; however, total colostral volume was higher in dams with female offspring than dams with male offspring. Dams with female offspring produced 5 % more milk during lactation than dams with male offspring. According to author's suggestion, hormones from the foetus and placenta may differ between fetal males and females, which subsequently enter the maternal bloodstream and affect the milk-producing cells in the mammary glands (Hinde *et al.*, 2014; Angulo *et al.* 2015).

2.14.5. Effect of dystocia

A difficult or dystocial birth often means that assistance must be provided during delivery. It is difficult to assess the internal state of the cow in terms of what pain or discomfort she experiences so, from a practical point of view, dystocia is normally described in terms of the level of assistance that is required (Mee, 2008). The most common cause of dystocia is a physical incompatibility between the pelvic size of the dam and the size of calve (fetal-pelvic incompatibility). Because of this, a high calve birthweight is known to be an important risk factor for dystocia, as well as the choice of sire, breed and length of gestation. It also follows that male calves are also more likely to experience a dystocial birth because of their higher birthweight. Pelvic size is influenced by the stage of maturity of the cow, so a smaller size of pelvis contributes to the higher prevalence of dystocia in heifers (Mee, 2008).

Calves born from dystocia calving's, especially those born from very difficult assisted or prolonged parturition, have a lower appetite when fed at will and take longer to suckle their dam for colostrums (Yeshwas, 2015). The welfare of the calves may be comprised through trauma to the musculoskeletal system, physiological stress, mortality, morbidity and pain

arising from assisted delivery and dystocia (Barrier *et al.*, 2012; Murray, 2014). And also, delaying and/or reduced colostrum consumption has been found to result in lower passive immune transfer and increase failure of passive immune transfer in calves born from difficult parturitions (Beam *et al.*, 2009; Barrier *et al.*, 2012).

Calving difficulty level is essential for benchmarking and monitoring calving performance or dystocia management plans and can be used as a sensitive indicator of welfare around calving time (USDA, 2010). It is recommended that calving difficulty is scored and recorded for each animal promptly post-parturition (Schuenemann *et al.*, 2011). The calving difficulty scoring scale should at least distinguish between unassisted, assisted, and veterinary or surgically assisted calving's (Fishwick, 2011).

2.14.6. Mothering instinct

Increased survivability of calves is very much dependent on mothering instincts of the dam which is characterized by stimulating the calve to stand and stimulate suckling behaviour (Hussain, 2011). Mothering has a tremendously beneficial effect on the efficiency of absorption of immunoglobulin. Mothered calves absorb 70 percent or more immunoglobulin from a standard feed than non-mothered calves (Selk, 2003). Poor mothering ability combined with reduced calvevigour could decrease the effectiveness of passive transfer (Sivula *et al.*, 1996). Grooming the calve by the cow may reduce stress and blood serum corticosteroid concentration and thereby improve absorption (Husband *et al.*, 1973).

2.14.7. Method of colostrum feeding

Recommendations for colostrum feeding have changed dramatically over the last decade. Before this, it was considered acceptable for all calves to run with their dams for one, two or even three days and for her to pass on passive immunity through natural suckling. Current advice to farmers is to ensure calves drink from their dam within the first three to six hours of life, and if not then to provide additional colostrum from its mother or another freshly calved cow (Moran, 2002). Leaving the calve with the dam to suckle exclusively may result in inadequate voluntary consumption of colostrum within the critical 4-hour window and so contributes to the development of FPT (McGuirk and Collins, 2004). American data indicate

that 61.4 % of calves that received colostrum via suckling developed FPT compared to 19.3 % that were bottle-fed and 10.8 % that were tube-fed (Beam *et al.,* 2009).

The reason is, many times, calves do not want to consume the recommended amount of colostrum right after birth because they do not have a strong drive to nurse (Mendonsa, 2011). Also, calves might be reluctant could be because the calve might not physically be able to consume colostrum because of the birthing process (Quigley, 2002). Even, it is not possible to determine accurately the intake of colostrum by suckling, and if inadequate suckling is anticipated or suspected, it is necessary to provide colostrum by bottle feeding or feeding by oesophageal intubation. A useful guideline is that (3 to 4 litres) of good quality colostrum should be given in the first 6 hours postpartum to ensure adequate passive transfer (APT).

The concentration of IgG in blood serum increased rapidly following colostrum consumption in calves given either by nipple bottle or oesophageal tube. Colostrum administered by oesophageal tube would be expected to be deposited in the rumen, rather than the abomasum as by nursing (Adams *et al.,* 1985). Oesophageal groove closure allows the colostrum to bypass the reticulum and rumen and deposit directly into the omasum and abomasum. This direct route shortens the time that the colostrum takes to reach the small intestine where absorption occurs. As well, the abomasum allows the colostrum to clot, and clotting is necessary as it allows whey proteins such as IgG to travel to the small intestine (Longenbach and Heinrichs, 1998). Colostrum feeding to newborn calves by means of oesophageal feeder, therefore, remains a labor-saving and effective method to obtain optimum levels of serum immunoglobulins and maximum protection against infectious diseases (Lateur-Rowet and Breukink, 1983). Others Molla (1978), Lateur-Rowet and Breukink (1983), support the use of oesophageal feeders to provide large amounts of colostrum without significant effect on serum IgG concentrations.

2.15. Colostrum Supplement

Maternal colostrum is almost always the preferred source of IgG. The IgG in maternal colostrum is derived from the dam's bloodstream and are based on the disease history to which the cow has been exposed. The industry has long recognized that management of colostrum on the farm is time-consuming, tedious and prone to error. Statistics, including neonatal morbidity, mortality and the proportion of calves with FPT are clear evidence that

colostral management is often inadequate (Quigley, 2002). The difficulties in ensuring adequate transfer of passive immunity in calves have led to commercial production of products aimed at supplementing colostral antibody and reducing the risk of FPT (Amoki, 2001). But, supplements cannot replace high-quality colostrum. Because, they do not contain sufficient quantities of antibodies to raise the blood level in calves beyond what average quality colostrum will do (Chester-jones, 2009). Maternal colostrum may be replaced by a supplement when it is unavailable, of poor quality (low IgG concentration) or may contain pathogenic organisms. The three readily available sources of IgG are lacteal secretions (colostrum and milk), blood and eggs (Quigley, 2002).

2.16. Mortality and Morbidity in Calves

Healthy calves form the basis of any successful cattle production system, from both an economic and an animal welfare point of view (Gulliksen *et al*., 2008). More importantly, rearing of dairy or beef calves for replacement or sale is an important source of income for producers (Azizzadeh *et al.*, 2012). Neonatal animal disease, morbidity and early mortality in the first days and/or weeks post-partum have been linked to a number of factors, including the lack of colostrum feeding, poor quality colostrum, insufficient quantity colostrum fed McGuire *et al.* (1976), and poor timing of colostrum feeding (Stott *et al.*, 1979).

The leading causes of calve morbidity and mortality reported worldwide are diarrhoea (scours) and respiratory diseases (Wudu *et al.*, 2008). Calve mortality rate of 20% can reduce net profit by 38% (Wold and Yehualashet, 1987). This arises from death loss, treatment cost, limits genetic selection, decreased lifetime productivity and survivorship (Mellado *et al.*, 2014). When compared to other countries, information on calve morbidity and mortality is scarce in Ethiopia. Those available are mostly from research and institutional herds, which do not properly represent the predominant smallholder production system existing in the country (Wudu *et al.*, 2008).

In Ethiopia most studies on calves reported mortalities. Except for some authors, Wudu *et al*. (2008), 62% crude morbidity rate, Megersa *et al.* (2009), 29.3% crude morbidity rate and Ferede (2015), 58.4% crude calve morbidity, and most other reports have covered specific morbidities. Mortality statistics in Ethiopia mostly ranges from 6.5 to 30.7% in pre-weaned calves (Ferede *et al.*, 2014; Megersa *et al.*, 2009; Shiferaw *et al*., 2002; Wudu *et al*., 2008).

Table: 2. Mortality rate in calves compiled from different studies in Ethiopia

Study area	Age of the calve	Mortality rate	Reference
Andassa ranch	Pre-weaning	6.5	(Amuamuta *et al.*, 2006)
Holleta	Pre-weaning	7	(Shiferaw *et al.*, 2002)
Around Holetta	Pre-weaning	14.2	(Amoki, 2001)
Adami Tulu	Pre-weaning	25	(Sisay and Ebro, 1998)
Gozamen & B. Zuria	Pre-weaning	30.7	(Yeshwas, 2015)
Abernosa ranch	Pre-weaning	17.3	(Dekeba *et al.*, 2006)

2.17. Method of Reducing Young Stock Mortality

Reducing calve mortality is a starting point for increasing the number of replacement heifers (McGuirk, 2007). Appropriate management in the peripartum period can substantially reduce morbidity and mortality in calves (Razzaque *et al.*, 2009). So, proper nutrition is fundamental for the growth of young animals and for the general profitability of livestock-rearing. A good nutritional strategy in young stock optimizes the development and growth while minimizing stress and disease. Experience indicates that young animal losses can be significantly reduced by introducing new techniques of management, including proper feeding and nutrition, housing, and hygiene (Razzaque *et al.*, 2009). The importance of colostral immunity to the health of immunocompetent, albeit immune-naive calves cannot be understated. While most dairy producers recognize that adequate colostrum volume, immunologic quality, timeliness of feeding and cleanliness are the key elements to reducing morbidity and mortality of calves (McGuirk, 2007).

2.18. Determination of Passive Transfer

Determination of successful transfer of passive immunity has been by measuring the concentration of IgG in the serum of the calve at 24 to 48 hours after birth (Quigley, 2002). Measurement of maternal immunoglobulin (Ig) is an important procedure to ensure adequate transfer of Ig to the new-born calve (Jones and Heinrichs, 2011). Many tests have been developed to assess passive transfer status in domestic animals can be diagnosed in calves one to seven days old by measuring serum Ig level concentration directly or indirectly. These include radial immunodiffusion (RID) and enzyme-linked immunosorbent assay (ELISA)

tests that directly determine the serum-plasma IgG concentration and the sodium sulphite turbidity test, zinc sulphate turbidity test, serum total protein concentration and digital or optical refractometer, which indirectly estimate the IgG concentration (Godden, 2008; Morrill *et al*., 2013).

2.18.1. Total protein

The serum chemistry panel can be performed to directly measure the amount of protein in the blood. As immunoglobulins are just one of many different proteins in the blood, this tells if there is moderate to several problems, but is not very specific. However, using an automated method such as biuret gives more accurate estimate (Quigley, 2002).

2.18.2. Zinc sulphate turbidity

This is another test used to gain a rough estimation of the amount of immunoglobulins present in the serum. A small of serum is added to zinc sulphate solution and incubate at room temperature for one hour. Zinc sulphate will cause precipitation of the Ig which makes the solution cloudy instead of being clear; thus lack of cloudiness signifies a lack of Ig. This test is fairly specific for Ig, but it is not very good in quantifying the Ig and if they are present and the solution is cloudy, it is difficult to distinguish a borderline problem (Pare *et al.*, 1993). Time, temperature, amount of dissolved carbon dioxide in the zinc sulphate solution and amount of haemolysis in the sample influence the amount of turbidity (McGuire and Scott, 1982).

2.18.3. ELISA

An Enzyme-Linked Immunosorbent Assay (ELISA) which directly measures serum IgG concentration is available for assessing passive transfer (Dawes *et al*., 2002). ELISA techniques are widely used in immunology for either the qualitative or quantitative detection of antigens. In this context, they rely on the interaction between the antigen (bovine IgG) and antibodies raised against the antigen. Many formats of ELISA are available (direct, sandwich and inhibition modes), depending on the sequence of antigen and antibody addition. For the quantification of bovine IgG, antibodies raised against bovine IgG (typically polyclonal) are bound to the plastic surface of an ELISA 96-well microtiter plate and samples are applied directly to the surface, resulting in IgG binding specifically to the antibody. Detection and

quantification are based on colorimetric measurement of bound antibody-enzyme conjugate and interpolation with a standard curve. Quantitative measurement of IgG using ELISA techniques is common in stability studies of IgG where the effects of various processing conditions such as thermal or pH treatments are examined (Dominguez *et al.* 2001; Gapper *et al.* 2007). The techniques are more flexible and complex. It has many advantages compared to other methods, including speed, accuracy and being able to measure very small quantities of IgG. It is a more complex method and requires greater technical skill and equipment compared to the other techniques. Therefore, it tends to be less widely used in measuring total IgG concentration in calve serum studies (Quigley, 2008).

