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ADDIS ABABA UNIVERSITY COLLEGE OF VETERINARY MEDICINE AND AGRICULTURE DEPARTMENT OF BIOMEDICAL SCIENCES

SERUM IMMUNOGLOBULIN G CONCENTRATION IN LAMB AND GOAT KID, AWASH FENTALE, AFAR NATIONS REGIONAL STATE, ETHIOPIA

MSc THESIS

BY

NEGASH DESTA

ADDIS ABABA UNIVERSITY, COLLEGE OF VETERINARY MEDICINE AND AGRICULTURE, DEPARTMENT OF BIOMEDICAL SCIENCES MSC PROGRAM IN ANIMAL PHYSIOLOGY

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SERUM IMMUNOGLOBULIN G CONCENTRATION IN LAMB AND GOAT KID, AWASH FENTALE, AFAR NATIONS REGIONAL STATE, ETHIOPIA

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

By

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College of Veterinary Medicine and Agriculture

Department of Biomedical Science

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As member of the Examining Board of the final MSc open defence, we certify that we haveread and evaluated the thesis prepared by: **Negash Desta** titled **Serum immunoglobulin concentration in lamb and goat kid, Awash Fentale, Afar Nations Regional State, Ethiopia** and recommend that it be accepted as fulfilling the thesis requirement for the degree of master of science in Animal Physiology.

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Final approval and acceptance of the dissertation is contingent upon the submission of its corrected copy to the CGC through the concerned departmental graduate committee. I hereby certify that I have read the revised version of this dissertation prepared under my direction and recommend that it be accepted as fulfilling the dissertation requirement.

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DEDICATION

This MSc thesis work is dedicated to the memory of my beloved and kind father, Teacher Desta Mekonnen, whom lost his life by car crush at June 3/2010 when he going to my DVM graduation to Jimma University. I never forget him, and my eyes are full of tears every moment I remember him and to my mother Debritu Melore survivor of the car crush when I lost my dad. I grew up under realm with love and care, as first son of my family. It is always surprising to me that they belief about education was so excited, though they did not receive any formal education. They allowed me to have a new life led by education. More importantly, they far sighted vision, dignified and disciplined personality, enhanced social relationship, positive attitude and hardworking spirit had shaped me and paved the way to my present situation. I wish if my dad could see my present situation. May GOD rest his soul in heaven!

STATEMENT OF AUTHOR

First, I declare that this thesis is my *bona fide* work and that all sources of material used for this thesis have been duly acknowledged. This thesis has been submitted in partial fulfillment of the requirements for an advanced (MSc) degree 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.

Name: Negash Desta Signature: _______________

College of Veterinary Medicine and Agriculture, Bishoftu

Date of Submission: ___________________________

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LIST OF ABBREVIATION

CSA	Central statistical agency
ELISA	Enzyme-linked immunosorbent assay
FAO	Food agricultural organization
GDP	Gross Domestic Product
IgA	Immunoglobulin A
IgG	Immunoglobulin G
IgM	Immunoglobulin M
MoARD	Ministry of Agriculture and Rural Development
PIT	Passive immunity transfer
RID	Radial immune diffusion
USDA	United States Department of Agriculture

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ABSTRACT

In this study a total of 143 head (97 lamb and 46 goat kid) were randomly selected from 3 villages, Awash Fentale district, Afar Regional State, Ethiopia. To determine the serum IgG concentration of lamb and goat kid in the study area and to determine effect of parity, age, birth type, sex and dam body condition in transfer of passive immunoglobulin concentration in the study area. From lamb and goat kid blood sample was taken at 2-7 days of age. 4 ml of blood was collected from jugular vein puncture in a sterile plain tube and serum collected by centrifuge. The species specific sheep and goat radial immuno diffusion kit analyzed serum result after 24 hrs showed as rang from 47.64 to 6071.632 mg/dl serum IgG concentration of lamb and goat kid ranges from 169.577 to 3559.875 mg/dl serum IgG concentration. The radial immuno diffusion result showed as 21.6 % of newborn lambs in the study area have failure of passive immunoglobulin concentration and the remaining 78.4 % had adequate passive immunity and 83.4 % of goat kids have adequate passive immunoglobulin concentration and 16.6 % kids had failure of passive immunoglobulin concentration from their dams. The result showed that failure of passive immunity important in the study area. Among the considered risk factors evaluated by simple and multiple logistic regression the age of the lamb and type of delivery was statistically significant P value ($P<0.05$) on lamb. Generally, passive immunity transfer increased by colostrum feeding and animal husbandry management intervention and significant for pastoralist whose livelihood is dependent on livestock.

Key words: *colostrum, concentration, immunoglobulin, IgG, RID, serum,*

1. INTRODUCTION

One of the many challenges of being a livestock producer is to produce healthy newborns that will survive and perform to their full genetic potential. The long term productivity of an animal is linked to its immune status and that the immune and nutritional status of pregnant dams can have consequences on the health and performance of their offspring. The colostrum contains important antibodies (Pattinson *et al*., 1995) which provides a defense mechanism for newborn ruminants until their own immune system is established (Ahmad *et al*., 2000; Yilmaz and Kasikci, 2013). There is a restricted time period for the transition of immunoglobulin into the small intestines of newborns as the intestinal epithelium (Galan-Malo *et al*., 2014). The quantity of colostrum produced by ewes is one of the determinants of colostrum intake by newborn lambs (O'Doherty and Crosby, 1997) and there are potential differences both between and within breeds in colostral IgG concentrations (Tabatabaei *et al*., 2013). Unsuccessful transfer of passive immunity in young animals results in higher susceptibility to diseases, increased mortalities and off-spring that do not perform to their full potential (Hashemi *et al*., 2008). Ahmad *et al*. (2000) found lambs that died during the neonatal period had significantly lower concentrations of immunoglobulin than lambs that survived the neonatal period. Furthermore, higher mortality rates were observed in lambs born to ewes that had a lower IgG content in their colostrum (Tabatabaei *et al*., 2013).

The diet consumed by pregnant ewes in late gestation can influence colostrum production and therefore the amount of colostral IgG available and absorbed by their lambs (O'Doherty and Crosby, 1997; Banchero *et al*., 2006). The maternal nutritional status of the ewe can influence the gut permeability to immunoglobulin of their newborn lambs (Hodgson *et al*., 1997). Newborn lambs have a greater absorption of immunoglobulin when the dam is healthier. Furthermore, nutritional supplementation of dams can enhance the erythropoietic response and therefore, improve offspring survival (Ahmad *et al*., 2000). The neonatal period is one of the most demanding adaptation periods during the life of any animal, including lambs and kids. The adaptation period to the extra uterine life begins immediately after parturition. First challenge lambs face, is fighting hypothermia. Suckling colostrum within the first few hours of life is also crucial for survival (Nowak & Poindron, 2006).

Failure of passive transfer of immunity prevalence varies with management condition and type of animal. In Ethiopia, there was one report Bekele *et al*. (1992) who report failure of passive transfer in Menz breed of lambs there was no previous, studies conducted in our country focused specifically on FPT prevalence in small ruminants. Regarding prevalence 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 lamb and goat kid, it is hypothesized that colostrum feeding and newborn management practices in the pastoral system have an influence on passive immunity transfer in newborn lamb and goat kid; based on these hypothesis the following objective are formulated.

Objective of the study

- To determine the concentration of lamb and goat kid serum IgG in Awash fentale.
- To determine effect of parity, age, birth type, sex, birth location, type of delivery, and dam body condition in transfer of serum IgG concentration in Awash fentale.

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 goats, 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, 2015). 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). Small ruminant production is a very significant component of livestock production throughout the world and more especially in the developing countries (Ketema, 2007; Thornton *et al.*, 2009).

The distribution of sheep population across different agro-ecology was reported to be an even distribution between highlands and lowlands but goat populations are found more dominant in the low land areas of the country (Aklilu and Catley, 2010). Sheep and goats contribute 25% of the domestic meat consumption; about 50% of the domestic wool requirements; about 40% of fresh skins and 92% of the value of semi-processed skin and hide export trade. It is estimated that 1,078,000 sheep and 1,128,000goats are used in Ethiopia for domestic consumption annually. The current annual off-take rate of sheep and goats is 33%and 35%, respectively (Hirpa and Abeba, 2008). There is also a growing export market for sheep and goat meat and live animal export. In 2010/11, the export value from sheep and goat meat and live animal were about 63 million and 148 million USD, respectively (USAID, 2011).

2.2. Pastoralist animal 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 socioeconomic 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 animals 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. The immune system

The immune system is a system of biological structures and processes within an organism that protects against disease. To function properly, an immune system must detect a wide variety of agents, from viruses to parasitic worms, and distinguish them from the organism's own healthy tissue. Pathogens can rapidly evolve and adapt, and thereby avoid detection and neutralization by the immune system, however, multiple defense mechanisms have also evolved to recognize and neutralize pathogens. Even simple unicellular organisms such as bacteria possess a rudimentary immune system, in the form of enzymes that protect against bacteriophage infections. Other basic immune mechanisms evolved in ancient eukaryotes and remain in their modern descendants, such as plants and insects. These mechanisms include phagocytosis, antimicrobial peptides called defenses, and the complement system. Jawed vertebrates, including humans, have even more sophisticated defense mechanisms (Gregory and Habicht, 1996), including the ability to adapt over time to recognize specific pathogens more efficiently. Adaptive (or acquired) immunity creates immunological memory after an initial response to a specific pathogen, leading to an enhanced response to subsequent encounters with that same pathogen. This process of acquired immunity is the basis of vaccination. Disorders of the immune system can result in autoimmune diseases, inflammatory diseases and cancer (Coussens and Werb, 2001; O'Byrne and Dalgleish, 2001).

2.4. Passive immunity

Lambs and goat kids are born agammaglobulinaemic (Wereme *et al*., 2001; Maden *et al*., 2003; Yilmaz and Kasikci, 2013) as ruminants have an epitheliochorial placenta (Pattinson *et al*., 1995; Sammin *et al*., 2009) whereby the fetal chorionic epithelium is in contact with the intact uterine epithelium. In this type of placentation, transplacental passage of immunoglobulins is prevented (Lazarevic *et al*., 2010; Yilmaz *et al*., 2011; Dudek *et al*., 2014) as there is a syncytium between the maternal endometrium and the fetal trophectoderm, thus separating the maternal and fetal blood supply (Weaver *et al*., 2000; Sammin *et al*., 2009).

2.5. Immunity of Lambs and Kids

Feeding of hyper immune colostrum to 24 hrs old lamb and kids was found to have great significance for their survivals. Three different classes of immunoglobulins including IgG, IgM, and IgA are usually synthesized during the last few weeks of pregnancy by the plasma cells located in the submucosa of the mammary gland epithelium (Yilmaz and Kasikci, 2013). These antibodies are transferred into the colostrum by selective receptor mediated intracellular route (Kacskovics, 2004). Fc is the specific receptor for transferring IgG antibody from the serum to mammary gland, and it is regulated by hormonal changes during the pregnancy period (Yilmaz *et al.*, 2011; Tabatabaei *et al*., 2013). Among three classes of immunoglobulins (IgG, IgM and IgA), only IgG was found to play main passive immunity functions in small ruminants (sheep, goats).

There are many functions of the Fc receptor, such as IgG metabolism and prevention of the degradation of IgG in maternal circulation. It also plays an important role in determining the concentration of IgG in the colostrum (Kacskovics, 2004; Baintner, 2007; Brujeni *et al.*, 2010; Lerias *et al*., 2014). The expression of FcRn receptor increased in dry ewes in late gestation period, and decreased during colostrogenesis (i.e., transfer of immunoglobulins from the dam blood circulation into the mammary secretions, which occurs in the last two weeks before parturition) and lactation period (Hine *et al.*,2010; Hernandez-Castellano *et al.*, 2014).

2.6. Neonatal period in lamb and goat kid

The neonatal period begins after parturition and it can be characterized as being an intensive period in time, when the newborn adapts to the extrauterine life (Mellor, 1988). As reviewed by Piccione *et al*. (2011) the neonatal period is the interval between birth and 28 days of age. Profound metabolic and morphological mechanisms, such as thermoregulatory system, cardiovascular system, respiratory system and metabolic homeostasis, complete maturation during the neonatal period. This period of time is also called as adaptive period (Piccione *et al*., 2007). It is one of the most vulnerable periods in animal's life and it is connected to the high mortality and morbidity rate, especially during the first few days of life (Piccione *et al*., 2008; Piccione *et al*., 2009).

Mortality in the neonate lambs appears in both intensive and extensive systems and remains approximately 15-25% worldwide (Nowak & Poindron, 2006). Nowak *et al*. (2000) found that neonatal deaths are highest, 21%, during the three first days of life. Dwyer (2008) estimated that half of all pre-weaning lamb deaths occur at the parturition day. This is a major economic loss and welfare issue in the sheep farming, and points out the importance of the post-partum periods role in lamb survival. Survival of newborn kids is an important determinant of profitability in a goat farm operation (Shokrollahi *et al.*, 2013). Newborns are exposed to a variety of environmental and physiological stress. Daniels *et al.* (2000) reported that mortality rates varied between 15% and 51% with rates as high as 35% supposed acceptable among large sheep farms. Losses approaching 100% have been observed in farms experiencing acute diseases (Shelton & Willingham, 2002). To prevent such disasters, it is critical to improve the immune system of newborn kids, particularly in the first few weeks of life in order to combat a variety of environmental and physiological stressors. This passive immunity works well to protect most kids against common infectious agents for the first ten to twelve weeks of life; it depends on the strength of the goat's immune system. Therefore, there can be a high degree of variability in the quality of passive immunity acquired by the kids (Rashid *et al.*, 2011).

2.7. Colostrum

Ruminant neonates rely entirely on colostrum and milk from their dam for survival (Wereme *et al*., 2001; Stelwagen *et al*., 2009). Ruminant colostrum is a mixture of lacteal secretions and constituents from blood serum, which consist of immunoglobulins and other serum proteins (Foley and Otterby, 1978). Newborns acquire passive immunity through ingesting immunoglobulins contained in colostrum (Stelwagen *et al*., 2009). There are five classes of immunoglobulins, IgG, IgM, IgA, IgD and IgE; with IgG, IgA, IgM (Ahmad *et al*., 2000; Gapper *et al*., 2007; Hashem *et al*., 2008) and IgE being actively transported into ovine colostrum (Hine *et al*., 2010). IgG is the most predominant (Thompson *et al*., 2013) and abundant (Hashem *et al*., 2008) immunoglobulin, accounting for approximately 80-90% of all immunoglobulins in colostrum (Ingvarsson, 1995; Mech *et al*., 2011). The transfer of immunoglobulins into ovine colostrum is most selective for IgG1, followed by IgA, IgE, IgM and finally IgG2 (Hine *et al*., 2010).

All immunoglobulin molecules are based around the same structure, consisting of two heavy polypeptide chains and two light polypeptide chains (Gapper *et al*., 2007). Each of the light chains is bound to one of the heavy chains by disulfide bridges, while the heavy chains are similarly bound to one another resulting in a Y shape. At the tip of each arm there are two identical antigen binding sites. These are unique for each different antibody so that it can only interact with a specific antigen (Sherwood *et al*., 2005). There are two subclasses of IgG, IgG1 and IgG2 (Gapper *et al*., 2007). IgG1 is the primary immunoglobulin found in ruminant colostrum (Weaver *et al*., 2000; Mayer *et al*., 2002; Hine *et al*., 2010) and is derived mainly from maternal circulation. IgG2 is derived from blood or can be synthesized in the cells of the mammary gland (Gapper *et al*., 2007). IgG is responsible for pathogen clearance (Mech *et al*., 2011) and protects the neonate against bacteria and viruses, neutralizes bacterial toxins, triggers the complement system and when bound to antigens enhances the effectiveness of phagocytic cells (Tortora *et al*., 2006).

2.8. Colostrogenesis

The development of the mammary gland includes different stages, mammogenesis, colostrogenesis, lactogenesis, galactopoiesis and involution (Dembiski and Shiu, 1987). Colostrogenesis is the prepartum transfer of immunoglobulins from maternal circulation into mammary secretions and it is a finite stage (Barrington *et al*., 2001). It is known that in ruminants, colostral immunoglobulin come from the bloodstream. However, in goat colostrum, the IgG concentration is about 2.8 times higher than those in blood serum. Although maternal concentration of IgG1 and IgG2 are approximately equal, goat blood serum concentration of IgG1 represents the 55% of total IgG, colostral IgG1 represents about 98% (Castro *et al*., 2006).

2.9. Colostrum composition

Colostrum is a unique food source of neonates and the lack or insufficient intake of colostrum is considered to be the second largest factor to affect neonatal survival, after body reserve. It is produced immediately before the parturition and it is a compilation of dense nutrients and high levels of immunoglobulins, growth factors, enzymes and hormones. Colostrum contains approximately 7% fat, 4% casein, 5% lactose and 82% water. It provides 2 kcal of energy for each ml (Nowak &Poindron, 2006). Mellor (1988) has determined that 180-290 ml of colostrum per kilogram of bodyweight is required for the lamb during the first 18 hours after birth to ensure adequate passive immune transfer. Colostrum contains a potent mixture of diverse components, some of which such as fat, proteins, lactose and minerals are of nutritional importance (Ontsouka *et al*., 2003).

In addition, colostrum contains vitamins, immunoglobulins, hormones, growth factors, cytokines, enzymes and many other peptides (Koldovsky, 1980; Campana and

Baumrucker, 1995; Blum and Hammon, 2000). Colostrum components are secreted by different mechanisms and the secretion is regulated by local and systemic factors. However most of them are still in discussion. Therefore, colostrogenesis is a process not fully understood at present (Dembiski and Shiu, 1987; Barrington *et al*., 2001).

2.10. Colostrum Immunoglobulins

In ruminants, colostrum is the sole source of acquired immunity (Stelwagen *et al*., 2009). According to Tizard (1987) sheep colostrum contains IgA 100-700 mg/ml, IgM 400-1200 mg/ml and IgG 4000-6000 mg/ml. IgG represents the major immunoglobulin class to provide the initial protection early on in life (Alves *et al*., 2015). The intestines of the ruminants are non-selective in their permeability and can therefore absorb all immunoglobulin isoforms from the colostrum (Yilmaz & Kasikci, 2013). However, as Nowak & Poindron (2006) states intestinal closure happen approximately 24 hrs after birth, meaning that the passive absorption of immunoglobulins in the intestine seize. Yilmaz & Kasikci (2013) found that the time of the intestinal closure for each immunoglobulin type in sheep was for IgG 26.4 hours, for IgM 25 hours and for IgA 26 hours postpartum.

Nowak & Poindron (2006) found that when suckling begins; the level of immunoglobulins in the blood starts to rise rapidly during the first hour and reaches a peak around 24 hours after parturition. Shubber *et al*. (1979) concluded that larger volumes of colostrum correlate with larger amounts of immunoglobulins. The immunoglobulins are resistant to enzymatic digestion in the intestine. There is also a trypsin inhibitor in the colostrum itself, which additionally adds to this resistance (Yilmaz & Kasikci, 2013). Colostrum is the only main source of proteins, necessary for the adaptation into extra uterine life for the neonate. Therefore, the total serum protein in both colostrum and serum contribute to neonate immunity and growth (Piccione *et al*., 2007). According to Ahmad *et al*. (2000) the serum of the lambs that survived the neonatal period was significantly higher than in those who died.

2.11. Concentrations in Colostrum and Milk—Physiological Conditions

The content of immunoglobulins in colostrum and milk is highly dependent on the animal species (Hurley, 2003; Butler *et al*., 2005). The same holds for the relative proportion of the immunoglobulin classes. These species differences are adaptations to the reproductive strategies of the animals and the degree of maturation of the offspring at birth. Animal species may be divided into three classes (Butler *et al*., 2005): (1) species where immunoglobulins are transferred mainly to the fetus via the placenta (humans and rabbits); (2) species where offspring are born agammaglobulinemic and immunoglobulin transmission occurs via mammary secretions (ungulates such as horses, pigs, cows, and goats); and (3) species where immunoglobulins are transferred both via placenta and mammary secretions (rats, mice and dogs).

These adaptations have several consequences both for the composition of immunoglobulins in colostrum and milk, and for the role of colostrum. Indeed, for animals like rats, mice, dogs and ungulates, uptake of colostrum of adequate quality and sufficient quantity is important for the offspring to boost the systemic immune function in the short term, whereas colostrum consumption in the human infant provides more protection for the gastrointestinal tract. This is reflected in a lower total immunoglobulin content in human colostrum as compared to colostrum from the other species (Figure 1) (Rouse, 1970; Butler *et al*., 2005). Human colostrum has a low content of IgG (2%), and the IgG required to provide systemic immunity is transferred across the placenta before birth. In contrast, colostral IgG content in many other species is typically greater than 75% of the total immunoglobulin content (Figure 1).