2.18.4. Refractometer

Recent work suggests thatevaluation of calves serum using Brix refractometer can provide a strong estimate of IgG concentration (Morrill *et al.*, 2013). Can be used digital and optical refractometers to evaluate colostrum quality and serum Ig (Bielmann *et al.*, 2010). The instrument used is a Brix refractometer, which measures the sucrose concentration in liquids such as fruit juice, molasses, and wine. When used in non-sucrose-containing liquids, percentage Brix (% Brix) approximates the total solids percentage. Considerable utility exists in using the same instrument to evaluate colostrum quality and assess passive transfer in calve. It works by allowing light to go through the sample of blood serum. The optics bends the light rays depending on the concentration of protein in the serum. The greater the protein concentration, the more light is bent. Thus, a high protein sample will have a smaller dark area at the top of the viewing area than a low protein one (Leadley, 2018). The digital Brix refractometer measures the index of refraction (Stojic *et al.*, 2017). When used the digital one, a microliter of serum sample was placed on the prism of the Brix refractometer, and % Brix value was read. The digital Brix refractometer has a range from 0 to 85% Brix Stojić *et al.* (2017), and suggest that a value of < 7.8% Brix may be used to identify FPT in 1-d-old calves (Morrill *et al.*, 2013).

2.18.5. Radial immunodiffusion assay (RID)

Single radial immunodiffusion (sRID) has been used extensively for quantitation of immunoglobulins (Ig) in blood serum (Beam *et al.*, 2009). In this method, agar or agarose melted in a suitable buffer is cooled to about 55_C and precipitating antiserum specific for the

protein to be measured is added to the appropriate concentration. The antiserum-containing gel is poured onto a glass or plastic plate or tray and allowed to cool, whereupon the gel solidifies. Wells are cut into the gel and each of these is filled with several microliters of test serum. A series of wells are each filled with reference serum (or reference solution) containing known concentrations of the protein to be assayed. The plate is left in a humid atmosphere to allow the proteins within the samples and reference solutions to diffuse into the surrounding antiserum-containing gel.As the protein molecules being assayed diffuse into the gel they form precipitates with the antiserum molecules (Mancini *et al.*, 1965). The area of the ring obtained is a measure of antigen concentration, and this can be compared to a standard curve obtained by using antigens of known concentration (Stevens and Miller, 2016).

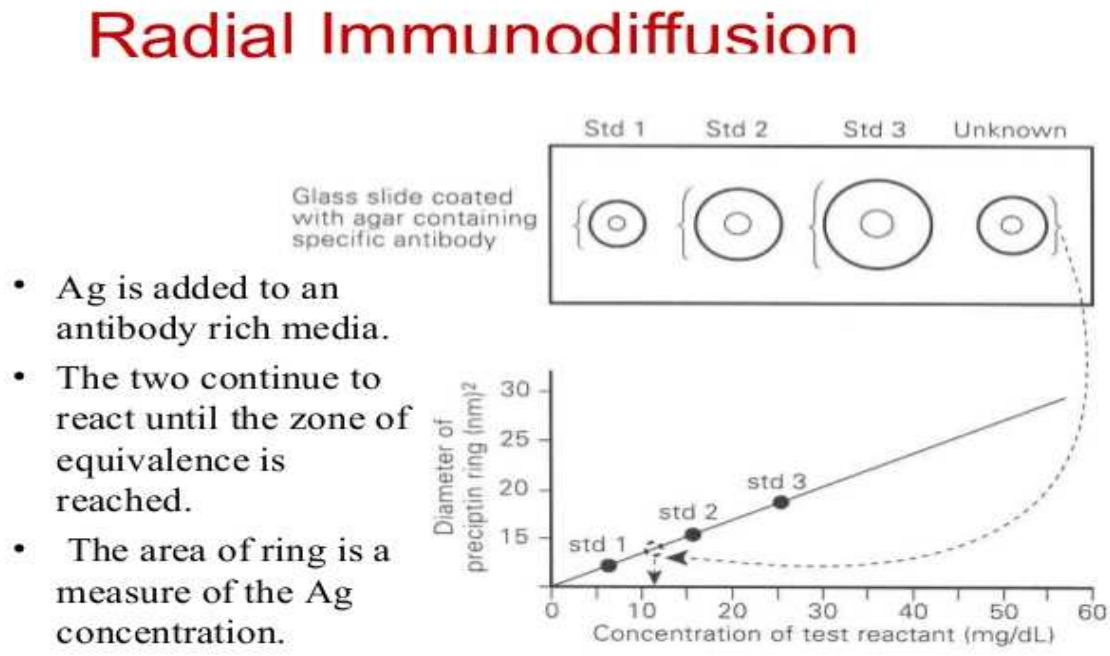

Figure: 2. Radial Immunodiffusion antibody-antigen precipitation

The molecular weight of antigen is the major determinant of time required for the performance of a RID assay. In practice, at least three antigen solution of known concentration (standards) are included with each assay, and their ring diameter is used to construct a standard curve, from which the concentration of an unknown solution is determined. Ring diameter can be measured used a ruler and magnifying glasses, although

more accurate measurement requires a special reading device. To compensate for irregularities in the well, it is customary to measure each ring diameter twice at the right angle and to calculate the average of measurement. Measurements of the ring diameter may be taken at the end point of the reaction (Mancini or endpoint diffusion) or at a fixed time before the endpoint (usually 18 hours) (Fahey or kinetic diffusion) in the endpoint diffusion method, the standard curve prepared on linear graph paper by plotting the concentration of antigen on the x-axis the diameter squared for precipitated ring diameter on the y-axis. If the standard curve is nonlinear a line of best fit is drawn (McClatchey, 2002).

The Fahey and McKelveymethod, also called the *kinetic method,* uses measurements taken before the point of equivalence is reached. In this case, the diameter is proportional to the log of the concentration (McClatchey, 2002). A graph is drawn on semi-log paper by plotting the antigen concentration on the log axis and the diameter on the arithmetic axis. Readings are taken at about 18 hours (Detrick *et al.*, 2006).

3. MATERIALS AND METHODS

3.1. Study Area

The study was conducted in Amibara District, part of the administrative zone three of Afar Regional state, Ethiopia. The District is located in the Middle Awash River Basin about 260 k.m Northeast of Addis Ababa. Amibara District is bordered on the south by Awash Fentale District, on the west by the Awash River, which separates it from Dulecha District to the southwest then on the northwest by the administrative Zone Five, on the north by Gewane, and on the east by the Oromia Region (Figure.1). The area is characterized by high temperature ranging from 25^0C to 39^0C with a mean annual precipitation less than 600 mm. May/June is the driest season of the year, *hagay.* It is said to be unsuitable for browsing since bushes dry up. The main rainy season *(Karima)* which extends from July to September accounts for above 60% of the annual total rainfall. The Dstrict has 18 kebelse of which 4 are urban, while the rests are pastoral, which hold large numbers of livestock population. The livestock populations of the Amibara District is composed of 103, 959 cattle, 122, 526 goats, 48,043 sheep, 3,888 donkeys and 39,995 camels (CSA, 2008).

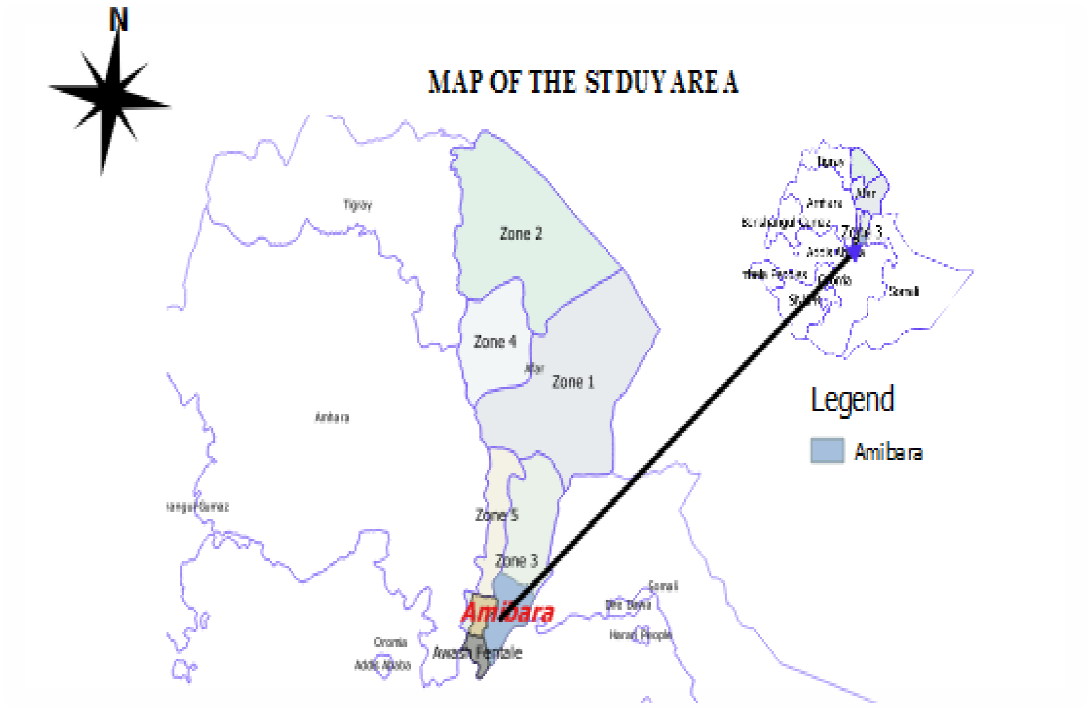

Figure: 3. Map of the study area

3.2. Study Design

A longitudinal study design was conducted from December 2017 to April 2018 in Amibara District, pastoral smallholder production system. Blood samples were collected from calves 24 hours to seven days of age to determine the amount of passive maternal immunity that had been obtained from colostrum. Failure of transfer of passive immunity test was conducted using radial immunodiffusion (RID) according to the manufacturer's instructions. The threshold of serum IgG concentrations was used to define the post-suckle passive immune status: classified as having FTP if serum IgG was < 800 mg/dL, calves categorized under partial FTP if serum IgG concentration is ranging from 800-1600 mg/dL and calves were categorized under adequate passive transfer of immunity if the serum IgG concentration was > 1600 mg/dL; the upper and lower limits of the assay detection were 3624.503 and 60.711 mg/dL, respectively.

3.3. Study Population and Sample Size

The study population was 112 calves which are between one to seven days old age. During the study period, all newly born calves and having 24 hours of age in the study area was included in the study population.

From slected study District, three kebeles namely Haliydege, Huleisi and Andido were selected using simple random sampling method.

3.3.1. Sample size determinations

The sample size was determined by using mathematical model of (Arshame, 2005). The sample size, N, can then be expressed as largest integer less than or equal to $0.25/SE^2$.

$$N=0.25/SE^2$$

SE is standard error (considering SE of 0.0472 with 95 % coefficient interval as follows, $N= 0.25/0.0472^2 = 112$. Accordingly, one hundred twelve (112) households were selected purposely based on the availability of newborn calves between one to seven days of age. Then, all calves which were born in selected Kebeles during sample collection from period (December to April) were included in the study.

3.4. Method of Sample Collection

Blood samples were collected through jugular veni-puncture in a sterile plain vacutainer tube during. For collecting whole blood firstly calve was restrained by hand, and then by pressing thumb or finger in the groove at the base of the neck flow of blood in the vein was stopped and creates pressure, thereby blood collection was performed. After collection of the whole blood, It was allowed to clot for at least 2-3 hours and then serum separated by centrifugation at 2000 g-force at room temperature for 10 minutes. Thereafter, the liquid component or blood

serum was transferred into a clean polypropylene tube using a micropipette and then the serum was labeled. Serum samples were maintained in ice box while handling and transported to Melka Werer Research Center and stored at –20 °C. The collected sample was stored at Melka Werer Research Center until the sampling collection process is completed from the study area, and when sample collection is completed, the sample was packed into the ice box and transported to Aklilu Lemma Institute of Pathobiology at Addis Ababa Universityand stored at -20 °C until laboratory analysis was performed. Samples were analyzed for serum total quantification by the RID kit (Dawes *et al.*, 2002).

After completing blood sample collection from each individuals,calve side and cow side retrospective data like (Calve ID, date of birth, sex of calve, birth time, birth site, dam parity, age at first colostrum ingestion, birth condition, presence or absence of attendant, mothering instinct and vaccination history) was collected on predesigned format. The sample of record format is available in Annex: 1

3.4.1. Key informant interview

Key informant interview was undertaken to collect qualitative data that play a role in the transfer of passive immunity. The key informant selection was undertaken based on the information provided by local administrators and elder people in the study area. Most of the participants were people who have a good knowledge of cattle production and calf management practice in the area. Also, these people own more livestock than others and are respected by the community. A checklist of containing nine open-ended questions was prepared on data related to pregnant cow care and management condition, calve management

and feeding method and perception towards colostrum (Annex 2 and 3). A total of nine key informants from the study area (three elderly people, three women's, two married young and one animal health technician) were enrolled during the interview.

3.5. Observational Study (calve health follow-up)

Follow-up for the smallholder calve morbidity and mortality were carried out for 90 days (three months). The mortality rate in the study ranges from (prenatal to early pre-weaning) meaning that prenatal mortality refers death of live-born animals within 24 hours of life after blood sample was taken. Early pre-weaning mortality refers death of young stocks within one to three month of age. Morbidity and mortality follow-up was performed for three month only, because the highest mortalities are reported during the first 30 days of life and this extends up to the first 3 months of life (Chenyambuga and Mseleko, 2009). According to Wudu *et al.* (2008) older calves above three months age were at lower risk of mortality than younger calves less than three months of age. In the study period, calves included in the study population were individually neck-tagged and regularly monitored in monthly basis for 90 days (up to three months). In this study, mortality will be defined as death of calves after blood sample has collected and calf morbidity is any sickness that has a recognizable clinical manifestation such as diarrhoea, respiratory disease, navel ill and other cases. The follow up was aimed to found morbidity and mortality condition of calves associated with failure transfer of passive immunity.

3.5.1. Method of measuring mothering instinct

To identify cows with good maternity, the researcher used the following criteria to measure maternity behaviour. Cow with poor mothering instincts are: kick her calves during suckling, detach her calves or move away, don’t lick her calve, lack behaviour of pushing her calve towards to the udder for supporting to suckle. Thereby, cows that having characteristics stated above were grouped with poor mothering instincts. Those that have no behaviours defined above were categorized under dams with good mothering instincts. Accordingly, dams those who have a poor mothering instinct, are coded [I], and dams with good mothering instinct were coded [II].

Dystocia was considered whenever the cow had difficulty of birth and such cow was designated-Code [1]. Calves born under such condition were closely followed for any health problem starting Day one. Normal parturition was designate Code [0].

3.6. Determination of Failure of Passive Transfer

A blood sample taken from one to seven-day-old calves because calves up to 7 days of age provide the most accurate indication of passive transfer Elizondo-Salazar and Heinrichs (2009), and blood serum total protein (BSTP) values being quite stable out up to 7 days but dropping significantly by Day 14 (Leadley, 2018). Collected samples were thawed at room temperature for an hour and vortexed for 5 seconds to mix serum sample thoroughly prior to analysis. The bovine radial immunodiffusion (RID) IgG test kit was used in accordance with manufacturer instructions (Triple J Farms Jorgensen Place Bellingham, WA USA). The plates were stored at 4^{o}C prior to use and the directions detailed by the manufacturer were followed. The manufacturer has included 0.25 ml containing three vial of pooled bovine reference sera of known IgG concentration (having, 2803 mg/dL, 1472 mg/dL, and 180 mg/dL); these three standard sera were included in all of the test plates. Ring diameter for these three standard solutions was used to construct a standard curve from which the concentration of unknown solution is determined. The plate contains 24 wells into three wells of each plate, 5μL of each standard solution was pipetted and serum was pipetted into the remaining 21 wells.

The plates were incubated in moist chamber at room temperature for 24 hours. After over night incubation, serum samples with a diffusion ring diameter smaller than that of the lowest standard and exceeded the ring diameter of the top standard solutions were removed out from the study. On regular graph paper, a standard curve of diameter versus antigen concentration constructed using a series of reference standards of known antigen concentration. From the standard curve, the unknown IgG concentration (mg/dL) of the serum samples was determined, by finding the diameter squared value on the Y-axis, finding the intersecting point on the standard curve line, and obtaining the value on the X-axis. The value on the X-axis is the concentration of antigen in the solution. Brief order and an illustration for the laboratory work is presented in (Annex: 4)

3.7. Data Management and Statistical Analysis

All data were entered into Microsoft Excel data sheet and was later analyzed using a statistical package STATA Version 12.0 Texas USA. The data was first summarized using descriptive statistics (means, SD, and percentages). Seven risk factors (Calf sex, parity number, birth time, birth site, birth condition, age at first colostrum ingestion and mothering instinct) were considered for their association with immunoglobulins concentration in blood serum, morbidity and mortality of calves. Chi-square ($\chi 2$) test was used to analyze the presence of an association between variables. P-value was held at $p<0.05$ to determine the presence of significant differences.

2.8. Ethical Statement

The study protocol was approved by the Ethical Clearance Committee of the Aklilu Lemma Institute of Pathobiology (ALIPB) (Reference no ALIPB/IRB/016/16/2017), Addis Ababa University Ethiopia.

4. RESULTS

A total of 112 samples, (65 females and 47 males) were collected from the study area. Age at sampling was ranging from one to seven days. The radial immunodiffusion assay results were not legible for 2 calves because of the ring diameter which is smaller than that of the lowest standard. There was no "low level" radial immunodiffusion plate to re-run the samples, so these two samples were eliminated from the study. Likewise, ring diameter for 16 samples was found above the top standard, and samples were removed out of the study because there is no extra RID plate to rerun the samples. For the remaining 94 calves, mean ± SD IgG concentration of the serum samples, determined with bovine radial immunodiffusion assay, was 1805.93 ± 718.68 mg/dL. Serum IgG concentration from those 94 calves ranged from 189.132 to 2783.4 mg/dL. Descriptive statistics of passive immunity transfer and IgG concentration in calves under different category are presented in (Figure 4).

Based on serum IgG concentration threshold, 18.09 % calves were considered to have FTP, 17.02% were considered to have partial passive transfer of immunity and the rest 64.89 % were considered to have adequate passive immunity.

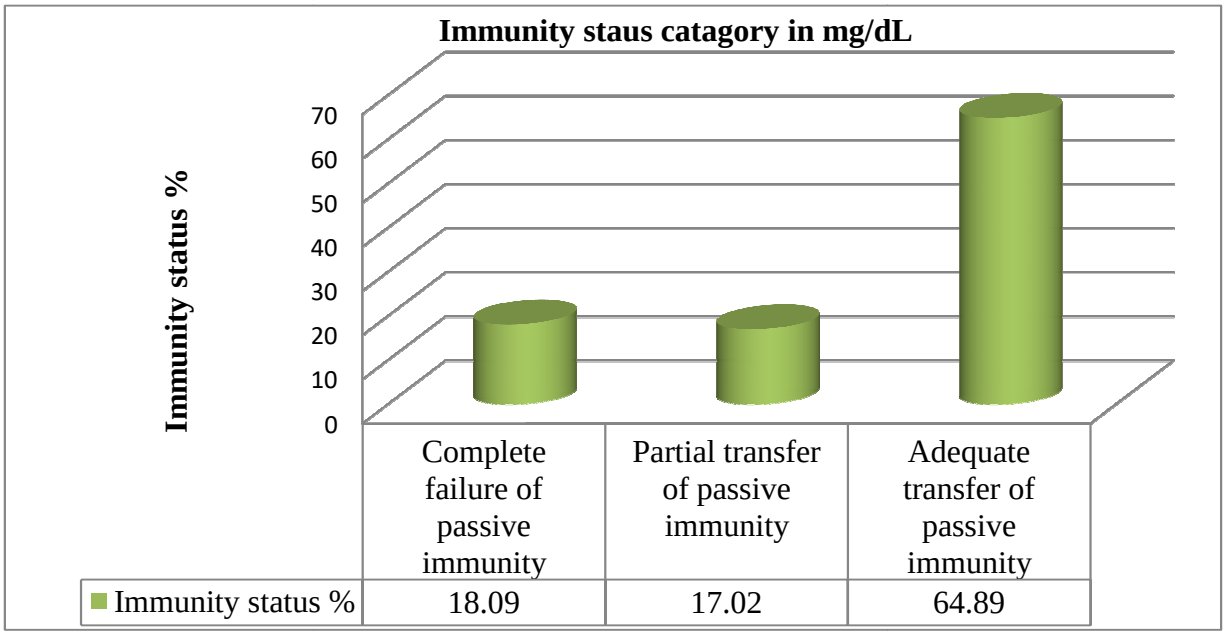

Figure: 4. Level of immunity status in the study area

4.1. Association Between Factors and FTP

Sexes of calve, cow parity, birth time, birth site, age at first colostrum ingestion, birth condition and mothering instincts were considered as risk factors to FTP are shown in (Table: 3). Their effect on calves serum IgG concentration as measured within a week after birth**.** Sexes of calve, cow parity, birth site, age ate first colostrum ingestion, dystocia and mothering instincts were found to be significantly associated with failure of passive transfer of immunity. Male calves had lower serum IgG concentrations than female calves. In the same way, low agammaglobulinemia were observed on those calves born from heifers birth site (when calves born out in the field), age at first colostrum ingestion, birth condition (when experienced dystocia) dam mothering instinct, were found to be significantly associated with FPT; whereas birth time was not significantly associated with serum IgG concentration.

Table: 3. Association between variables and FTP condition in calves

Variables	Variable category	No	Immunity level			$\chi 2$	P-Value
			FPTI %	PPTI%	APTI%		
Sex	Male	37	58.82	56.25	29.51	7.0910	0.029
	Female	57	41.18	43.75	70.49		
Cow parity	One parity	16	47.06	25	6.5	25.3719	0.013
	More than one parity	78	52.94	75	93.44		
Birth time	Daytime	70	94.12	68.75	70.49	4.2346	0.120
	Night time	24	5.88	31.25	29.51		
Birthsite	At home	74	52.94	68.78	88.52	11.1952	0.004
	Out in the field	20	47.06	31.25	11.48		
Age at first colostrum ingestion	< 6 hours	77	70.59	75	88.52	35.0003	0.004
	≥ 6 hours	17	29.41	25	11.48		
Mothering instinict	Good [I]	82	64.71	62.50	86.89	9.7539	0.008
	Poor [II]	12	35.29	37.50	13.11		
Birth difficulties	Normal [0]	74	70.59	75	95.08	6.9345	0.031
	Assisted [1]	20	29.41	25	4.92		

IgG: serum immunoglobulin concentration; p-value: calculated probability; $\chi 2$**:** *Chi-square test; ATPI: adequate passive transfer of immunity; PPTI: partial passive transfer of immunity; FPTI: failure of passive transfer of immunity.*

Table: 4. Mean and Standard deviation (SD) of serum IgG concentration for different variables and variables category

Variables	Variable Category	Serum IgG		
		No	Mean	SD
Sex	Male	37	1611.08	744.68
	Female	57	1932.41	678.15
Cow parity	One parity	16	1169.45	619.68
	More than one	78	1963.49	669.01
Birth time	Daytime	70	1740.31	758.76
	Night time	24	1997.33	556.76
Birthsite	At home	74	1934.37	684.01
	At field	20	1330.69	654.62
Age at first colostrum	< 6hours	77	1852.15	701.58
Ingestion	>6 hours	17	1596.60	779.29
Birth condition	Normal [0]	82	1882.87	679.18
	Assisted [1]	12	1280.15	790.13
Mothering instinct	Good [I]	74	1898.10	697.82
	Poor [II]	20	1428.04	680.94
FPTI	< 800 mg/dL	17	589	200.97
PPTI	800- 1600 mg/dL	16	1404.71	195.60
ATPI	> 1600 mg/dL	61	2250.15	342.79

SD: Standard deviation; ATPI: adequate passive transfer of immunity; PPTI: partial passive transfer of immunity; FPTI: failure of passive transfer of immunity.

4.2. Calve Morbidity and Mortality

Morbidity and mortality rates of calves were significantly associated with serum IgG concentration ($\chi 2 = 30.7698$; (*P = 0.000*), rate $\chi 2 = 17.5835$, (*P = 0.000*); respectively). Other risk factors such as sex of the calve, cow parity, birth site, mothering instinct, birth condition and time at first colostrum ingestion were known to highly influence morbidity and mortality condition in calves (*P <0.031 to 0.004*). The mean serum Ig level for the nine died calves was 736.31 mg/dL and the ability of calves surviving under morbidity and mortality highly depend upon their serum IgG concentration (Table: 5). Calves with small serum IgG concentration were more vulnerable to disease and death.

Table: 5. Mean and Standard Deviation value of serum IgG for calves under morbidity and mortality condition

Variables	Variable Category		Serum IgG	
		No	Mean	SD
Morbidity	Diseased	21	1069.30	599.14
	Not diseased	73	2017.84	603.02
Mortality	Died	9	736.31	584.13
	Not died	85	1991.18	635.13

Calves with FPTI and PPTI are more likely to get disease and die before weaning. Morbidity and mortality risk for calves with adequate transfer of passive immunity are extremely small when compared to calves with FPT and PFTP (Table. 6).