An additional consequence of different routes of immunoglobulin transmission relates to the changes in relative contents of immunoglobulins that occur in the transition from colostrum to milk within certain species (Figure 1). For example, the profile of immunoglobulins in human colostrum is similar to that found in milk, where the IgA level is high in both colostrum and milk (88–90% of total immunoglobulin). This is in contrast to the bovine mammary secretions where the high concentration of IgG in colostrum declines rapidly with successive milking. For animals like rats, mice, dogs and ungulates, the role of colostrum and milk immunoglobulins is to provide immune protection both systemically and for the gastrointestinal tract, which is reflected in large changes in the profile of immunoglobulins during the transition from colostrum to mature milk (Figure 1). Thus, for many species the proportion of IgA increases between colostrum and milk

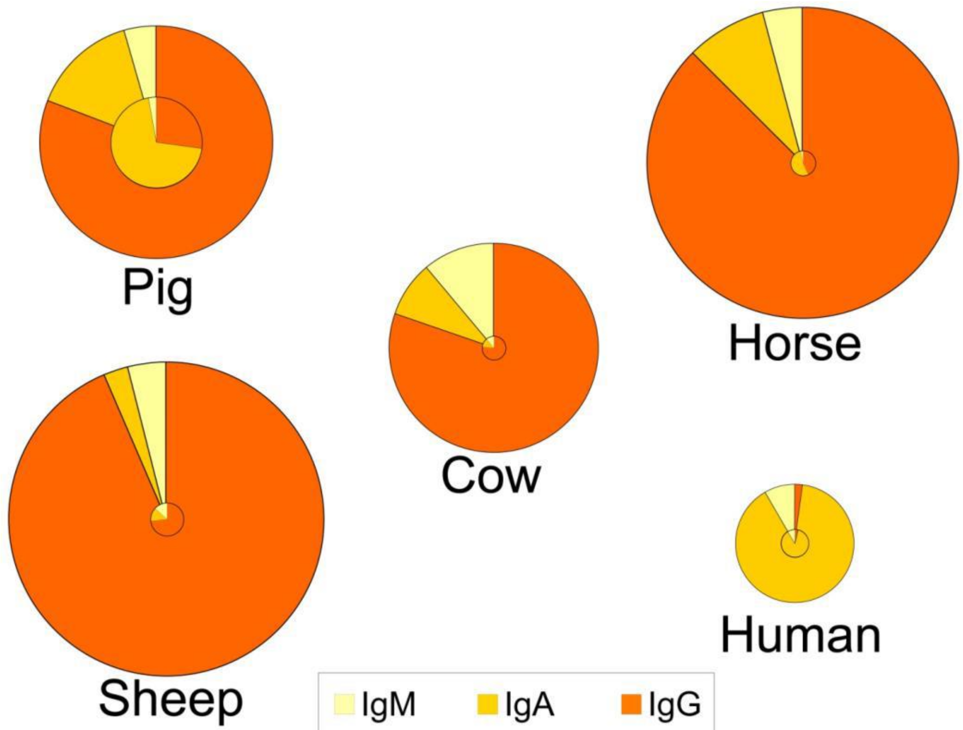

Figure 1: Picture of immunoglobulins IgM, IgA and IgG

2.12. Source and transport of immunoglobulin (IgG)

Immunoglobulins found in mammary secretions arise from systemic and local sources. In the case of IgG in milk, the major portion comes from the serum (Hurley, 2003). While IgG producing plasma cells may occur within the mammary tissue, their contribution to the IgG in colostrum is minor compared with the IgG derived from the serum. Although limited paracellular passage of immunoglobulins may occur during inflammation (mastitis), uptake and transport of immunoglobulin across the mammary epithelial barrier is thought to occur primarily through an Fc-receptor-mediated process (Larson, 1992; Butler *et al*., 2005). Immunoglobulins are thought to bind to receptors at the basolateral surfaces of the mammary epithelial cell. These receptors are specific for the Fc portion of the immunoglobulin molecule. The receptor-bound immunoglobulin is internalized via an endocytic mechanism (He *et al*., 2008) transported to the apical end of the cell and released into the alveolar lumen. Recent studies have shed additional light on the details of this process (Cervenak and Kacskovics, 2009).

In the case of IgG, the receptor responsible for transcytosis of IgG into colostrum is referred to as FcRn, or the neonatal Fc receptor, because it was initially identified in the neonatal rodent intestine as the receptor responsible for the specific uptake of maternal IgG (Simister and Rees, 1985; Simister, 1997). The FcRn also has been implicated in the trans-placental transport of IgG in humans and other species, which may involve an endocytic and transcytotic process (Fuchs and Ellinger, 2004). Since its initial discovery, FcRn has been described in many tissues (Cervenak and Kacskovics, 2009). The receptor is a heterodimer composed of a membrane-bound α-chain similar to MHC class-1 molecules and a smaller MHC class-1 protein, β2-microglobulin. Binding of IgG to FcRn is pH-dependent, with high affinity binding occurring at acidic pH, but only weak binding at neutral or basic pH (Cervenak and Kacskovics, 2009). This observation suggests that IgG taken up by the epithelial cells may bind to FcRn within an acidic environment in the endosomes. The precise mechanism of transport across the epithelial cell and release into the colostrum or milk remains to be demonstrated.

The half-life of IgG in serum is typically longer (1–3 weeks) than that for IgA or IgM (1–2 days), and the half-life of IgG2 is slightly longer than for IgG1. Evidence suggests that IgG2 has a higher affinity for FcRn than IgG1 (Cervenak and Kacskovics, 2009). In bovine colostrum, IgG1 is many fold greater in concentration than IgG2 (Guidry *et al*., 1980), although they are of approximately equal concentrations in serum. It may be that the majority of the IgG2 taken up by the mammary epithelial cell during colostrum formation is not passed on to the alveolar lumen, but rather is recycled back to the extracellular fluid. The FcRn is thought to have a major role in the recycling of IgG in various tissues in the body (Telleman and Junghans, 2000). That is, IgG that potentially may be lost through various tissues is recycled by the respective cells by binding to FcRn and recycled back to the blood or lymph. This is supported by studies of over expression of FcRn in transgenic mice where there is an extension of the half-life of serum IgG (Bender *et al*., 2007; Lu *et al*., 2007), as well as a boosting of the overall humoral immune response of the mice (Cervenak *et al*., 2010).

Localization of FcRn in bovine, sheep and water buffalo mammary tissue indicates that the receptor is homogeneously distributed throughout the epithelial cells prior to parturition, but primarily localized at the apical surface of the mammary epithelial cells after (Mayer *et al*., 2002; Mayer *et al*., 2005; Sayed *et al*., 2010). While this type of observation corroborates the conclusion that FcRn plays an important role in IgG transport during colostrum formation, at least in ruminant species, the precise meaning of this redistribution of FcRn staining in mammary cells remains to be determined. It is also interesting to note that the initial report of this distribution pattern in sheep mammary epithelium included the observation that the staining pattern became diffuse within the cells during mammary involution (Mayer *et al*., 2002). Transport of IgG also may increase transiently in mammary secretions during involution in cattle (Zou *et al*., 1988).

2.13. Factors affecting colostrum quality and quantity

Colostrogenesis, the transfer of immunoglobulins from the dam’s circulation into mammary secretions, begins one or two weeks’ pre-partum (Baintner, 2007) and is under hormonal control by estradiol and progesterone (Castro *et al*., 2011). High plasma progesterone is negatively associated with colostrum yield, and low nutrition during gestation can result in higher plasma progesterone concentrations, hence affecting the survival of lambs due to inadequate colostrum production (Nowak and Poindron, 2006) and subsequent transfer of passive immunity. The IgG specific receptor on alveolar epithelial cells, involved in transporting IgG from blood into colostrum, is inactivated when prolactin (lactogenic hormone) increases during lactogenesis, thereby down regulating the transfer of immunoglobulins (Weaver *et al*., 2000; Castro *et al*., 2011). Shortly after parturition the secretion of immunoglobulins in colostrum ceases (Baintner, 2007).

In the ewe, serum IgG concentration peaks during late gestation and significantly decreases post-partum (Mayer *et al*., 2002; Hine *et al*., 2010). The highest IgG concentration in colostrum is at 1 h post-partum and then rapidly declines at 12 and 24 h post-partum (Hashemi *et al*., 2008). However, the relationship between ewe (serum) IgG concentrations and IgG concentrations in colostrum is unclear. Tabatabaei *et al*. (2013) found colostral IgG concentrations were not associated with ewe serum IgG concentration whereas Hashemi *et al*. (2008) found that the IgG concentrations in ewe serum are significantly correlated with IgG concentrations in colostrum. The concentration of IgG in colostrum can be influenced by various factors including breed, lactation number, age, health status, nutrition, body condition score at parturition and genetics as well as environmental factors (Gilbert *et al*., 1988; Hart *et al*., 2009).

Variations in colostrum quality (concentration of IgG) due to breed differences have been reported in both cattle and sheep (Castro *et al*., 2011). Tabatabaei *et al*. (2013) found considerable variation in colostral IgG concentrations between ewes and suggested the breed differences in the quality of colostrum produced may be due to polymorphism in the FcRn gene. Age of the dam can influence the quantity and quality of colostrum. Both ewes and cows have been reported to produce more colostrum with a higher content of immunoglobulins than younger animals (Yilmaz and Kasikci, 2013). Colostrum from heifers is of a poorer quality when compared to older cows (Bielmann *et al*., 2010). However, there appears to be an interaction between the age and breed on colostrum quality. Most studies report a tendency for the older animals to produce higher quality colostrum.

2.14. Hypothermia, body weight and energy reserves

The first couple of hours after birth are highly important for the survival. Hypothermia is one of the first battles the newborn must counteract. During the first 15 minutes after birth the body temperature decreases 1 to 2°C from the intrauterine temperature of 39°C (Nowak & Poindron, 2006). Normal body temperature for lambs is considered to be 39-40°C (Eales *et al*., 2004). Alexander (1962) concluded that depending on the lamb size and prenatal nutrition, the body reserves are sufficient to maintain the lamb body temperature for 5 to 12 hours in cold weather and up to 3 days in good weather. The one greatest factor affecting to the lamb mortality is birth weight outside of optimum range, which varies between breeds (Fogarty *et al*., 2000). Lambs with low birth weight might die because of exposure and starvation. Especially single lambs with higher birth weight are more predisposed to death caused by dystocia due to bigger size (Dwyer, 2008).

2.15. Role of globulins during the neonatal period

Globulin proteins are serum proteins that are classified into three groups in ruminants; α-, β-, and γ- globulins (Tizard, 1987). The α-fraction includes two major acute phase proteins, serum amyloid A (SAA) and haptoglobin (Hp), which both increase during inflammatory process and stress. The most important component in β-fraction is C-reactive protein (CRP), which is also involved in the stress response (Kaneko, 1997). The γ-globulin fraction contains mainly immunoglobulins, which are proteins with antibody activity (Tizard, 1987; Kaneko, 1997; Thrall, 2004). Tizard (1987) stated, that sheep have four different isotypes of immunoglobulins; IgG, IgA, IgM and IgE. Part of the immunoglobulin isotypes includes several sub-isotypes. Of these isoforms, IgG is the immunoglobulin found in highest concentration in serum.