Table: 6. Association between immunity status and morbidity and mortality in calves

Variables	Variable category	No	Immunity Satus			$\chi 2$	P value
			FPTI%	PPTI%	APTI%		
Morbidity	Diseased	21	58.82	50	4.92	30.7698	0.000
	Not diseased	73	41.18	50	95.08		
Mortality	Died	9	35.29	12.50	1.64	17.5835	0.000
	Not died	85	64.71	87.50	98.36		

According to this finding, morbidity and mortality risk in calves decreased substantially as serum IgG concentrations increased (Figure 5). The highest mortality was observed among calves whose IgG concentration was less than 800 mg/dL. Calves having a partial transfer of passive immunity serum IgG concentration from 800-1600 mg/dL are better protected from mortality than those having complete failure transfer of immunity < 800 mg/dL IgG and calves having an adequate transfer ≥ 1600 mg/dL IgG have well protected from mortality than others.

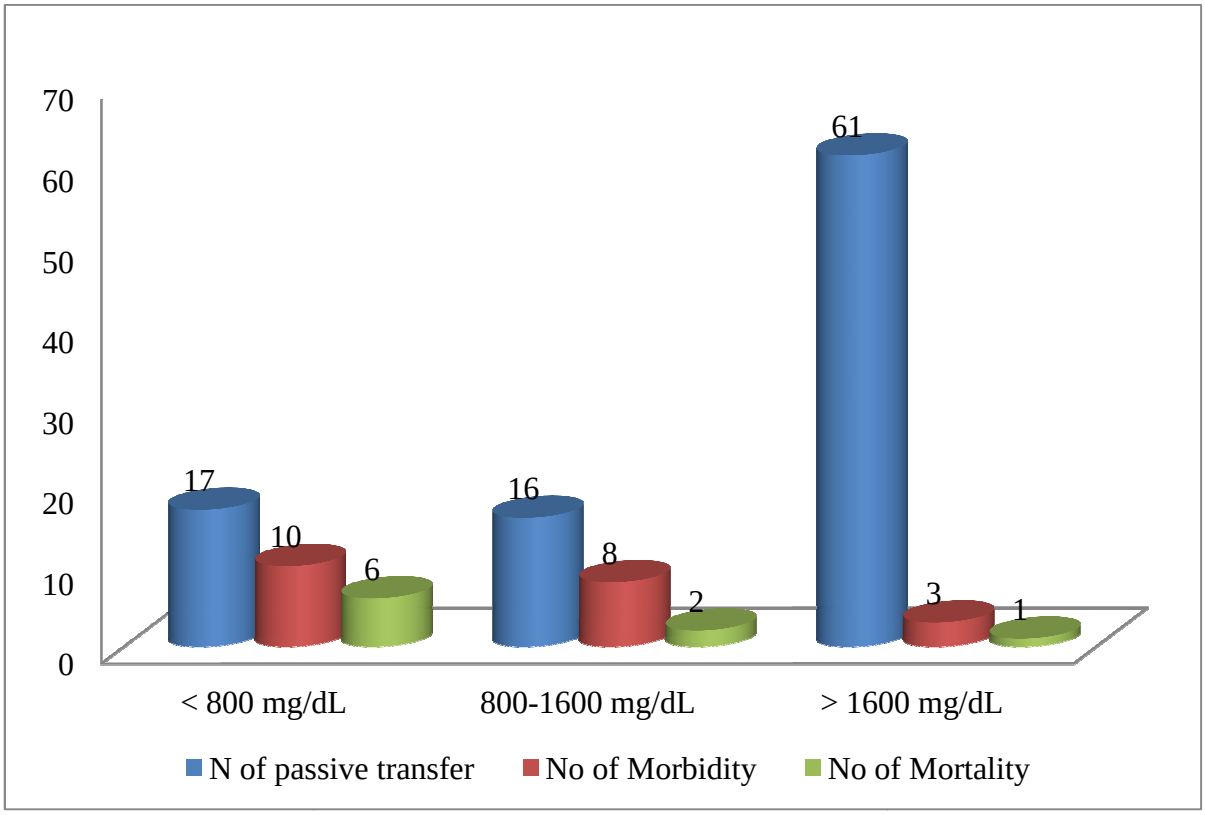

Figure: 5. Association between passive immunity status and calve morbidity and mortality.

4.3. Key Informant Interview Result

According to the response from key informant interview, unlike most other cows no special care and attention are given to the pregnant cows near to giving parturition. As well, the exact month and week of pregnant cows going to give birth is not known. Sometimes, pregnant cows are engaged in grazing and given birth out in the field or at grazing area. According to the pastoralist community, if calve was born out in the field and able to suckle little amount of colostrum at there, it is considered as a sufficient, and then calves come back to home by following the dam. Feeding of calve high amount of colostrum before six hours of age is not known by knowledge level. If one cow deliver in early in the morning and removed fatal membrane, then the cow was engage for grazing with other cows. On the other hand, key informants indicate, male and female calves are not equally treated. In the case of problem associated birthing difficulties, when their pregnant cows are unable to give birth and need assist, they will be assisted by some body who has experience before.

5. DISCUSSION

In this study, radial immunodiffusion assay result for two calves were unreadable because of the ring diameter is smaller than that of lowest standard and there are no "low level" radial immunodiffusion plates to re-run the sample, this low-level plate is developed under the manufacturer especially to be tested for low concentration so, these two samples were eliminated from the study. During field work 13 blood samples were collected from calves with 24 hours of age. Unfortunately, from these calves eight of them are ingested colostrum almost between 5-6 hours postpartum and the rest five were ingesting before six hours. Those two calves with smaller ring diameter than of the lowest standard can be from one of those animals. Similarly, ring diameter size for 16 samples was found above the top standards pecified by the manufacturer. The ring diameter result may exceed than the size of the top standard, due to overfilling of wells with serum samples. According to manufacturer manual reference, when such kind of result is attained, it is appropriate to rerun the samples using another new kit. But there was no extra RID kit is available to rerun those samples again. Hence, these 16 sampleswere removed out of the study. In terms of assay reliability, the result obtained for 94 sample is not outside of the limit range of RID which is between 60.711 to 3624.503 mg/dL.

Mean ± SD of serum IgG concentration in the present report was 1805.93 ± 718.13 mg/dL respectively and rangs from 189.132 to 2783.4 mg/dL. The results of this study were closer to the study of Vandeputte *et al.* (2011), who conducted the study on 108 calves and found mean ± SD serum IgG concentration of 2,310 ± 750 mg/dL, ranging from, 730-4,530 mg/dL. Difference was observed in mean ± SD value between the current and previous reports of Corke (2010), who reported mean ± SD of 2,550, ± 1909 mg/dL, and IgG concentration range from 120-3900 mg/dL. Animal breed, management condition, the environment where the animal lives, previous exposure of animals to various diseases and pathogen may cause a difference in serum IgG concentration.

The radial immunodiffusion assay result showed 35.11% of newborn calves in the study area have less immunoglobulin and the remaining 64.89 % had adequate passive immunity. The result was closer to the findings of Amoki (2001), who found 32% of total failure of FTP. Ibrahim and Lemma (2009), reported 30.2% of total FTP on market-oriented dairy farms

which is lower than the result of the current study. On the other hands, big difference was observed between the current study and the report of Yeshwas (2015), in Bahir, Dar milk-shed, in that higher percentage of calves (65.2%) were not immunologically protected. The highest prevalence of passive failure in yeshiwas study could be due to the small sample size. The report of Razzaque *et al.* (2009) also found, 50 % of low level of IgG in calves serum, which is deferent from the current study report. In fact, it is not hard to find out why this difference is coming; calve and colostrum management condition under different production system is never being same, therefore; it plays a significant role in such a peculiarity. In the study, birth site, age at first colostrum ingestion, mothering instincts, birth condition and dam parity were key factors that were associated with management condition and aggravating the problem.

The RID assay method classifies the degree of Ig protection level in to three main categories (complete failure, partial transfer and adequate transfer of passive immunity). It could be better to group those partially protected calves in to complete failure of passive transfer as they are not fully protected and hence the likelihood of infection is certainly higher either in neonatal or older life. McGuirk and Collins (2004), indicates partial or complete failure of passive transfer of colostral immunoglobulins is a primary cause of disease and mortality in neonatal calves. The current study result showed 18.09 % calves had FPT (serum IgG concentration < 800 mg/dL), and 17.02 % were considered to have partial passive transfer of immunity (serum IgG concentration 800-1600 mg/dL) and 64.89 % had adequate passive immunity transfer (serum IgG concentration was ≥ 1600 mg/dL). Similarly, Amoki (2001), and Yeshwas (2015), stated their study result by grouping FTP condition into three catagories. Generally, issue of failure of passive transfer in the pastoral production system is crucial matter, but no published reports are found in Ethiopia so far. Thus, this finding can be considered as the first one, which was applied at pastoral production system. To conclude, when comparing those two groups (complete failure and partial transfer), calves having complete failure in transfer are more prone to disease and mortality while calves with partial passive transfer are somewhat protected than calves with complete failure. On the other hand calves with adequate passive transfer are well protected than calves with complete failure and partial transfer.

Different management practice, cow and calve side factors are closely associated with development of low agammaglobulinemia. Among these, several factors such as sex (male), heifer as primiparous, birth at out in the field, the presence of birth difficulties, poor mothering instinct (when first calve heifer) and late colostrum consumption (after 6 hours) are the main factors for the occurrence of FTP in calves.

Higher serum IgG concentration was observed in female calves than male calves. The result agrees with a previous study by Ibrahim and Lemma (2009), and Angulo *et al.* (2015), who indicated that cows with female calve produced higher amounts of colostrum and milk during lactation than cows with male calve. Roy (1990), suggested that the gender of calve might be related to blood volume than to apparent efficiency of absorption (AEA) and the larger size of bull calves may influence the metabolic state of the calves, thereby affecting immunoglobulin absorption. Another possibility that was observed in the study was management practice, which is the pastoral production system, male calves have less future replacement value, and they also given less attention and care at the very beginning compared to female calves. Most of the smallholder community use one or two superior male bulls only for breeding purpose while other male calves in the herd are culled out from the herd by selling or slaughtering.

In this study calves from primiparous cow have lower IgG concentration than calves from multiparous. The influence of parity on IgG concentration in the present study agrees with the study of Godden *et al.,* (2009) and Ibrahim and Lemma, (2009). Similarly, Angulo *et al.* (2015), found that cows with one to two parity produced lower amounts of colostrum than cows with more parity, this is due to the fact that primiparous animals have not had the time to accumulate and build up resistance to the various herd-specific pathogens. Older cows build up a larger amount of antibodies because of a greater exposure time to pathogens. This exposure provides to calve attain higher amounts of antibodies from the maternal colostrum during ingestion. Immunoglobulin concentrations in primiparous cows significantly differed from older cows, with multiparous cows secreting 1.3-1.6 times higher antibody concentration (Liu *et al.*, 2009). This suggests the importance to establish a favourable condition for the calves to gain adequate serum IgG by vaccinating their dam prior to calving.

High statistical association was observed between mothering instincts and failure of passive transfer of immunity. The study agrees with Selk (2003), who reported on disease protection for newborn calves. He concludes that mothering instinct problem is observed more in heifers; cows have better maternal instincts than heifers. Mothered calves absorb 70 percent or more immunoglobulin than non-mothered calves. Poor mothering ability combined with reduced calve vigour could decrease the effectiveness of passive transfer in calves (Sivula *et al.*, 1996). It is possible to give some explanations for this study, calves that suckle colostrum from cow with poor mothering instinct are likely to ingest less colostrum than the recommended amount during the first feeding. Furthermore, the ability of calves to absorb IgG decreases with time, and some calves do not suckle quickly enough because of dam's poor mothering instinct. Based on the results of the study, it is advisable to milk the dam early after birth and feeding the colostrum to the newborn calve using another alternative like nipple feeding or bottle feeding.

Statistically significant associations was observed between FTP and age at first colostrum ingestion. Higher serum IgG concentration is observed in calves feeding colostrum before six hours than calves with delayed colostrum ingestion. The current report was agrees with previous reports of Ibrahim and Lemma, (2009); Bartier (2013) and Muller, (2017). The timing of colostrum feeding is very crucial. Waldhalm *et al.* (1971), indicated that suckling within 6 hours of birth attained higher levels of all sub-classes of Ig than calves failing to suckle. Every half hour after birth that colostrum feeding is delayed, antibody transfer decreases by about 5 % (Moran, 2002). Similarly, for each hour delay in colostrum feeding in the first 12 hours of life, the chance of a calve becoming ill increase by 10 % (Wudu, 2004). Calves that do not suckle within 6 hours of their life have already lost the opportunity for 30 % of the possible antibodies entering its bloodstream (Moran, 2002). By six hours post-partum, the gut wall's ability to absorb Ig is already reduced by 33% and at 24 hours post-partum this absorption is decreased by around 90 % (Heinrichs and Jones, 2003).

In the same way, low serum IgG concentration was observed in calves born from assisted dam which agrees with report of Ibrahim and Lemma, (2009). Similarly, the study was consistent with Yeshwas (2015), who explained that calves born from difficult birthing, especially those born from very difficult assisted or prolonged parturition, have a lower appetite when fed. Association between calving difficulty and passive immune transfer may be mainly due to

reduced vigour in calves born from assisted calvings. In low vigour calves standing up, walking and consuming colostrum may be challenging. Calves with low vigour have an increased risk of failure of passive transfer due to low volume of ingested colostrum (Barrier *et al.*, 2012). Delayed and/or reduced colostrum consumption raised from lack of vigour has been found to result in lower passive immune transfer and increase failure of passive immune transfer in calves born from difficult parturitions. However, Murray (2014), did not find any association between calving ease and passive immune transfer. Early colostrum feeding for such kind of calves using another best alternative may solve the problem.

In the study, higher association was observed between birth site and FTP condition in calves. Calves born outside in the field are more prone to develop lower immunoglobulin than calves born at home. When explaining this, calves born out in the field unable to suckle by them, due to lack of opportunity to get assistance. If, the cow has at the risk of birthing dificulty, may not have opportunity to get help. This situation will reduce the calves' vigour. This suggests that routine monitoring of pregnant cows will reduce the chance of giving birth out in the field or in grazing area. If this is so, cow can get assist right away, if the cow has birthing difficulties. Alao the newborn calve was not exposed to develop FTP arising from low colostrum ingestion.

Highly significant association were observed between morbidity rate and presence of failure of passive transfer in calves. The report of the present study agrees with previous reports Amoki, (2001); Ibrahim and Lemma, (2009). Particularly, calves with inadequate colostrum intake, leading to low immunoglobulin concentration within the first week of life exhibit a greater risk of neonatal morbidity and mortality (Wittum and Perino, 1995). Ibrahim and Lemma (2009), explain that presence of failure transfer of passive immunity in calves was directly associated with early calve morbidity. In this study, calves with complete failure and partial transfer of passive immunity exhibit 58.82 % and 50 % morbidity condition respectively; and both groups have possibility to be exposed to disease. In this study, the greatest relative risk of morbidity is observed in calves whose serum total IgG concentration is lower than 800 mg/dL. Similarly, calves with partial transfer had more chance to be exposed to disease than calves with adequate transfer. This means that calves having adequate passive immunity are well protected from morbidity as supported by previous studies in Ethiopia (Amoki, 2001; Ibrahim and Lemma, 2009; Yeshwas, 2015).

In this study, high significance association was observed between FTP and mortality. This report agrees with Ibrahim and Lemma, (2009). According to Yeshwas (2015), calves with history of partial colostrum feeding were at higher risk of mortality than those calves with complete colostrum feeding. Similarly, Tyler *et al.* (1999), used population mortality and relative risk of mortality in each serum protein concentration stratum to determine the population baseline mortality and mortality due to inadequate passive transfer and observed that 39 % of the observed mortality was attributed to inadequate passive transfer or FTP. The present study showed 35.29 %, 12.5 % and 1.64 % mortality were due to failure, partial and adequate transfer of passive immunity respectively. Mortality rate in calves decreased substantially as serum IgG concentrations increased. Particularly, mortality problem was higher in calves with serum IgG < 800 mg/dL than calves with partially protected; for example nine calves withdrawn from the study because of death have mean serum IgG of 736.31 mg/dL meaning that the value falls under complete failure of transfer. On the other hand, calves with adequate transfer are well protected against mortality. A young stock mortality survey by Tsegaw *et al.* (2016), indicate that 26–29.2% mean annual young stock mortality was observed in the pastoral production system.

According to answer from key informants interview, there is no special care and attention given to pregnant cows that are near to give birth. Likewise, the exact month and week of birth for pregnant cows are not known. Kerr (2013), described that pregnant animals very important needs that are different from those of other livestock. Without good record keeping system or management calendar, livestock producers can easily overlook crucial management task and disaster can happen.

Sometimes, pregnant cows engaged in grazing and given birth out in the field or at grazing area. For calves born in such situation it cannot be sure that calve have ingested adequate amount of maternal colostrum. As explained in the above discussion, when calves are born out in the field, they are less likely to develop adequate immunity. The pastoralists believed that if calve are born out in the field and are able to suckle little amount of colostrum, it is considered as sufficient. But Donna (2017), indicated that calves which do not ingest and absorb adequate IgG antibodies have lower weight gain, increased risk for disease and death, and decreased milk production during their first lactation. In addition, within a few hours after birth the newborn calves go back to home by following their dam. When newborn calves

obligated to travel a long distance following their dam, they might be exposed to high physiological stress. This stress condition may be able to cause other health problems lasts until death. Hulbert and Moisa (2016), mentioned that, psychological stress is known to suppress immune function and increase susceptibility to infections and if stress condition is severe, it may impede immunocompetence

In fact, there is one good cultural custom in the study area regarding colostrum feeding perception, the community does not use the milk for food purpose until a week and only the calves will allowed suckling the dam. Moran (2002), described the term "colostrum" is all the milk produced by cows up to five days after calving. Therefore, it is thought to be good to allow calves to ingest colostrum for up to five days. This helps the newborn calves to get better nutrition from the milk. Quigley (2002), described that after about 24 hours of age, the chance to provide the calf with antibodies is gone. However, it is important to continue to feed colostrum for 2 to 3 days after birth, thereby the colostrum will bathe the calf's digestive tract and make it difficult for bacteria to attach to the intestinal wall. This "local effect" can reduce the incidence of scours during the first several weeks of life.

The community is not familiar in the feeding of calves with ample amount of colostrum before six hours of age. If once the cows deliver in the morning and removed fetal membrane, then the cow will be engaged in grazing with other cows, therefore, calves do not have the chance to ingest colostrum until the dam returns from grazing. This would mean, the calve will no longer have enough milk before six hours and thus results in agammaglobulinemia in calves. Merricks (2005) stated that calves should consume a minimum of 2 liters of colostrum within the first hour after birth, preferably within the first 30 minutes. Similarly, Chigerwe *et al.* (2009) recommend that calves have to ingest 3 litres of colostrum in first feeding before six hours and again will need to ingest 1 litre of colostrum within 12 hours to have an optimum colostral intake. Feeding high-quality colostrum to calves as soon as possible after birth will provide them not only with immunoglobulins to help fight disease but also with other nutritive values such as energy and high levels of vitamins and minerals and non-nutritive growth factors.

On the other hand, key informants stated that male and female calves are not treated equally, because male calves are not used as future stock unlike females, so female calves get more care than male.

An interesting finding was that he pastoralists use traditional medicines to solve poor mothering instinct associated with first calving heifers. The traditional medication is placed in the cow's reproductive organ. From that moment on it is believed by the pastoralist that the heifer/cow with poor mothering instincts will begin to show a good motherly behaviour. Though not proven scientifically, it believed that this medication is associated with oxytocin hormone. Thomas (2015) describes first-calf heifers produce less oxytocin than cows that have previous calves and this may explain why heifers may be less motherly and reject or detach their calves. Oxytocin can switch off the heifer’s aggression, reluctance or fear, and turn it into good mothering.

6. CONCLUSION AND RECOMMENDATION

The present study conducted in pastoral production system has revealed presence of high failure of passive transfer of immunity than most previous reports. This will, in the long run, influence the survival of newborn calves and contribute significant role to an increase in young stock mortality. Among factors that were studied for their effect on the failure transfer of passive transfer, calf sex, dam parity, birth site, birth condition, mothering instinct and age at first colostrums ingestion were found statistically significant associated with failure of passive transfer of immunity, morbidity and mortality. The incidence of morbidity and mortality was apparently higher in calves having lower serum IgG concentration. The highest percentage of failure of passive transfer of immunity in the study area could be associated with; improper colostrum feeding practice, improper handling of newborn calves and absence of regular care for pregnant cows. However, the existing management difficulty in the pastoral production system can be controlled and improved with a coalition of governmental and pastoralist communities almost at minimum a cost. Based on the above conclusion the following recommendations are forwarded:

- Improving knowledge of pastoralists concerning calve and colostrum management practices help to ensure the newborn calve to ingested sufficient amount of colostrum just after birth for achieving adequate passive transfer of immunity.

- Caring for pregnant cows, adoption of regular observation have great significance in reducing the FTP prevalence.

- Further studies are needed in the future, which recommends the right time at which health and management intervention will needed.

REFERANCES

Adams, G. D., Bush, L. J., Horner, J. L. and Staley, T. E. (1985): Two methods for administering colostrum to Newborn Calves1, *Journal of dairy science*. Elsevier **68**: 773–775.

Alberghina, D., Casella, S., Vazzana, I., Ferrantelli, V., Giannetto, C. and Piccione, G. (2010): Analysis of serum proteins in clinically healthy goats (Capra hircus) using agarose gel electrophoresis, *Veterinary Clinical Pathology*. Wiley Online Library **39**: 317–321.

Amoki, O. T. (2001): Management of dairy calves in Holleta area, central highlands of Ethiopia, *Unpublished MSc thesis, Addis Ababa University*.

Amuamuta, A., Asseged, B. and Goshu, G. (2006): Mortality analysis of Fogera calves and their Friesian crossesin andassa cattle breeding and improvement ranch, Northwestern Ethiopia, *Revue de médecine vétérinaire*. Ecole nationale vétérinaire de Toulouse **157**: 525.

Angulo, J., Gómez, L. M., Mahecha, L., Mejía, E., Henao, J. and Mesa, C. (2015): Calf's sex, parity and the hour of harvest after calving affect colostrum quality of dairy cows grazing under high tropical conditions, *Tropical animal health and production*. Springer **47**: 699–705.

Arsham H (2005) Questionnaire design and survey sampling. http://homeubalt.edu/ntsbarsh/stat-data/surveys.htm

Arthington, J. D. (2001): Technologies for delivering passive immunity to newborn calves, in *Proceedings 12lh Annual Florida Ruminant Nutrition Symposium* 80–90.

Arnold M. (2014): Colostrum Management for Dairy Calves. University of Kentucky, College of Agriculture. http://www.afsdairy.ca.uky.edu/extension/nutrition/calves/colostrummanagement

Asresie, A., Zemedu, L. (2015): Contribution of Livestock Sector in Ethiopian Economy : A Review, *Advances in Life Sciences and Technology* **29**: 79–91.

Azizzadeh, M., Shooroki, H. F., Kamalabadi, A. S. and Stevenson, M. A. (2012): Factors affecting calf mortality in Iranian Holstein dairy herds, *Preventive veterinary medicine*. Elsevier **104**: 335–340.

Baldwin, R. L., McLeod, K. R., Klotz, J. L. and Heitmann, R. N. (2004): Rumen development, intestinal growth and hepatic metabolism in the pre-and postweaning ruminant, *Journal of dairy science*. Elsevier **87**: E55–E65.

Barrier, A. C., Haskell, M. J., Macrae, A. I. and Dwyer, C. M. (2012): Parturition progress and behaviours in dairy cows with calving difficulty, *Applied animal behaviour science*. Elsevier **139**: 209–217.

Barrington, G. M., McFadden, T. B., Huyler, M. T. and Besser, T. E. (2001): Regulation of colostrogenesis in cattle, *Livestock Science*. Elsevier **70**: 95–104.

Barrington, G. M. and Parish, S. M. (2001): Bovine neonatal immunology, *Veterinary Clinics of North America: Food Animal Practice*. Elsevier **17**: 463–476.

Barrington, G.M. and Parish, S.M., (2002): Ruminant immunodeficiency diseases. *Large animal internal medicine*, *3*, pp.1600-1602.

Bartier, A. (2013): Evaluation of Colostrum Quality, Passive Immunity and Dairy Calf Management Practices in Alberta.

Baumrucker, C. R., Burkett, A. M., Magliaro-Macrina, A. L. and Dechow, C. D. (2010): Colostrogenesis: Mass transfer of immunoglobulin G1 into colostrum, *Journal of Dairy Science*. Elsevier **93**: 3031–3038.

Beam, A. L., Lombard, J. E., Kopral, C. A., Garber, L. P., Winter, A. L., Hicks, J. A. and Schlater, J. L. (2009): Prevalence of failure of passive transfer of immunity in newborn heifer calves and associated management practices on US dairy operations, *Journal of Dairy Science*. Elsevier **92**: 3973–3980.

Behnke, R. and Metaferia, F. (2013): The contribution of livestock to the Ethiopian economy, *Nairobi: IGAD Centre for Patoral Areas and Livestock Development*.

Besser, T. E. and Gay, C. C. (1994): The importance of colostrum to the health of the neonatal calf, *Veterinary Clinics of North America: Food Animal Practice*. Elsevier **10**: 107–117.

Besser, T. E., Gay, C. C. and Pritchett, L. (1991): Comparison of three methods of feeding colostrum to dairy calves., *Journal of the American Veterinary Medical Association* **198**: 419–422.