Nowak & Poindron (2006) found that, as sheep have an epitheliochorial placenta, the immunoglobulins do not cross the placental barrier and the lamb is born without any circulating antibodies. The passive immune transfer from the ewes' colostrum to the lamb is of utmost importance for the survival of the offspring, providing it with some resistance against infectious diseases. Studies measured blood serum parameters in foals, calves (Bauer *et al*., 1985; Piccione *et al*., 2009) and lambs (Nowak & Poindron, 2006) state a trend for total protein and serum immunoglobulin levels, having a peak on the first day of life (Piccione *et al*., 2013). Immunoglobulin synthesis is initiated at approximately 3 weeks of age in neonatal lambs (Klobasa *et al*., 1985).

2.16. Mortality of new born

The mortality of neonatal farmed livestock is a source of wastage, affects farm profitability, impacts on animal welfare and, frequently, farmer morale. Animals are most vulnerable on the day that they are born, with up to 50% of all pre-weaning mortality occurring on this day in sheep (Nowak *et al*., 2000) and goats (Singh *et al*., 2008). Newborns move from the warm and protected uterus into a more challenging extrauterine environment, which can occur in a cold or wet environment.

2.17. Physiology and neonatal survival

At birth, ambient temperatures can drop from 39°c in utero to 10°c or lower. Maintenance of body temperature depends on the balance between heat loss and heat production. Heat loss is mainly affected by body surface area (small lambs have a higher surface area/body weight ratio than large lambs, thus they lose heat faster and are more at risk of hypothermia) and the insulation value of the coat (short, fine birth coats have lower insulation value than long, coarse coats). A wet coat, by amniotic fluid, reduces insulation value but removal of the fluid by maternal licking or grooming contributes to the lamb's ability to maintain normal body temperature. Within 10 min of birth the lamb increases heat production but important inter individual variations are observed (Dwyer and Morgan, 2006), and in the most extreme cases, where lambs are unable to generate sufficient heat, hypothermia is irreversible. Heat production comes from two main mechanisms in the neonate: non-shivering thermogenesis, mostly if not all attributable to brown adipose tissue, and shivering thermogenesis, which usually takes place under cold conditions, mainly in older, dry lambs, and metabolizes muscle glycogen.

Colostrum contains nutrients, immunoglobulins and other elements such as enzymes, hormones, growth factors and neuroendocrine peptides. Colostrum contains fat (7% to 13%), non-immunoglobulin protein (4% to 10%) and lactose (2% to 5%), and provides 6 to 7kJ of energy/ml (Nowak and Poindron, 2006; Banchero *et al.*, 2015). At 18°C to 26°C, lambsrequire50mlcolostrum/kg within the first18hrs of life to make up for lipid energy deficit and prevent hypothermia, at 0°C to 10°C requirements increase to 280ml colostrum/kg. Early ingestion of colostrum has an additional benefit in that it increases heat production by 17% even if body energy reserves are still replete, enhancing resistance to hypothermia. Under optimal conditions, neonates would consume sufficient colostrum to meet their carbohydrate requirement for 14 h of the first 24 h of life (Mellor and Cockburn, 1986).

Utilization of glycogen is therefore essential to make up the difference, and lambs face the first few days with largely depleted liver and muscle glycogen. Colostrum yield is dependent on adequate supplies of both energy and protein in the last 3 weeks of gestation. Although twin bearing ewes generally yield more colostrum than single-bearing ewes, their onset of lactation is slower and they do not produce as much colostrum per lamb. Thus, multiple-born lambs, compared with singles, are disadvantaged, in addition to lower birth weights and energy reserves, and higher surface area/body weight ratios. Inadequate nutrition during gestation can delay the onset of lactogenesis, reduce colostrum and milk production, and affect colostrum viscosity (Banchero *et al.*, 2015). As a consequence, delayed suckling may lead to energy reserves being exhausted within 6h of birth and result in depressed heat production. Insufficient intake of colostrum is the second major factor affecting neonatal survival after body reserve depletion.

More than 80% of losses occur within 48 h of life (Hinch and Brien, 2014). Failure of transfer of passive immunity is associated with increased risks of morbidity and mortality, lower growth rates and poor productivity (Alley *et al*., 2012). Around 14% of lamb mortalities, due to failure or inadequate transfer of passive immunity, occur between 2-10 days of age and 5% after 10 days of age (Hart *et al*., 2009).

2.18. Absorption of colostrum mechanism

Colostral absorption is defined as the passage of substances in colostrum into blood from the intestinal lumen. Newborns transfer colostrum to the intestinal canal by absorbing it. Lambs are able to absorb antitoxins of different species, ruminant colostral proteins, and substances such as egg proteins within 48 h after birth (Lecce and Morgan, 1962). Proteins enter the intestinal epithelium of neonates through the agency of the tubule vesicular system located at the edge of epithelial cells. From here, they pass into the intestinal lymphatics and capillary vessels. Thus, these absorbed immunoglobulins, which are the structure of protein, pass into circulation and the newborn completes the transfusion of maternal immunoglobulins. However, this situation changes for each domestic animal according to selectivity and intestinal permeability. The intestine of ruminants has nonselective permeability and therefore may absorb all immunoglobulin isotypes (Tizard, 1992).

2.19. Factors affecting absorption of colostral immunoglobulins

The efficiency of colostral immunoglobulin absorption through the intestinal epithelium decreases linearly with time from birth to 24 h of age (Weaver *et al*., 2000). This is due to the maturation of intestinal epithelial cells (Jochims *et al*., 1994) and the secretion of digestive enzymes. After approximately 12 h enzyme secretion increases thus reducing the ability of IgG to reach the small intestine without being degraded (Quigley *et al*., 2013). For these reasons it is critical that the timing of the first maternal colostrum feeding is appropriate in order to ensure successful transfer of immunity (Yilmaz and Kasikci, 2013).

Premature birth has been associated with decreased ability of newborns to absorb immunoglobulins from colostrum. This is because intestinal enterocytes do not reach their full endocytotic potential, which is due to the lack of the specific maturational process that occurs close to parturition to allow newborns to take up and transfer intact proteins (Sangild, 2003). Intestinal closure can have an effect on immunoglobulin absorption; the average time of intestinal closure for IgG, IgM and IgA is 26.4 h, 25 h and 26 h, respectively. The time colostrum is initially taken by the newborn is thought to influence intestinal closure (Yilmaz and Kasikci, 2013) and therefore the level of antibodies absorbed. The ability for lambs to absorb colostral immunoglobulins lasts between 24 to 36 h post-partum, before intestinal closure and activation of the neonate's digestive system (Dominguez *et al*., 2001).

Breed may also have an effect on the absorption of immunoglobulins; IgG1 concentrations (at 24 to 48 h of age) have been found to be higher in Angus compared to Simmental (Engle *et al*., 1999), Hereford and Red Poll (Muggli *et al*., 1987) new born. Differences in IgG profiles have been observed in different breeds of lambs. Brujeni *et al*. (2010) found that at day 1 post-partum the serum IgG concentration. Gender may also influence immune status. Ahmad *et al*. (2000) found that male lambs had higher albumin and globulin concentrations than female lambs whilst Brujeni *et al*. (2010) reported there was no interaction between sex of lambs, serum IgG and total immunoglobulin concentrations. The nutritional status of the dam can influence the intestinal permeability of newborn lambs to immunoglobulins (Hodgson *et al*., 1997). Newborn lambs have been found to have greater absorption of immunoglobulins when the dam is healthier. Furthermore, nutritional supplementation of dams can enhance the erythropoietic response and therefore improve offspring survival (Ahmad *et al*., 2000).

2.20. Failure of passive immunity transfer

Failure of passive transfer (FPT) predisposes the neonate to disease (Brujeni *et al*., 2010) due to them being hypogammaglobulinaemic (Britti *et al*., 2005; Mech *et al*., 2011). Low absorption of immunoglobulins often results in an increase in diseases such as diarrhea, enteritis, septicemia, arthritis, pneumonia (Thompson *et al*., 2013) and high risk of mortality (Ahmad *et al*., 2000; Wereme *et al*., 2001; Zarrilli *et al*., 2003; Stelwagen *et al*., 2009; Furman-Fratczak *et al*., 2011). Failure to acquire passive immunity can be directly responsible for half of all neonatal mortalities (Murphy *et al*., 2014). Factors influencing colostral IgG transfer include the quantity of IgG ingested, time period between birth and colostrum intake, the feeding of colostrum and also genetic, physiological and environmental factors (Wereme *et al*., 2001; Thompson *et al*., 2013). Serum IgG concentration in the ruminant newborn is an important indicator of FPT (Yalcin *et al*., 2010). The prevalence of FPT in lambs has been reported to range from 3.4% to 20%, with mortality rates of 45% to 50% in lambs from birth up until they are two to three weeks of age (Britti *et al*., 2005).

Timing of colostrum feeding has a profound effect on the transfer and absorption of colostral immunoglobulins (Morin *et al*., 2001). Multiple births, mastitis, low birth weights, breed, parity and inadequate production of colostrum are all factors that can impact on the newborn's access to colostrum (Wereme *et al*., 2001). Chirstley *et al*. (2003) found there was a linear relationship between the birth weight of lambs and their serum immunoglobulin status. Newborns that have a lower birth weight tend to be weaker and therefore are unable to or have more trouble suckling adequate amounts of colostrum to achieve adequate serum IgG concentrations for initial immune protection (Ahmad *et al*., 2000; Hashemi *et al*., 2008). Therefore, mortality rates are generally higher in newborns with low birth weights. Brujeni *et al*. (2010) found lambs with birth weights lower than 3 kg had significantly lower IgG concentrations than lambs that weighed greater than 3 kg at birth. Adequate passive immune transfer (PIT) has been determined in some studies to be reached when the lambs IgG intake was above 30 g during the first 24 h of life (Alves *et al*., 2015). Consequently, some studies suggest that the failure of passive immune transfer (FPIT) for the neonatal lamb has a significant effect on neonatal mortality and losses because of infectious causes correlate positively with low concentrations of serum immunoglobulins (Ahmad *et al*., 2000).

2.21. Assessing colostrum quality

The quality of colostrum is based on the concentration of IgG (Wereme *et al.,* 2001; Tabatabaei *et al.,* 2013; Yilmaz and Kasikci, 2013) and several diagnostic tools have been developed to assess colostrum quality. The colostrometer (hydrometer) measures colostral specific gravity (Bielmann *et al.,* 2010; Zarrilli *et al.,* 2003). Several factors have been found to influence the results including breed (Morin *et al*., 2001; Quigley *et al.,* 2013), season of the year (Quigley *et al.,* 2013) and colostral temperature (Bielmann *et al.,* 2010; Quigley *et al.,* 2013). Quigley *et al*. (1995) and Fleenor and Stott (1980) found that bovine colostral specific gravity was more strongly correlated with total protein concentration rather than immunoglobulin concentration. This may result in the specific gravity reading providing false information regarding the actual concentration of immunoglobulins in the colostrum.