Bielmann, V., Gillan, J., Perkins, N. R., Skidmore, A. L., Godden, S. and Leslie, K. E. (2010): An evaluation of Brix refractometry instruments for measurement of colostrum quality in dairy cattle, *Journal of dairy science*. Elsevier **93**: 3713–3721.

Birara, E. and Zemen, A. (2016): Assessment of the Role of Livestock in Ethiopia : A Review, *American-Eurasian Journal of Scientific Research* **11**: 405–410.

Blowey, R. W. (1999): A veterinary book for dairy farmers. Farming Press.

Blum, J. W. and Hammon, H. M. (2000): Bovine colostrum: more than just an immunoglobulin supplier, *Schweizer Archiv fur Tierheilkunde* **142**: 221–228.

Catley, A. (2007): Impact Assessments of Livelihoods-based Drought Interventions in Moyale and Dire Woredas, *A Pastoralist*.

Chenyambuga, S. W. and Mseleko, K. F. (2009): Reproductive and lactation performances of Ayrshire and Boran crossbred cattle kept in smallholder farms in Mufindi district, Tanzania, *Livestock Research for Rural Development*. CIPAV Foundation **21**: 100.

Chester-jones, H. (2009): Colostrum 5 C ' s for a Healthy Start 1–30.

Chigerwe, M., Tyler, J. W., Summers, M. K., Middleton, J. R., Schultz, L. G. and Nagy, D. W. (2009): Evaluation of factors affecting serum IgG concentrations in bottle-fed calves, *Journal of the American Veterinary Medical Association*. Am Vet Med Assoc **234**: 785–789.

Concha, C., Holmberg, O. and Morein, B. (1980): Characterization and response to mitogens of mammary lymphocytes from the bovine dry-period secretion, *Journal of Dairy Research*. Cambridge University Press **47**: 305–311.

Conneely, M., Berry, D. P., Sayers, R., Murphy, J. P., Lorenz, I., Doherty, M. L. and Kennedy, E. (2013): Factors associated with the concentration of immunoglobulin G in the colostrum of dairy cows, *Animal* **7**: 1824–1832.

Connor, E. E., Baldwin, R. L., Li, C., Li, R. W. and Chung, H. (2013): Gene expression in bovine rumen epithelium during weaning identifies molecular regulators of rumen development and growth, *Functional & integrative genomics*. Springer **13**: 133–142.

Corke, M. J. (2010): The use of colostrum and colostrum supplements in neonatal calves., *Cattle Practice*. British Cattle Veterinary Association **18**: 216–219.

Costello, R., Specialist, D. T. and Groove, E. (2010): Bottles vs . Pails : Are There Differences Between Calf Feeding Methods ?www.merricks.com accessed april 26/2018 1–8.

CSA (2008) :CSA (Centeral Statstics Agency): Federal Democratic Republic of Ethiopia Agricultural Sample Survey 2007 / 08 [2000 E. C.] Volume II report. **II**.

CSA (Central Statistics Authority) (2012): Federal Democratic Republic of Ethiopia, Agricultural sample survey on livestock and livestock production (Private peasant holdings), Statistical Bulletin No. 532, Addis Ababa, Ethiopia. 2: 9-20.

Dawes, M. E., Tyler, J. W., Hostetler, D., Lakritz, J. and Tessman, R. (2002): Evaluation of a commercially available immunoassay for assessing adequacy of passive transfer in calves, *Journal of the American Veterinary Medical Association*. Am Vet Med Assoc **220**: 791–793.

Day, M. J. and Schultz, R. D. (2014): Veterinary immunology: principles and practice, Immune System Ontogeny and Neonatal Immunology. Edited by S. Edition. CRC Press.

Dekeba, A., Ayalew, W., Hedge, P. B. and Taddese, Z. (2006): Performance of the Abernossa ranch in the production of Ethiopian Boran x Holstein crossbred dairy heifers in Ethiopia, *Ethiopian Journal of Animal production* **6**: 33–53.

Detrick, B., Hamilton, R. G. and Folds, J. D. (2006): Manual of Molecular and Clinical Lab Immunology. American Society for Microbiology Press.

Devery-Pocius, J. E. and Larson, B. L. (1983): Age and Previous Lactations as Factors in the Amount of Bovine Colostral Immunoglobulins1, *Journal of Dairy Science*. Elsevier **66**: 221–226.

Doepel, L. and Bartier, A. (2014): Colostrum Management and Related to Poor Calf Immunity: Faculty of Veterinary Medicine, University of Calgary, 3330 Hospital Drive NW, Calgary, AB T2N 4N1 **26**: 137–149.

Dominguez, E., Perez, M. D., PUYOL, P., SÁNCHEZ, L. and CALVO, M. (2001): Effect of pH on antigen-binding activity of IgG from bovine colostrum upon heating, *The Journal of dairy research*. Cambridge University Press **68**: 511.

Donna M (2017): Dairy Calf Management Practices Impact Future Production Colostrum intake: University of Kentucky Extension Dairy Nutritionist.

Duguma, B., Tegegne, A. and Hegde, B. P. (2012): Smallholder livestock production system in Dandi district, Oromia Regional State, central Ethiopia, *Read and write* **20**: 25–26.

Eshetu, E. and Abraham, Z. (2016): Review on live animal and meat export marketing system in Ethiopia : challenges and opportunities, *Scientific & Innovative Research* **5**: 59–64.

FAO (Food and Agricultural Organization), (2012: Crop and Food Security Assessment Mission to Ethiopia. Special report. Food and Agriculture Organization of the United Nations, Rome, Italy http://www.fao.org/giews/html

Fetcher, A., Gay, C. C., McGuire, T. C., Barbee, D. D. and Parish, S. M. (1983): Regional distribution and variation of gamma-globulin absorption from the small intestine of the neonatal calf., *American journal of veterinary research* **44**: 2149–2154.

Ferede, Y. (2015): Epidemiological determinants and magnitude of calf morbidity and mortality in Bahir Dar milk-shed, north west Ethiopia. Addis Ababa University.

Fishwick, J. (2011): Welfare issues associated with calving, *Cattle Practice*. British cattle Veterinary Association. The Green, Frampton-on-severn Gl2 7ep, Glos, England **19**: 1–2.

Foley, J. A. and Otterby, D. E. (1978): Availability, Storage, Treatment, Composition, and Feeding Value of Surplus Colostrum: A Review1, 2, *Journal of Dairy Science*. Elsevier **61**: 1033–1060.

Fonty, G., Gouet, P. and Nebout, J. M. (1989): Development of the cellulolytic microflora in the rumen of lambs transferred into sterile isolators a few days after birth, *Canadian journal of microbiology*. NRC Research Press **35**: 416–422.

Gapper, L. W., Copestake, D. E. J., Otter, D. E. and Indyk, H. E. (2007): Analysis of bovine immunoglobulin G in milk, colostrum and dietary supplements: a review, *Analytical and bioanalytical chemistry*. Springer **389**: 93–109.

Garry, F., Adams, R. and Aldridge, B. (1993): Role of colostral transfer in neonatal calf management: current concepts in diagnosis, *The Compendium on continuing education for the practicing veterinarian (USA)*.

Godden, S. (2008): Colostrum management for dairy calves, *Veterinary Clinics: Food Animal Practice*. Elsevier **24**: 19–39.

Godden, S. M., Haines, D. M., Konkol, K. and Peterson, J. (2009): Improving passive transfer of immunoglobulins in calves. II: Interaction between feeding method and volume of colostrum fed, *Journal of Dairy Science*. Elsevier **92**: 1758–1764.

Gulliksen, S. M., Lie, K. I., Solverod, L. and Osteras, O. (2008): Risk factors associated with colostrum quality in Norwegian dairy cows, *Journal of Dairy Science*. Elsevier **91**: 704–712.

Guy, M. A., McFadden, T. B., Cockrell, D. C. and Besser, T. E. (1994): Effects of unilateral prepartum milking on concentrations of immunoglobulin G1 and prolactin in colostrum, *Journal of dairy science*. Elsevier **77**: 3584–3591.

Hassen, A., Tessema, Z. K. and Tolera, A. (2017): Seasonal variations in chemical composition, in vitro digestibility and ruminal degradation of browse species in the Rift Valley of Ethiopia, *Livestock Research for Rural Development* **29**.

Heinrichs, A. J., Kehoe, S. I., Gehman, A. M., Jones, C. M. and Tricarico, J. M. (2007): Case Study: Effects of Amylase on Rumen Development in Neonatal Dairy Calves1, *The Professional Animal Scientist*. Elsevier **23**: 64–69.

Heinrichs, A. J. and Jones, C. M. (2003): Feeding the newborn dairy calf. PennState, College of Agricultural Sciences, Agricultural Research and Cooperative Extension.

Hinde, K., Carpenter, A. J., Clay, J. S. and Bradford, B. J. (2014): Holsteins favor heifers, not bulls: biased milk production programmed during pregnancy as a function of fetal sex, *PloS one*. Public Library of Science **9**: e86169.

Hulbert, L. E. and Moisá, S. J. (2016): Stress, immunity, and the management of calves 1, *Journal of Dairy Science*. American Dairy Science Association **99**: 3199–3216.

Husband, A. J., Brandon, M. R. and Lascelles, A. K. (1973): The effect of corticosteroid on absorption and endogenous production of immunoglobulins in calves, *Immunology and Cell Biology*. Nature Publishing Group **51**: 707.

Hussain, K. (2011): Health Management of New Born Calf, *SMVS'Dairy Year Book* 47–48.

Ibrahim, A. and Lemma, A. (2009): Relationships between serum protein concentration and passive transfer of immunity, morbidity and mortality of dairy calves in market oriented urban dairy farms, *Revue de Medecine Veterinaire* **160**: 394–399.

Jainudeen, M. R. and Hafez, E. S. E. (2000): Gestation, prenatal physiology, and parturition, *Reproduction in Farm Animals, 7th Edition*. Wiley Online Library 140–155.

Jami, E., Israel, A., Kotser, A. and Mizrahi, I. (2013): Exploring the bovine rumen bacterial community from birth to adulthood, *The ISME journal*. Nature Publishing Group **7**: 1069.

Jones C. and Heinrichs J. (2011): Colostrum supplements and replacer. *Penn State Cooperative Extension*, DAS 11-180.

Kehoe, S. I., Jayarao, B. M. and Heinrichs, A. J. (2007): A survey of bovine colostrum composition and colostrum management practices on Pennsylvania dairy farms1, *Journal of dairy science*. Elsevier **90**: 4108–4116.

Kerr, S. (2013): Pregnant Animal Care, Extension, Wsu-klickitat County: http://www.smallfarms.oregonstate.edu/sfn/pregnantanimla

Kirk, J. H. Colostrum (2011): The key to control of early calfhood diseases and death loss. http://www.vetmed.ucdavis.edu/vetext/Inf-Da/Inf- Da_Colostrum.html

Klobasa, F., Werhahn, E. and Butler, J. E. (1987): Composition of sow milk during lactation, *Journal of animal science*. Oxford University Press **64**: 1458–1466.

Lang, B. (2008): Colostrum for the dairy calf. Ministry of Agriculture, Food and Rural Affairs, Ontario.

Langer, P. (2009): Differences in the composition of colostrum and milk in eutherians reflect differences in immunoglobulin transfer, *Journal of Mammalogy*. American Society of Mammalogists 810 East 10th Street, Lawrence, KS 66044-8897 **90**: 332–339.

Lascelles, A. K. (1963): A review of the literature on some aspects of immune milk, in *Dairy Sci Abstr* 359–364.

Lateur-Rowet, H. J. and Breukink, H. J. (1983): The failure of the oesophageal groove reflex, when fluids are given with an oesophageal feeder to newborn and young calves., *The Veterinary quarterly* **5**: 68–74.

Leadley, S. (2018): When to Test for Passive Transfer of Immunity, Calf and Heifer Management Specilaists.

Liu, G. L., Wang, J. Q., Bu, D. P., Cheng, J. B., Zhang, C. G., Wei, H. Y., Zhou, L. Y., Zhou, Z. F., Hu, H. and Dong, X. L. (2009): Factors affecting the transfer of immunoglobulin G1 into the milk of Holstein cows, *The Veterinary Journal*. Elsevier **182**: 79–85.

Lilius, E.-M. and Marnila, P. (2001): The role of colostral antibodies in prevention of microbial infections, *Current opinion in infectious diseases*. LWW **14**: 295–300.

LMP (2015): Ethiopia livestock master plan Roadmaps for growth and transformation.

Longenbach, J. I. and Heinrichs, A. J. (1998): A review of the importance and physiological role of curd formation in the abomasum of young calves, *Animal feed science and technology*. Elsevier **73**: 85–97.

MacDonald, M. and Simon, J. (2011): Climate, Food Security & Growth: Ethiopia's Complex Relationship with Livestock. Brighter Green.

Mancini, G. A., Carbonara, A. O. t and Heremans, J. F. (1965): Immunochemical quantitation of antigens by single radial immunodiffusion, *immunochemistry*. Elsevier **2**: 235-IN6.

Margerison, J. and Downey, N. (2005): Guidelines for optimal dairy heifer rearing and herd performance, *Calf and Heifer Rearing: Principles of Rearing the Modern Dairy Heifer from Calf to Calving, Nottingham University Press, Nottingham* 307–338.

Marnila, P. and Korhonen, H. (2011): Colostrum, *Encyclopedia of Dairy Sciences: volume three Editorss. John W. Fuquay, Patric F. Fox and Paul LH McSweeney*. Academic Press.

McClatchey, K. D. (2002): Clinical laboratory medicine. Lippincott Williams & Wilkins.

McEwan, A. D., Fisher, E. W. and Selman, I. E. (1970): Observations on the immune globulin levels of neonatal calves and their relationship to disease, *Journal of comparative pathology*. Elsevier **80**: 259–265.

McGuire, T. C., Pfeiffer, N. E., Weikel, J. M. and Bartsch, R. C. (1976): Failure of colostral immunoglobulin transfer in calves dying from infectious disease., *Journal of the American Veterinary Medical Association* **169**: 713–718.

McGuirk, S. M. (2007): Reducing dairy calf mortality, in *Proceedings of the 40th Annual Conference of the American Association of Bovine Practitioners, Vancouver, BC, Canada* 126–131.

McGuirk, S. M. (2010): Herd-Based Problem Solving: Failure of Passive Transfer, *University of Wisconsin, Madison*.

McGuirk, S. M. and Collins, M. (2004): Managing the production, storage, and delivery of colostrum, *Veterinary Clinics: Food Animal Practice*. Elsevier **20**: 593–603.

Mee, J. F. (2008): Prevalence and risk factors for dystocia in dairy cattle: A review, *The Veterinary Journal*. Elsevier **176**: 93–101.

Megersa, B., Yacob, A., Regassa, A., Abuna, F., Asmare, K. and Amenu, K. (2009): Prevalence and incidence rates of calf morbidity and mortality and associated risk

factors in smallholder dairy farms in Hawassa, Southern Ethiopia, *Ethiopian Veterinary Journal* **13**: 59–68.

Mehra, R., Marnila, P. and Korhonen, H. (2006): Milk immunoglobulins for health promotion, *International Dairy Journal*. Elsevier **16**: 1262–1271.

Mellado, M., Lopez, E., Veliz, F. G., De Santiago, M. A., Macias-Cruz, U., Avendaño-Reyes, L. and Garcia, J. E. (2014): Factors associated with neonatal dairy calf mortality in a hot-arid environment, *Livestock Science*. Elsevier **159**: 149–155.

Mendonsa, K. M. (2011): Factors affecting passive transfer in neonatal calves.

Merricks's, I. (2005): Colostrum -- The key to calf Survival Animal Nut: 1–12.

Meyer, H., Kröger, H. and Sagel, B. (1976): Studies on the various causes of birth weight in piglets as well as the body and blood composition of low-birth weight piglets, *DTW. Deutsche tierarztliche Wochenschrift* **83**: 438–448.

Mirkena, T., Duguma, G., Haile, A., Tibbo, M., Okeyo, A. M., Wurzinger, M. and Sölkner, J. (2010): Genetics of adaptation in domestic farm animals: A review, *Livestock Science*. Elsevier **132**: 1–12.

MoARD (2015): Ethiopia ' s Agriculture Sector Policy and Investment Framework (2010 – 2020) External Mid-term Review.

Molla, A. (1978): Immunoglobulin levels in calves fed colostrum by stomach tube., *The Veterinary record* **103**: 377–380.

Moran, J. (2002): Calf Rearing: A Practical Guide 226.

Moran, J. (2012): Rearing young stock on tropical dairy farms in Asia. CSIRO PUBLISHING.

Moran J (2002): The importance of colostrum to newborn calves, *Calf rearing: a practical guide, second edition* 211.

Morrill, K. M., Polo, J., Lago, A., Campbell, J., Quigley, J. and Tyler, H. (2013): Estimate of serum immunoglobulin G concentration using refractometry with or without caprylic acid fractionation, *Journal of dairy science*. Elsevier **96**: 4535–4541.

Morton, J. (2013): Livestock Emergency Guidelines and Standards (LEGS) Climate Change Briefing Paper. Livestock Emergency Guidelines and Standards.

Muller, J. (2017): Dehydration as a risk factor for calf mortality in northern Australia 1–120.

Murray, C. (2014): Characteristics, risk factors and management programs for vitality of newborn dairy calves.

Musayeva, K., Sederevičius, A., Želvytė, R. and Stankevičius, R. (2015): Analysis of factors influencing Immunoglobulin G concentration in Milk if Dairy Cows., *Veterinarija ir Zootechnika* **72**.

Mussa, M. (2004): A comparative study of pastoralist parliamentary groups: Case study on the pastoral affairs standing committee of Ethiopia. Natural Resources Institute, UK.

NMSA (2001): Initial National Communication of Ethiopia to the United Nations Framework Convention on Climate Change, Adis Ababa Ethiopia, pp: 1-113. 1–113.

O'Lakes, L. (2010): The next stage in dairy development for Ethiopia: Dairy value chains, end markets and food security, *Addis Ababa, Ethiopia: Land O'Lakes Inc*.

Pare, J., Thurmond, M. C., Gardner, I. A. and Picanso, J. P. (1993): Effect of birthweight, total protein, serum IgG and packed cell volume on risk of neonatal diarrhea in calves on two California dairies., *Canadian Journal of Veterinary Research*. Canadian Veterinary Medical Association **57**: 241.

Poulsen, K. P., Foley, A. L., Collins, M. T. and McGuirk, S. M. (2010): Comparison of passive transfer of immunity in neonatal dairy calves fed colostrum or bovine serum-based colostrum replacement and colostrum supplement products, *Journal of the American Veterinary Medical Association*. Am Vet Med Assoc **237**: 949–954.

Quigley, J. (2002a): Passive Immunity in Newborn Calves Absorption of Immunoglobulins **14**: 273–292.

Quigley, J. (2008): Calf Notes . com: On methods of IgG analysis, http://www.calfnotes.com

Quigley, J. D. (2002b): Effects of spray-dried whole egg and biotin in calf milk replacer, *Journal of dairy science*. Elsevier **85**: 198–203.

Quigley, J. (2004): The role of oral immunoglobulins in systemic and intestinal immunity of neonatal calves, *Cedar Rapid, Iowa, USA: Diamond V Mills*.

Radostits, O. M., Gay, C. C., Blood, D. C. and Hinchelift, K. W. (2000): Disease caused by bacteria–Mycobacterium, *Veterinary Medicine: A Text Book of Disease of Cattle, Sheep, Pig, Goat and Horses. 9th ed. Harcourt Publisher Ltd., London* 909–918.

Razzaque, M. A., Al-Mutawa, T., Abbas, S. and Bedair, M. (2009): Performance of pre-weaned dairy calves under hot arid environment: effects of immunoglobulins and age on diseases and mortality, *American Journal of Applied Sciences* **6**: 1885.

Robison, J. D., Stott, G. H. and DeNise, S. K. (1988): Effects of passive immunity on growth and survival in the dairy heifer1, 2, *Journal of Dairy Science*. Elsevier **71**: 1283–1287.

Roy, J. H. B. (1990): The calf: management of health, Vol. 1. Butterworts. London.

Salazar, J. A. E. (2008): Heat treatment of bovine colostrum: Effects on physical and chemical properties and on neonatal blood and growth parameters. The Pennsylvania State University.

Sandford, S. (1983): Management of pastoral development in the Third World. Chichester (UK) Wiley.

Sasaki, M., Davis, C. L. and Larson, B. L. (1976): Production and Turnover of IgG1 and IgG2 Immunoglobulins in the Bovine around Parturition1, *Journal of Dairy Science*. Elsevier **59**: 2046–2055.

Shen, R. L., Thymann, T., Ostergaard, M. V, Stoy, A. C. F., Krych, L., Nielsen, D. S., Lauridsen, C., Hartmann, B., Holst, J. J. and Burrin, D. G. (2015): Early gradual feeding with bovine colostrum improves gut function and NEC resistance relative to infant formula in preterm pigs, *American*

Sasaki, M., Larson, B. L. and Nelson, D. R. (1977): Kinetic analysis of the binding of immunoglobulins IgG1 and IgG2 to bovine mammary cells, *Biochimica et Biophysica Acta (BBA)-General Subjects*. Elsevier **497**: 160–170.

Schuenemann, G. M., Nieto, I., Bas, S., Galvão, K. N. and Workman, J. (2011): Assessment of calving progress and reference times for obstetric intervention during dystocia in Holstein dairy cows, *Journal of dairy science*. Elsevier **94**: 5494–5501.

Selk, G. E. (2003): Disease Protection for Baby Calves, *Division of Agricultural Sciences and Natural Resources F-3358*.

Shiferaw, Y., Yohannes, A., Yilma, Y., Gebrewold, A. and Gojjam, Y. (2002): Dairy husbandry and health management at Holleta, in *Proceeding of the 16th conference of the Ethiopian veterinary association. Addis Ababa, Ethiopia* 103–119.

Shitarek, T. (2012): Ethiopia Country Report:Economic Research-Ethiopia country Report, Pp 1-34. 1–34.

Singh, a, Chandra, G., Huozha, R. and Kushwaha, R. (2011): Bovine Col Ostrum and Neona Te Immunity - a Review Colostrum Neonate, *Agricultural Reviews* **32**: 79–90.

Singh, K. M. and Shivahre, P. R. (2015): Importance of colostral immunoglobulins in calf's health.

Sisay, A. and Ebro, A. (1998): Growth performance of Boran and Their Semmintal cross calves Proceeding of 6 th national conference of the Ethiopian society of animal production (ESAP), *Addis Ababa, Ethiopia* 157–162.

Sivula, N. J., Ames, T. R. and Marsh, W. E. (1996): Management practices and risk factors for morbidity and mortality in Minnesota dairy heifer calves, *Preventive Veterinary Medicine*. Elsevier **27**: 173–182.

Staley, T. E. and Bush, L. J. (1985): Receptor Mechanisms of the Neonatal Intestine and Their Relationship to Immunoglobulin Absorption and Disease1, 2, *Journal of dairy science*. Elsevier **68**: 184–205.

Stelwagen, K., Carpenter, E., Haigh, B., Hodgkinson, A. and Wheeler, T. T. (2009): Immune components of bovine colostrum and milk, *Journal of animal science*. Oxford University Press **87**: 3–9.

Stevens, C. D. and Miller, L. E. (2016): Clinical Immunology and Serology: A Laboratory Perspetive. FA Davis.

Stojić, M., Fratrić, N., Kovačić, M., Ilić, V., Gvozdić, D., Savić, O. and Đoković, R. (2017): Brix Refractometry of Colostrum from Primiparous Dairy Cows and New-Born Calf Blood Serum in the Evaluation of Failure of Passive Transfer, *Acta Veterinaria*. De Gruyter Open **67**: 508–524.

Stott, G. H., Marx, D. B., Menefee, B. E. and Nightengale, G. T. (1979): Colostral Immunoglobulin Transfer in Calves I. Period of Absorption1, *Journal of Dairy Science*. Elsevier **62**: 1632–1638.

Thomas, S. (2015): Motherly Instinct 42–43.

Tsegaw Fentie, Wudu Temesgen, Achenef Melaku, Shimelis Tsesfaye, Feyussa Fufa, Zemene Adane, Ayalew Niguse, Bosenu Alemu, Fikire Zeru, Birhanu Hailu, Sentayehu Guta, Gebreyes Mokonen (2016): Assessment of Young Stock Mortality in major Livestock Production Systems of Ethiopia.

Tyler, J. W., Hancock, D. D., Wiksie, S. E., Holler, S. L., Gay, J. M. and Gay, C. C. (1998): Use of serum protein concentration to predict mortality in mixed-source dairy replacement heifers, *Journal of Veterinary Internal Medicine*. Wiley Online Library **12**: 79–83.

Tyler, J. W., Steevens, B. J., Hostetler, D. E., Holle, J. M. and Denbigh, J. J. L. (1999): Colostral immunoglobulin concentrations in Holstein and Guernsey cows., *American journal of veterinary research* **60**: 1136–1139.

USDA (2010): USDA (United States Department of Agriculture): Passive Transfer Status of Heifer Calves on U . S . Dairies , Veterinary Services Centers for Epidemiology and Animal Health.