2.22. Assessment of passive immunity

Passive immune transfer can be accessed through the serum total protein concentration, which reflects the amount of albumin and globulins, although this technique is nonspecific (Flaiban *et al*., 2009). The confirmatory tests considered the gold standard for IgG measurement include single radial immunodiffusion and ELISA. Enzyme-linked immunosorbent assay offers advantages in terms of cost, time, and ability to quantify the IgG level in a large number of samples at once, and this approach has been used to confirm the diagnosis of failure of PIT (FPIT) in cattle (Weaver *et al*., 2000; Hurley *et al*., 2004; Lee *et al*., 2008). Authors used reference values in calves to characterize FPIT in lambs (Hunter *et al*., 1977; Flaiban *et al*., 2009; Silva *et al*., 2009; Turquino *et al*., 2011).

There are many tests available for the testing of passive immunity transfer; however, there are only two quantitative passive immune transfer tests (Thompson *et al.,* 2013) that directly measure IgG concentrations and these are radial immunodiffusion (RID) and enzyme-linked immunosorbent assay (ELISA) (Weaver *et al.,* 2000). Both RID and ELISA is species specific; they have been found to have a 94% agreement in relation to serum IgG concentrations (Thompson *et al.,* 2013). The other tests, which include refractometry, zinc sulfate turbidity test, sodium sulfite turbidity test, serum GGT activity and whole blood glutaraldehyde gelation, measure serum total solids (Weaver *et al.*, 2000; Britti *et al.*, 2005) and do not quantitatively measure serum IgG concentration (Lee *et al.*, 2008).

2.22.1. Radial immunodiffusion

Radial immunodiffusion (RID) involves samples being applied to wells in an agarose gel that contains an antibody raised to IgG for a particular species. The sample being analyzed then diffuses through the gel and forms a precipitin ring complex with the antibody, and it is the diameter of the ring which is proportional to the concentration of IgG present (Gapper *et al.*, 2007). RID is considered the 'gold-standard' for measuring serum IgG concentrations (Dawes *et al.,* 2002; Morrill *et al.,* 2013; Deelen *et al.,* 2014) and is a commonly used method for analysis of IgG in newborn's serum (Quigley *et al*., 2013) as it is accurate (Britti *et al.*, 2005; Yalcin *et al.,* 2010). However, RID can be expensive and time-consuming because it requires 18-24 h for results from laboratory analysis (Lee *et al.*, 2008; Yalcin *et al.*, 2010; Tabatabaei *et al*., 2013) and discrepancies do exist between RID kits (Gapper *et al.*, 2007; Morrill *et al.*, 2013). RID technique has been used for IgG analysis in many species, including bovine, ovine, equine, caprine, porcine and human (Gapper *et al.,* 2007).Radial immunodiffusion (RID) is an immune-based technique that can be used to determine colostral IgG concentration in humans, sheep, goats, horses, pigs and cattle. Colostrum samples are applied to wells in an agarose gel with antibody raised to the species IgG. The sample then diffuses through the gel and the IgG forms a precipitin complex with the antibody. The amount of IgG present in the sample is proportional to the ring diameter (Gapper *et al.,* 2007).

3. MATERIALS AND METHODS

3.1. Study area

The study was conducted in Awash fentale district, zone 3, Afar Regional State of Ethiopia. The zone is located in the part of the middle Awash basin about 225 km north east of Addis Ababa. Geographically, the study area Awash fentale located between 99◦12to 9◦15 North latitude and $39^0$58'- 40^0 3'East longitude. The altitude range is from 736 – 801m above sea level and the majority of the land is rocky with annual rain fall range 150–500mm per year. The average daily ranges of minimum and maximum temperature are 25^0C and 39^OC, respectively. Awash fentale is bordered on the south by the Oromia Region, on the west by the Amhara Region, on the north by Dulecha, and on the east by Amibara (Figure 1). Towns in Awash Fentale include Awash Sebat Kilo and Sabure. Rivers in this woreda include the Awash and its tributary the Germama. A large portion of this woreda is occupied by the Awash National Park. The soil texture of the area is characterized under sandy loam which accounts 55% sand, 40% silt (mud) and 5% of clay. The water holding capacity of such soil is very poor (Mesfin, 2017). The dry season of the year from May to June while the main rainy season extends from July to September accounts for above 60% of the annual total rainfall. The livestock populations of the Awash fentale district are composed of Cattle 86085, sheep 35389, goat 83691 horses, asses 45260, mules 4355, camels 41245, poultry 4355 (CSA, 2012).

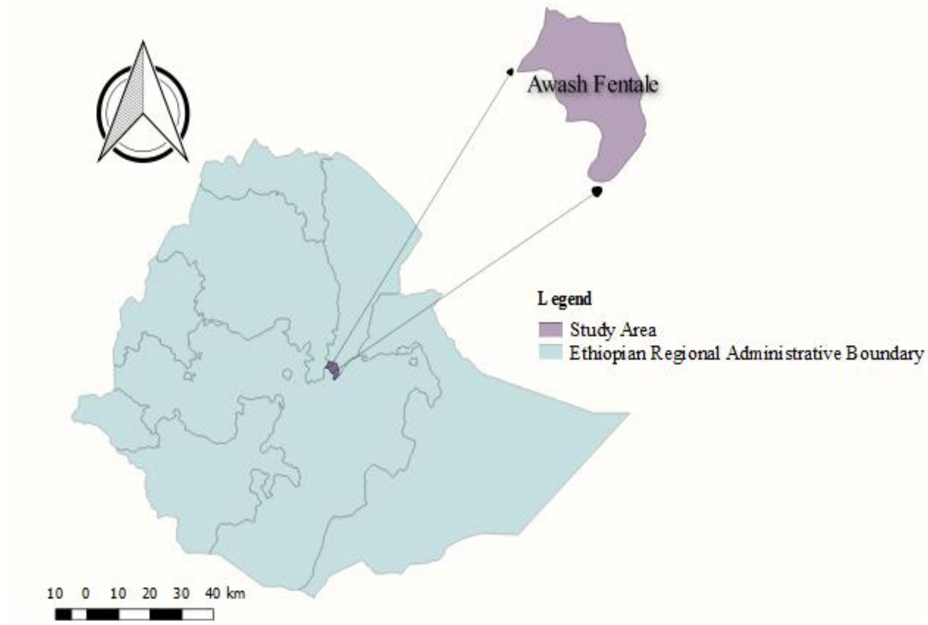

Figure 2: Spatial location of study site (source: QGIS)

3.2. Study population and recruitment criteria

Lamb and goat kid of indigenous Afar breed of both sexes aged from 2-7 days constitute the study population. Lamb and goat kid less than 2-7 days at the study were recruited. Besides, during study all newly born and having 2-7 days of age experimental animals were in the selected villages recruited. Individual risk factors were identified by means of check-off forms attached (Annex 2).

3.3. Study design

Cross sectional study was conducted from January to April, 2019 in Awash fentale district of Afar region, Ethiopia in order to determine the concentration of lamb and goat kid serum IgG. A well-structured questioner (Annex 2 and 3) was designed to assess the young stock and house hold risk factors about the lamb and goat kid, also their dam management practice. Key informants from the owner of animals, elders and community leaders, one district animal extension officer, one veterinarian from Werer research center were selected and invited for pre-testing the questioner. The questioner was administered to the new born holder pastoralist. Determination of passive Immunity transfer to the new born lamb and goat kids conducted by species specific sheep and goat radial immune diffusion kit according to the manufacturer's instructions (Annex 4).

3.4. Sampling and sample size determination

From the district, random selection of villages was employed based on the lists of villages obtained from the district office. 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 0.32/SE2.

$N=0.32/SE^2$

SE is standard error considering SE of 0.0472 with 95 % coefficient interval as follows, N= 0.32/0.04722 = 143. Accordingly, one hundred forty-three (143) lamb and goat kid were selected purposely based on the availability and between 2-7 days of age. Three villages namely Qebana, Doho and Dudubi were selected from the five villages.Households from the villages were selected purposely based on availability of lamb and goat kid, facilities and ease of access from villages. Then, all lamb and goat kid born in the selected villages during field visits from January, 2018–April, 2019 was included in the study. From the selected villages, all the lambs and goat kids were enrolled.

3.5. Method of sample collection

3.5.1. Physical and clinical examination of new-born

Physical and clinical examination was performed by examining the normal physiology of the newborn. The health status of the lambs and goat kids was monitored on farms by examination made during blood samples collection. The newborns examined for temperature, pulse rate, respiratory rate, diarrhea, presence of umbilical infection, fecal score, joint and umbilicus infection. At the sample collection period, ewes and doe determined to have disease (mastitis, pneumonia, enteritis, pregnancy toxemia, abortion etc.) were eliminated from the study. The examinations performed with the manner of (Annex 1). Information about parameters including age, parity, birth witness, birth location, birth time, type of delivery, birth type and related once were collected by interviewing owners or persons directly responsible for handling of animals (Annex 2).

3.5.2. Body condition score

The newborns body condition score performed by according to the manner of annex 5. Dams body condition score were assessed by the palpation of the lumbar region, specifically on and around the backbone (spinous and transverse processes) in the loin area, immediately behind the last rib and above the kidneys to examine the degree of sharpness or roundness (Russel, 1984). Body condition score (BCS) was determined using a five points scale according to (Santucci *et al*., 1991). Body condition scoring has been widely adopted based on a subjective assessment of the fat level and muscle thickness on the back bone behind the last rib (Treacher *et al.*, 2000).Body condition score was assessed at farm during sample collection according to manual of body condition score located at annex 5 and scored on scale from 1-5 categorized as medium (BCS 3), good (BCS 4and 5) or poor (BCS 1 and 2) (Annex 5).

3.5.3. Blood and data collection

Lamb and goat kid and other putative factors were recorded during blood sample collection. A total of 43 house hold information was obtained from questioner survey concerning young stock enrolment household risk factors and related activities; both the lamb and goat kid and dam related management practices (Annex 2 and 3). Blood samples were collected through jugular vein from 143 lamb and goat kid from 2-7 days of age. For collecting whole blood firstly, the animal restrained by the help of assistant and by pressing thumb or finger in the groove at the base of the neck flow of the vein and blood stopped and creates pressure, thereby blood collection was performed. The collected samples were individually labeled with identification number and placed in icebox containing icepack. The whole blood allowed to clot for at least 2-3 hours and then serum separated by centrifugation (2000r/m) at room temperature for 10 minutes. Thereafter, blood serum transferred into a clean 2ml cryovial tube using a micro pipette. The samples stored at –20°C Melka Werer research center until shipment to Aklilu Lemma Institute of Pathobiology (ALIPB) using portable refrigerator where laboratory analysis was performed.

3.6. Approach of determining IgG concentration

A blood sample taken from 2-7 day-old lamb and goat kid because 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 specie specific sheep and goat radial immuno diffusion (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^{0}C prior to use and the directions detailed by the manufacturer were followed. The manufacturer has included 0.25 ml containing three vials of pooled sheep and goat reference sera of known IgG concentration; these three standard sera were included in well of 1-3 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 (Annex 4). After overnight incubation, serum samples with a diffusion ring diameter was measured by digital caliper and correlated with the table of reference. Ring diameter smaller than that of the lowest standard and higher diameter that exceeded the ring diameter of the top standard results were considered as lower and higher concentration from the study.

3.6. Method of determining failure of passive immunity

Passive transfer immunity determined by quantification of IgG concentration through taking a blood sample from the lamb and goat kid. The species specific sheep and goat radial immuno diffusion (RID) IgG (Triple J Farms Jorgensen Place Bellingham, WA USA) test kit was used to determine IgG in serum samples in accordance with manufacturer instructions (Annex 4). Samples are typically applied to wells cut in an agarose gel set with antibody raised to Sheep and goat IgG. During incubation, the sample diffuses through the gel, with the IgG forming a precipitin complex with the antibody. Serum IgG concentration of test samples were determined by comparing the diameter of the zone of precipitation. The diameter of precipitated rings was measured using a hand-held caliper after 18 to 24 h of incubation at room temperature. Determination of failure of passive immunity in goat kids defined as serum IgG concentrations <1200 mg/dl Hunter *et al*. (1977); O'Brien and Sherman (1993); Turquino *et al*. (2011) Alves *et al*. (2015) and Massimini *et al*. (2006) in lambs used 1500 mg/dl as the threshold of passive immunity. For calculation of sensitivity and specificity, a positive test result was considered to be indicative of failure of passive transfer, and a negative test result considered as to be indicative of adequate passive transfer of immunity (Dawes *et al*., 2002).

3.7. Data management and statistical analysis

All data was entered in to Ms-Excel 16 after the completion of the study from the study area to make the information easier for software integration with R Studio Version 1.1.383 2009-2017 R Studio, Inc. Simple and multiple logistic regression models were used to evaluate the association between the risk factors and the concentration of serum

IgG on lamb and goat kid. Factors parity, age of lamb, sex, body condition of newborn and dam, birth type was considered for their association with immunoglobulins concentration and evaluated for the association. Logistic regression was used to describe association between variables based on a calculated odd ratio (OR), which is a measure of the association between an exposure and an outcome (Edwards, 1963).Final data was summarized using descriptive statistics (means, variance and percentages).

4. RESULT

A total of 97 (56 males and 41 females) of lamb and 46 goat kid (19 females and 27 males) blood samples were collected during study time. All samples were collected from day7 of age from both species. The single radial immuno diffusion test performed on the 97 samples of lamb serum and 42 serum samples of kid. Among the lamb sRID result 10 samples (2 below and 8 above the standard) were considered as low concentration and higher concentration respectively. From goat kid 6 samples exceed the concentration level of the standard. The samples below standard considered as failure and higher considered as adequate passive immunity in case of absence of low and higher concentration IgG measure kit. Result of the single radial immuno diffusion showed as range from 47.64 to 6071.632 mg/dl serum IgG concentration in lambs where as in goat kids it ranges from 169.577 to 3559.875 mg/dl serum IgG concentration. Based on the radial immuno diffusion result 21.6 % of newborn lambs in the study area have failure of passive immunoglobulin concentration and the remaining 78.4 % had adequate passive immunity. In goat kids 83.3 % have adequate passive immunoglobulin concentration and 16.7% kids had less or failure of passive immunoglobulin concentration from their dams (Graph 1 and Table 1).

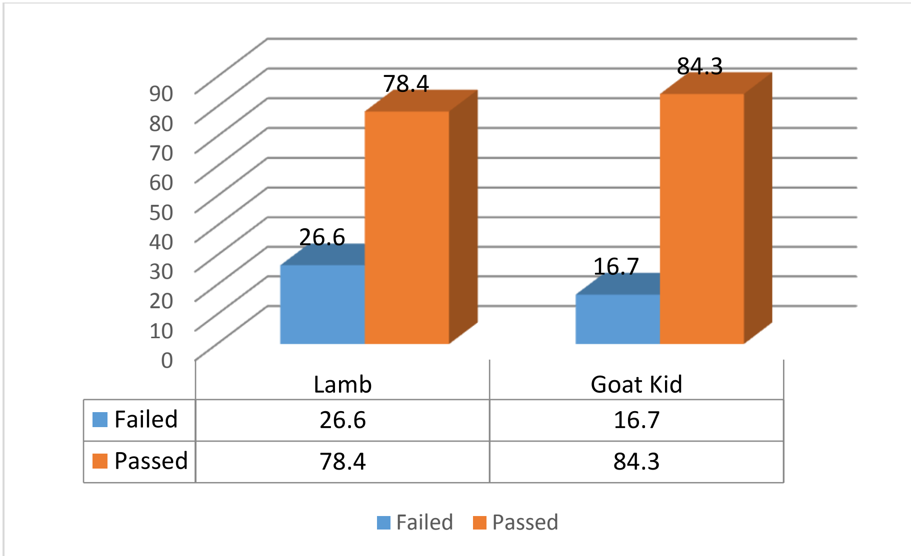

Figure 4: Level of lamb and goat kid immunity status of the study

Result on the effect of parity number on IgG concentration among the 5 primiparous doe 4 (80%) kids were having adequate immunity and 1 (20%) kid with FPI and from 37 multiparous does 31 (83.7%) kids gained adequate passive immunity and 6 (16.3%) with failure of passive immunity. Kids of primiparous doe were seen to have failure of passive transfer but in multiparous doe the transfer likelihood better. According to birth type single born kids were prone to failure of immunity than multiple births. From the 24 single born kids 18 (75%) kids gained adequate passive immunity and 6 (25%) kids gained inadequate passive immunity. Belong to the 18 twin born kids 17 (94.4%) with sufficient passive immunity and 1 (5.6%) did not gain adequate passive immunity. Result of sex of the kid shows that from 17 females 12 (78.6%) kids gain sufficient passive immunity and 5 (21.4%) did not gain adequate passive immunity, among the 25 males 23 (92%) gain adequate passive immunity and 2 (8%) did not gain sufficient passive immunity (Table 1).

The better transfer of passive immunity was observed from 74 multiparous ewes 56 (75.6%) lambs gain adequate passive immunity in 8 ewes did not found sufficient and from 23 primiparous ewes 18 (78.8%) passes adequate passive immunity and 5 ewes passes insufficient passive immunity to their offspring. This study result shows that single born 47 lambs 33 (70%) gain adequate and 14 (29.8%) inadequate passive immunity. Primiparous ewes born lambs considered more tend to failure passive immunity. From 41 female lambs 30 (73.1%) lams gain sufficient and 11 (26.9%) did not gain required amount of IgG concentration. From 56 male twin born lambs 44 (78.6%) gain sufficient amount of IgG concentration and 12 (21.4%) did not gain required amount of IgG concentration (Table 1).

Table 1: Immunity status of the lamb and goat kid and factors

Newborn	Sex	Total examined	Passed	Failed	Immunity level	
					Passed	Failed
Lamb	Female	41	30 (73.1%)	11 (26.9%)	74.4%	21.6%
	Male	56	44 (78.6%)	12 (21.4%)		
Birth type	Single	47	33 (70.2%)	14 (29.8%)		
	Twin	50	41 (82%)	9 (18%)		
Ewe parity	Primiparous	23	18 (78.8%)	5 (21.2%)		
	Multiparous	74	56 (75.6%)	8 (24.4%)		
Goat kid	Female	17	12 (78.6%)	5 (21.4%)	83.3%	16.7%
	Male	25	23 (92%)	2 (8%)		
Birth type	Single	24	18 (75%)	6 (25%)		
	Twin	18	17 (94.4%)	1 (5.6%)		
Doe parity	Primiparous	5	4 (80%)	1 (20%)		
	Multiparous	37	31 (83.7%)	6 (16.3%)		

4.1. Goat kid variables associations with serum IgG concentration

The result were analyzed to show the correlation of the IgG concentration and observed variables (Table 2). Sex of kid, doe parity, birth time, birth site, type of delivery, birth type, kid body condition, doe body condition, birth location, type of delivery and birth witness effect on kid serum IgG concentration were considered as risk factors and measured within 7 days after consumption of colostrum with in first 24 hrs of birth. These factors were analyzed to found significant associated with failure of passive transfer of immunity with serum IgG concentration. Based on the statistical result there was no significant importance between factors variables on the concentration of IgG of goat kid. The concentration of IgG was not affected between age differences on goat kid ($P>0.05$). Similarly, no significance association was found between the serum IgG concentrations of the male kid and female kids.

Table 2: Risk factors association and IgG concentration of goat kid and doe in Awash fentale, Afar regional state.

Variables	Variable category	Number	Immunity		Serum IgG		X^2	P value
			Passed	Failed	Mean	SD		
Age goat kid (Day)	Two-three	9	6	3	2219.65	1040.18	2.4857	0.2886
	Four-five	12	11	1	2301.03	1064.38		
	Six -Seven	21	18	3	2342.29	1075.99		
Sex	Female	17	12	5	2304.29	1062.2	4.002	0.1352
	Male	25	23	2	2219.65	102426		
Kid BCS	Poor	31	26	5	2342.29	1062.20	1.5207	0.4675
	Medium	7	5	2	2383.55	1089.28		
	Good	4	4	0	2219.65	1054.89		
Birth location	In burn	22	19	3	2219.65	1080.16	0.0190	0.8901
	Out side	20	16	4	2260.34	1114.62		
Birth type	Single	24	18	6	2219.65	1035.98	1.575	0.2095
	Twin	18	17	1	2425.37	1036.34		
Doe BCS	Poor	4	4	0	2260.34	1084.19	1.1926	0.5508
	Medium	7	5	2	2342.29	1074.61		
	Good	4	4	0	2260.34	1063.22		
Doe parity	One-three	13	9	4	2342.29	1055.05	6.96	0.541
	Four-six	14	11	3	2383.55	1075.99		
	Seven-nine	12	11	1	2219.65	1043.84		
	Don't know	3	2	1	2637.937	1010.87		
Birth witness	Yes	23	19	4	2383.55	1063.22		
	No	19	16	3	2301.03	1053.42	2.7836	1
Type of delivery	Normal	23	21	2	2301.03	1053.42		
	Assisted	15	11	4	2383.55	1075.99	2.3322	0.3116
	Don't know	4	3	1	2425.37	1078.56		
Time of birth	Day	15	13	2	2219.65	1089.44		
	Night	27	22	5	2342.29	1062.2		

BCS= Body condition score X^2= Chi-square P-value=Precision valu

Within the same sex association with IgG concentration female sex was found important on the serum IgG concentration of goat kid (Table 3). Analysis of between within age, parity number, body condition score doe and lamb, obtained result showed there was no significant association.