Vandeputte, S., Detilleux, J. and Rollin, F. (2011): Comparison of four refractometers for the investigation of the passive transfer in beef calves, *Journal of veterinary internal medicine*. Wiley Online Library **25**: 1465–1469.

Waldhalm, D. G., Frank, F. W., Meinershagen, W. A., Philip, R. N. and Thomas, L. A. (1971): Lambing performance of ewes inoculated with Chlamydia sp. before and after breeding., *American journal of veterinary research* **32**: 809.

Walter L. Hurley: (2010): Intestinal Absorption of Immunoglobulins, http://ansci.illinois.edu/static/ansc438/Motherneonate/absorption.html

Weaver, D. M., Tyler, J. W., VanMetre, D. C., Hostetler, D. E. and Barrington, G. M. (2000): Passive transfer of colostral immunoglobulins in calves, *Journal of Veterinary Internal Medicine*. Wiley Online Library **14**: 569–577.

Wittum, T. E. and Perino, L. J. (1995): Passive immune status at postpartum hour 24 and long-term health and performance of calves., *American journal of veterinary research* **56**: 1149–1154.

Wold, A. G. and Yehualashet, T. (1987): Note on calf mortality rate at two IAR Livestock Stations: Holetta and Adami Tulu, in *1. National Livestock Improvement Conference, Addis Ababa (Ethiopia), 11-13 Feb 1987*. IAR.

Wudu, T. (2004): Calf morbidity and mortality in dairy farms in Debre Zeit and its envirions, Ethiopia. Msc thesis. Faculty of Veterinary Medicine, Addis Ababa University, Ethiopia.

Wudu, T., Kelay, B., Mekonnen, H. M. and Tesfu, K. (2008): Calf morbidity and mortality in smallholder dairy farms in Ada'a Liben district of Oromia, Ethiopia, *Tropical animal health and production*. Springer **40**: 369–376.

Yami, A. and Sileshi, Z. (2001): Contribution of animal science research to food security, *Livestock in Food Security–Roles and Contributions* 31.

Yeshwas, F. A. (2015): Epidemiological Determinants and Magnitude of Calf Morbidity and Mortality in Bahir Dar Milk-Shed, North West Ethiopia 1–96.

ANNEXES

Date _____

Study area: **Amibara**

Annex: 1. Record format use collect cows and calves birth information from the study area

No	Date of sample collection	Kebele	ID	Date of birth	Calve sex	Dam parity	Birth time	Birth site	Birth condition	Age at first colostrum ingestion	Mothering Instinct
1											
2											
3											
4											
5											
6											
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Date _____

Study area: Amibara

Annex: 2. Sample format used to collect information about key informants

No	Kebele	Name	Age	Sex	Category	Pho. No
1						
2						
3						
4						
5						
6						
7						
8						
9						

Date of Interview _____

Annex: 3. Sample format used to collect information about key informants

Key Informants Question

1. You know the exact month and week to when you're pregnant cows that have been born?
2. Unlike most other cows, do you give special care and attention to your pregnant cow, especially when they are about to give parturition (two weeks before their birth)?
3. If your cows give birth out in the field, how would you take newborn calf to a home?
4. If your newly born calf are unable to suckle after a birth, what kind of help/assist is given to they get enough colostrum early after birth?
5. Do you believe that feeding large amount of colostrum to newborn calf before six hours postpartum to keep the newborn calves from getting sick and death?
6. After the birth of your cow, by what time of day you will begin to use their milk for the purpose food?
7. If your cow was born in the early morning and did not engage for grazing with other animal, from where they get fed for that day?
8. Do you give equal care and attention for newly born male and female calfs?
9. What kind of help is provided for pregnant cows if the cow has a problem of birthing difficulties during parturition?

Annex: 4. Radial Immunodiffusion Laboratory work procedures

❖ **Material required**

1. Bovine radial immunodiffusion plate kept between +4^{o}C to +8^{o}C with reference Sera: containing 3x0.25 mls
2. Serum sample (kept at – 20 until the day of analysis)
3. Microliter dispenser (5 µl)
4. 5 µl pipet tips
5. Digital caliper providing accurate measurement in millimeter
6. Normal graph paper
7. Gown and glove
8. Marker
9. Vortex machine

Serum, RID plate, and Vortex

Three type reference standards and 5µl pipet

High (2803 mg/dL)

Medium (1472 mg/dL)

Low (180 mg/dL)

❖ **Laboratory application procedure**

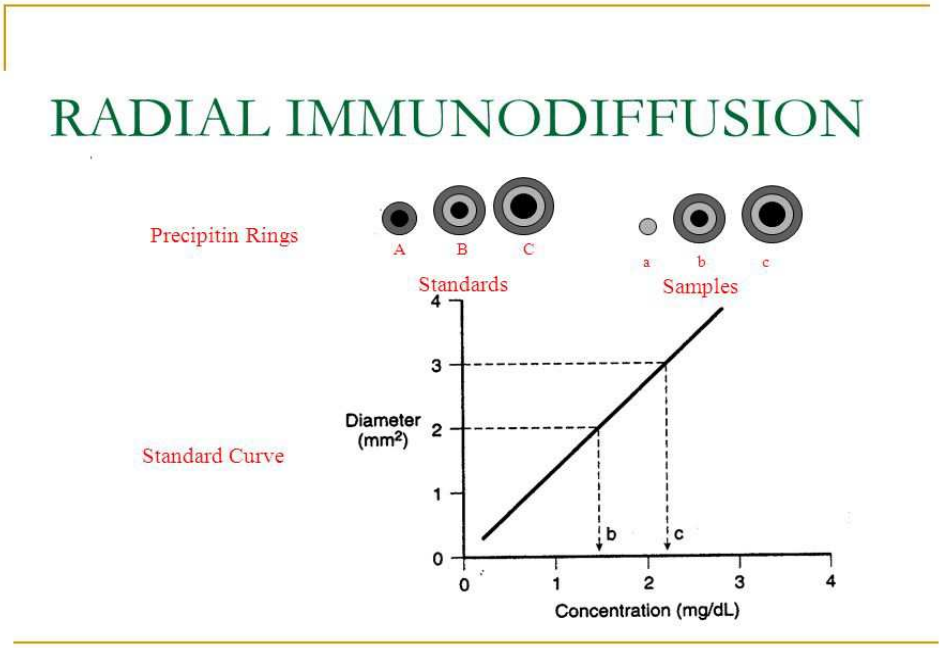
1. The plate was removed from refrigerator to room temperature approximately 30 minutes before filling wells.
2. Plate bag is not opened until ready for use.
3. When ready for use the plate were removed from bag and checked for presence of excess moisture. Unfortunately three plates have moisture, and so plate cover were removed until evaporation has dried from the surface and wells and then the cover replaced until used.
4. Dark back ground were used for best visualization of wells during deliver specimen and reference serum
5. Firstly each vial of reference serum and test sera was vortexed to mix thoroughly. Then three wells were filled with reference sera for each plate using 5 microliter pipette. Location of each well is noted. The rest 21 wells filled by serum sample by placing the pipette tip at the bottom of the well.

6. Each test sample pipetted in to plate well is recorded on a card, times of completion were marked on plate cover and then the cover replaced to the plate.
7. The plate was replaced in bag and reseal carefully, and then incubated upright on flat surface at room temperature over 24 hours for end point readings.

8. After 24 hours period of incubation the precipitated ring diameter measured in mm using digital hand held caliper and obtained value for each sample was recorded in format.

9. To get unknown concentration serum IgG standard curve is generated using normal graph paper, from the standard curve, the concentration was determined, by finding the diameter squared value on the Y- axis, finding the intersecting point on the standard curve line, and obtaining the value on the X-axis.

- **Constructed standard curve sample picture**

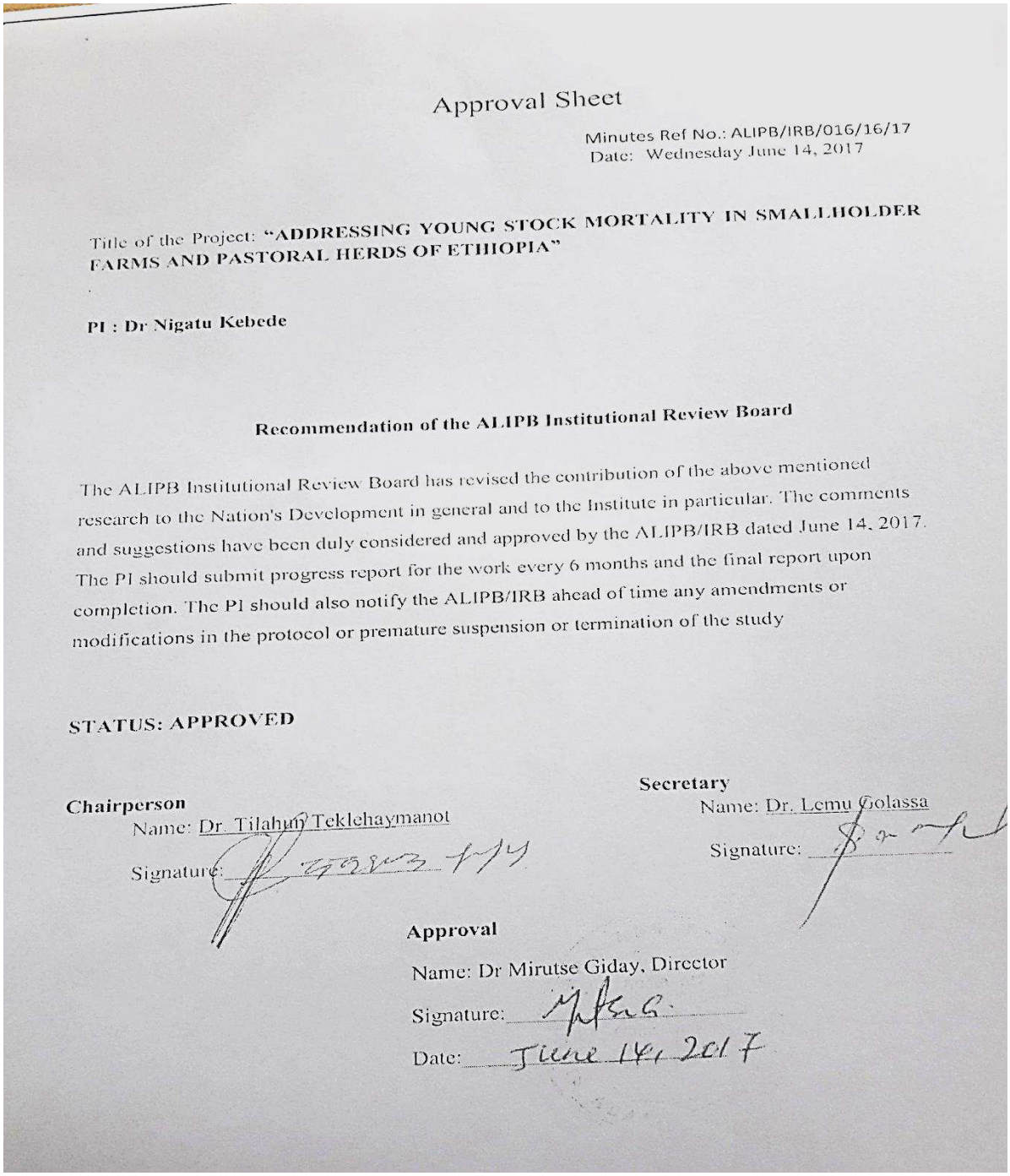


- **Sample format used to record lab result**

❖ Bovine radial immunodiffusion (RID) lab result for end point reading Lab date_____/_____/_____ Plate lot #_____

		ID_____	ID_____	ID_____	ID_____	ID_____
Plate No _____	End point ring diameter					
	Squared R/Diameter					
	Concentration in mg/dL					
	Immunity status of calve					

Ethical Approval Certificate



Approval Sheet

Minutes Ref No.: ALIPB/IRB/016/16/17
Date: Wednesday June 14, 2017

Title of the Project: **"ADDRESSING YOUNG STOCK MORTALITY IN SMALLHOLDER FARMS AND PASTORAL HERDS OF ETHIOPIA"**

PI : Dr Nigatu Kebede

Recommendation of the ALIPB Institutional Review Board

The ALIPB Institutional Review Board has revised the contribution of the above mentioned research to the Nation's Development in general and to the Institute in particular. The comments and suggestions have been duly considered and approved by the ALIPB/IRB dated June 14, 2017. The PI should submit progress report for the work every 6 months and the final report upon completion. The PI should also notify the ALIPB/IRB ahead of time any amendments or modifications in the protocol or premature suspension or termination of the study

STATUS: APPROVED

Chairperson
Name: Dr. Tilahun Teklehaymanot
Signature:

Secretary
Name: Dr. Lemu Golassa
Signature:

Approval
Name: Dr Mirutse Giday, Director
Signature:
Date: June 14, 2017