Table 3: Sex of kid association within the same sex and IgG concentration

Variable	Category	Number	Immunity		P-value
			Passed	Failed	
Sex	Female	17	12	5	0.0285
	Male	25	23	2	0.1297

In the same fashion in kid serum IgG concentration association analysis with time of birth, birth type, type of delivery there was no statistical importance.

4.2. Lamb variables associations with serum IgG concentration

Similar to goat kids there was no recognized significant association of birth type, lamb body condition, sex of lamb, birth location, time of birth on failure of passive immunity in lamb serum IgG concentration. The result of lamb age and type of delivery was important factor on the transfer of serum IgG concentration and was found statistically significance ($P<0.05$). Birth type, lamb body condition, time of birth and birth location did not show statistical significance association IgG concentration. Lamb serum IgG were not affected by ewe body condition, ewe parity and birth witness ($P>0.05$) (Table 4).

Table 4: Risk factors association and IgG concentration of lamb and ewe in Awash fentale, Afar regional state

Variables	Variable category	Number	Immunity		Serum IgG		X^2	P value
			Passed	Failed	Mean	SD		
Age lamb (Day)	Two-three	23	21	2	2856.35	1853.11	5.8574	0.05345
	Four-five	33	21	12	2540.29	1825.92		
	Six-seven	41	32	9	2665.16	1863.06		
Sex	Female	41	30	11	2665.16	1822.95	0.14149	0.7068
	Male	56	44	12	1826.05	1826.05		
Lamb BCS	Poor	58	43	15	2602.72	1826.05	1.21.7	0.5459
	Medium	34	28	6	2602.72	1810.63		
	Good	5	4	1	2920.72	1704.67		
Birth location	In burn	42	34	8	2540.29	1822.66	2.1393	0.3431
	Out side	52	47	15	2665.16	1849.04		
	Don’t know	3	3	0	2792.94	1167.13		
Birth type	Single	47	33	14	2540.293	1820.11	1.2662	0.2605
	Twin	50	41	9	2602.72	1826.05		
Ewe BCS	Poor	23	16	7	2665.16	1934.77	1.6257	0.4436
	Medium	53	40	13	2540.29	1817.02		
	Good	21	18	3	2665.16	1838.62		
Ewe parity	One-three	54	41	13	2602.72	1810.63	0.14385	0.9306
	Four-six	28	21	7	2665.16	1840.94		
	Seven-nine	15	12	3	2920.72	1805.35		
Birth witness	Yes	58	31	8	2665.16	1835.22	1.6257	0.4436
	No	39	43	15	2540.29	1810.63		
Type of delivery	Normal	41	34	7	2665.16	1817.02	7.7995	0.02025
	Assisted	43	34	9	2602.72	1835.31		
	Don’t know	13	6	7	2665.16	1846.94		
Time of birth	Day	27	18	9	2665.16	1835.22	2.13893	0.3431
	Night	69	55	14	2602.72	1810.62		
	Don’t know	1	1	0	1610.9	1610.9		

BCS= Body condition score X^2= Chi-square P-value=Precision value

5. DISCUSSION

Failure in transfer of passive immunoglobulin to neonatal lambs has a significant effect on neonatal morbidity and mortality rates, and losses due to infectious diseases are possibility correlated with low concentrations of serum immunoglobulin (Ahmad *et al*., 2000). Hodgson *et al*. (1992) reported that morbidity and mortality rates were higher in colostrum deprived lambs (80 and 67%) than colostrum fed lambs (20 and 13%). The estimates of the immunoglobulin concentration contain in colostrum which fed to the lambs are necessary for knowledge of immunoglobulin levels transferred to neonatal lambs (Weaver *et al*., 2000).

The performed study on lamb serum by single radial immuno diffusion kit on present study shows that IgG concentration range from 47.64 to 6071.632 mg/dl. This finding agrees with Hashemi *et al.* (2008), Vatankhah (2013) and Erhan *et al*. (2014) (6078 ± 2526 mg/dl) who reported an overall comparable mean IgG concentration. In contrast this finding disagrees with Bekele *et al*. (1992) (3660 mg/dl) who observed on Ethiopian Menz breed, Gilbert *et al*. (1988) (3100mg/dl), Ahmad *et al*. (2000) (2890 mg/dl), Boland *et al.* (2005) (680-1960 mg/dl) who reported an overall lower IgG concentration. Alves *et al*. (2015) reported 39.5% and Turquino *et al*. (2011) 12.4% of the lamb's IgG concentration is found to be failed passive immunity transfer. This might be due to the difference of the nutrition (O'Doherty and Crosby 1997; Mazzone *et al*., 1999), ewe body condition score (BCS) at lambing (Thomas *et al*., 1988; Al-Sabbagh *et al*., 1995), genetic and environmental factors (Gilbert *et al*., 1988). It is also known that poor quality colostrum or inadequate colostrum intake causes failure of passive transfer of immunity (Vatankhah, 2013).

The Present study showed sex had no effect on the transfer of serum IgG concentration of the lamb. This is in agreement with Bekele *et al*. (1992), Kaymaz *et al*. (2000), Gilbert *et al*. (1988), and Ahmad *et al*. (2000) who stated sex had no effect on passive immunity. With regard to lamb IgG concentration on birth type was not affected IgG of twin and single in this study. On contrary to this finding Turquino *et al*. (2011) and Filteau *et al*. (2003) reported that twin lambs had lower IgG concentration than single lambs. Gilbert *et al*. (1988) observed a linear increase in

IgG concentration as litter size increased from singles to triplets. Halliday (1974; 1978) and Gilbert *et al*. (1988) contrarily reported birth type to be a source of variation in lamb serum IgG. They found that as litter size increased, the lamb serum IgG concentration decreased. This might be due to the difference of the nutrition Mazzone *et al*. (1999), genetic and environmental factors Gilbert *et al*. (1988).

Body condition of the lamb did not affect the concentration of serum IgG on present study. The result agrees with Al-Sabbagh *et al*. (1995) serum IgG concentrations were not affected by body condition score of lamb. In agreement with this result Vatankhah (2013) stated that the effects of age of ewe, type of parturition and body condition score on considered immunity traits were not significant. Also similarly, with this finding Thomas *et al*. (1988), Al-Sabbagh *et al*. (1995) and Al-Sabbagh (2009) observed that the concentrations of IgG were not affected by BCS and had no significant effect on immunity traits. In contrast to this study Yılmaz *et al*. (2011) observed body condition at the time of breeding and lambing can affect the performance of ewes and lambs, as well as productivity. Studies have reported direct impacts of ewe BCS on reproductive performance colostrum production (Jalilian and Moeini, 2013), survival and growth rates of lambs (Sarı *et al*., 2013; Corner-Thomas *et al*., 2015a, b).

Present study found that ewe parity did not affect the serum IgG concentration of lamb. Ewe parity did not influence the colostral energy content, whereas the IgG concentration of the colostrum obtained from primiparous ewes was higher than that of multiparous ewes. Among the multiparous ewes, the colostrum obtained from ewes carrying twins showed higher energy content and IgG concentrations than that from ewes carrying a single lamb Higaki*et al*. (2013) and Turquino *et al*. (2011). In a study conducted on sheep, no difference was observed in IgG concentrations of colostrum in multiple births; however, parturition number was reported to be effective on IgG concentration (Tabatabaei *et al*., 2013). Gallo and Davies (1987), however, reported that ewes giving birth to twins or multiples produced a greater volume of colostrum than ewes giving birth to single lambs. Turquino *et al*. (2011) also found no difference in serum IgG, total protein, and gamma-globulin concentrations in lambs from ewes of different parity. According to these previous authors, the parity did not seem to be important in the success or failure of passive immunity transfer. Other authors have also reported no relationship between parity and serum IgG concentration in lambs (Gilbert *et al*., 1988; Ahmad *et al*., 2000).

The immunoglobulin stays in the newborn blood circulation for a certain period until their immune systems start to develop (Jaster, 2005).The rate and pattern of colostral immunoglobulin in blood serum of the kids were dependent on the concentration of IgG in the colostrum they consumed (Matte *et al.*, 1982). A delay in this period results in decreased viability and increased susceptibility to diseases (Hernandez *et al*., 2011). Another important issue is the decreased viability due to insufficient colostrum intake during the first couple of hours after birth, which may cause increased death rates (Quigley and Drewry, 1998).

In this study the result of RID species specific goat kit showed serum IgG concentration ranges from (169.577-3559.875 mg/dl). Similar results were observed on the study of Argüello *et al.* (2006) who found IgG blood serum concentration of (3500 mg/dl) and Ubertalle *et al*. (1987) found at birth but not at 24 hrs postpartum in Saanen (3600 mg/dl) and on other study Ubertalle *et al*. (1987) observed lower result on Camosciata goats (1691 mg/dl), O'Brien and Sherman (1993) who found serum IgG concentration of (1439mg/dl) on grouped 39 kids and also Mellado *et al*. (2008) observed lower result in 21 kids ingesting maternal colostrum freely and found (1011± 1140 mg/dl) at 24 hours of birth.

O'Brien and Sherman (1993) observed lower IgG blood serum concentrations result of kids on 1 and 2 kid litters ranged from (1550 to 2310 mg/dl) and from 3 kid litters, which had values ranging from (620 to 1630 mg/dl) the result observed had lower IgG concentration on single, twin and triple birth the result showed as lower concentration than observed on present study. The rate and pattern of colostral immunoglobulin in blood serum of the kids were dependent on the concentration of IgG in the colostrum they consumed (O'Brien and Sherman, 1993). In contrast to this study Suraya and Yaakub (2011) reported colostrum fed 100 ml at 0 and 8 h later (9526 mg/dl) and fed 200 ml of colostrum at one time (7599 mg/dl) which was higher result than present study.

The result of RID performed on goat kid serum IgG confirmed that sex of goat kid didn't affect concentration of IgG. Similarly, to this study Castro *et al*. (2009), Khan and Ahmad (1997), Mehmet *et al*. (2017), Pakkanen and Aalto (1997), Chen *et al*. (1999) and Argüello (2000) reported no differences between sexes in Nubian or Majorera kids. In contrary to this study O'Brien and Sherman (1993) observed that males tend to present a higher IgG blood serum concentration than females. The reason for these differences might be different managements of colostrum feeding periods; thus, in Chen *et al*. (1999) and Argüello (2000) male and female kids received the same colostrum, whereas in O'Brien and Sherman (1993) female kids were fed with pasteurized colostrum.

Litter size did not affect the concentration of goat kid IgG. In agreement with this study Ethiopian Bekele *et al*. (1992) did not find differences between single and twin lambs from the Menz breed, Nedim *et al*. (2018), Auad *et al*. (2016), Pattinson *et al*. (1991) and Pakkanen and Aalto (1997)litter size did not affect the concentration of IgG. In contrast to these study Gilbert, 1988), Chen *et al*. (1999) observed lower concentrations of IgG in the blood of single born kids than for twins. and also O'Brien and Sherman (1993) who contrary found failure of IgG from litters of 3 kids showed IgG concentrations indicative of FPT at 1, 3, 4, and 5 d post-birth, Castro (2009) who observed differences with triplets.

Present study showed age of the doe did not found significant effect on the transfer of IgG concentration. In contrast to present study Gilbert (1988) observed that age of the doe found significant on transfer of IgG. Parity and effects of birth type were not significant among the investigated properties Nedim *et al*. (2018) and Pakkanen and Aalto (1997). Nedim *et al*. (2018) did not observed sufficient previous reports regarding to age of the dam, body condition score on concentration of neonatal kids. The effects of birth type, kid gender and parity were not significant. Colostrum intake is of great importance in the viability and passive immunity development in newborn animals (Stelwagen *et al*., 2009; Hernandez *et al*., 2014a, b). Goat kid mortality is closely associated with the IgG level in blood (Moreno *et al*., 2012).

6. CONCLUSION AND RECOMMENDATION

The present study has observed failure of passive immunity in small ruminant newborns of pastoral herd. This indicates that failure of passive immunity is significant that can expose newborn to weakness in the immune system makes it easy for disease and infections in the body. For the fight against these factors, the antibodies in colostrum provide the greatest support to newborns. This will, influence the newborn lambs and goat kid viability in the long run, influence the survival and contribute significant role to an increase in young stock mortality. Important factors that affect passive immunity of newborn include; type of delivery and age of the lamb.

Based on the above conclusion the following recommendations are forwarded:

- ❖ Extension of pastoralists concerning newborn, colostrum and dam management practices.
- ❖ Caring for pregnant ewes and doe has great significance in reducing the failure of passive immunity.
- ❖ Pastoralist need to be aware of the importance of colostrum feeding.

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8. ANNEXES

Annexes 1. Physical examination form

HOUSEHOLD ID: ______________________ DATE: (dd) _____(mm)_____(yyyy)__________
YOUNG STOCK ID: ____________________Examiner Name: _____________________
Before restraining the animal, make observations about general attitude.

1) Attitude (circle one)	Bright/Alert	Depressed	Lethargic	Comatose

2) General notes if necessary (emaciation, in pain, etc.)

Once you are able to restrain the animal, record the following:

3) Rectal Temperature		Celsius
Use rectal temperature key to give a temperature score below		

37.7- 38.2 = 0

38.3-38.8 = 1

4) Respiratory Score – USE SCORING CARD:

Temperature Score (^{o}C) (0-3)	Cough Score (0-3)	Nasal Discharge Score (0-3)	Eye Score (0-3)	Ear Scoe (0-3)	Increased Respiratory effort? 0 – no1-yes

Thoracic Auscultation:

5) Respiratory Rate		Breaths per minute	
Are wheezes present?	Yes	No	Don't know
Are crackles present?	Yes	No	Don't know
Are lung sounds muffled?	Yes	No	Don't know
Is animal coughing?	Yes	No	Don't know
6) Heart Rate		Beats per minute	
Are there any abnormal heart sounds?	Yes	No	Don't know
7) Notes on Thoracic Auscultation			

Abdominal Auscultation:

8) Ruminal Contractions		Number per minute

Are peristaltic sounds increased?	Yes	No	Don't know
Are there any ping sounds?	Yes	No	Don't know
9) Notes on Abdominal Auscultation			

10) Body Condition Score (1-5 for calves/kids/goats ; 0-5 for camels)			
11) Umbilicus Examination (Use thumb as reference)	Normal	Abnormal	Don't know
12) Notes on umbilicus exam			
13) Limb Joints Examination	Normal	Abnormal (Enlarged, painful, fluid accumulation)	Don't know
14) Notes on joints exam			

15) Fecal Score - USE SCORING CARD (circle one)

NORMAL	Pasty, normal color	Loose	Watery
0	1	2	3

16) Blood in stool?	Yes	No	Don't know
17) Notes on fecal exam			

18) Samples Collected (circle)

Sample Type				Guidance	
				Who?	STORAGE
1	Blood, EDTA tube	Yes	No	ALL	Cooler

Annexes 2. Young stock enrollment form

HOUSEHOLD ID: ____________________ DATE: (dd)_____(mm)_____(yyyy)_________
YOUNG STOCK ID: _________________ Examiner Name: ________________

Use this form for each animal less than 7 days old that you enroll in the household. Different animals should have different forms. Each household should have a folder and all its forms should be kept together within the household folder.

1)YOUNG STOCK ID ASSIGNED (Per ID protocol)	

2) Age	(days)	(Weeks)	(months)	Don't know

3) Sex (circle one)	Male	Female

4) Predominant Breed (circle one)	Local	Improved/Crossbred	Don't know

5) Name of Breed		Don't know

Animal History
For the following questions, circle one option.

6) Sire	Artificial Insemination	Live Cover		Don't know
7) Time of Birth	Day	Night		Don't know
8) Birth witnessed	Yes	No		Don't know
9) Birth location	In barn	outside	Other	Don't know
10) Type of delivery	Normal	assisted		Don't know
11) Colostrum fed within 24 hours?	Yes	No		Don't know
12) If answer to question 11 is no, was this because				
Lamb and kid but unable to suck	New born depressed, unable to suck	Other		Don't know
13) Navel dipped	Yes	No		Don't know
14) Did animal have continuous access to dam for the first 24 hours of life?	Yes	No		Don't know
15) Separate housing from herd?	Yes	No		Don't know
16) Animal housed with dam?	Yes	No		Don't know
17) Animal housed with other young stock?	Yes	No		Don't know

18) Any vaccinations given to	Yes	No	Don't know

animal since birth?			
19) If yes, what type of vaccines?			
20) Any medications given to animal since birth?	Yes	No	Don't know
21) If yes to question 20, what type of medications? Mark from the following below			
Dewormers	Yes	No	Don't know
Vitamins	Yes	No	Don't know
Oral electrolytes	Yes	No	Don't know
Other, specify	Yes	No	Don't know

22) Has this animal shown any signs of illness since birth?	Yes	No	Don't know

23) If yes to question 22, mark what symptoms of illness has been observed			
Nasal discharge	Yes	No	Don't know
Cough	Yes	No	Don't know
Difficulty breathing	Yes	No	Don't know
Ocular discharge	Yes	No	Don't know
Cloudy eyes	Yes	No	Don't know
Head tilt	Yes	No	Don't know
Circling	Yes	No	Don't know
Not suckling	Yes	No	Don't know
Diarrhea (not bloody)	Yes	No	Don't know
Diarrhea (bloody)	Yes	No	Don't know
Bloat	Yes	No	Don't know
Lameness	Yes	No	Don't know
Swollen joints	Yes	No	Don't know
Umbilical infections or swelling	Yes	No	Don't know
Difficulty urinating	Yes	No	Don't know
Straining to defecate	Yes	No	Don't know
Birth defects	Yes	No	Don't know
Collapse	Yes	No	Don't know
Predation	Yes	No	Don't know
Trauma	Yes	No	Don't know
Bleeding from orifices	Yes	No	Don't know
Other, describe			

24) Have any of the following procedures been done to this animal?			
Castration	Yes	No	Don't know
Dehorning	Yes	No	Don't know

Branding	Yes	No	Don't know
Ear notch	Yes	No	Don't know
Tail docking	Yes	No	Don't know

Animal Environment

Inspect animal housing place. Look at where this animal is housed and answer the following questions.

36) Is animal housed on bedding?	Yes	No	Don't know
37) Does the area where the animal lies down look clean and dry?	Yes	No	Don't know
38) If all the animals housed together were going to stand up at the same time, would they have enough room to do so and turn around without touching each other?	Yes	No	Don't know

Dam History

For the following questions, circle one option

39) Age of dam at parturition		(years)	Don't know
40) Dam gestation number			Don't know
41) Were any vaccines given to the dam within 2 months of delivery	Yes	No	Don't know
42) If yes to vaccines, specify type			

43) Is dam alive?	Yes	No	Don't know
If yes, answer the following			
44) Dam Body Condition Score (cows/sheep/goats use scale 1 -5; camels use scale 0-5)			Don't know
45) Dam milk production (include all milk, i.e., milk given to calf and milk sold /human consumption)		(Liters/day)	Don't know
46) Is dam mothering instinct adequate?	Yes	No	Don't know

If no to question #43, answer the following			
47) Did the dam die within the first 2 weeks after parturition?	Yes	No	Don't know

Annex 3. House hold risk factor identification form

HOUSEHOLD ID: ____________________   DATE: (dd)_____(mm)_____(yyyy)_________

Fill out this form ONCE per household, at the very first time the household is enrolled in the study. Write NA if the question is not applicable, NR when the respondent declines to do.

1)Household ID ASSIGNED (Per ID protocol, i.e., G-F001)	
2) Interview performed by (full name):	
3) Date (Western Calendar):	DD: MM: YYYY:

Owner information

4) Livestock Ownername (optional)			
5) Primary telephone contact number			
6) Gender	MALE	FEMALE	
7) District/Woreda			
8) Kebele			
9) GPS coordinates of farm:	Latitude	Longitude	
10) Predominant Production System (use WGS 1984)	Peri-Urban Dairy	Mixed Crop Livestock	Pastoral
11) Owner educational status 0=none or preschool 1=primary 2=secondary 3=higher 8=don't know9= no response			

Annexes 4. Radial immuno diffusion laboratory work procedures

□□Material required

Sheep and Goat radial immuno diffusion plate kept between +4oC to +8oC with reference Sera: containing 3x0.25 ml

1. Serum sample (kept at – 20 until the day of analysis)
2. Microliter dispenser (5 µl)
3. 5 µl pipet tips
4. Digital caliper providing accurate measurement in millimeter
5. Normal graph paper
6. Gown and glove
7. Marker
8. 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. If plates have moisture plate cover were removed until evaporation has dried from the surface and wells and then the cover replaced until used.

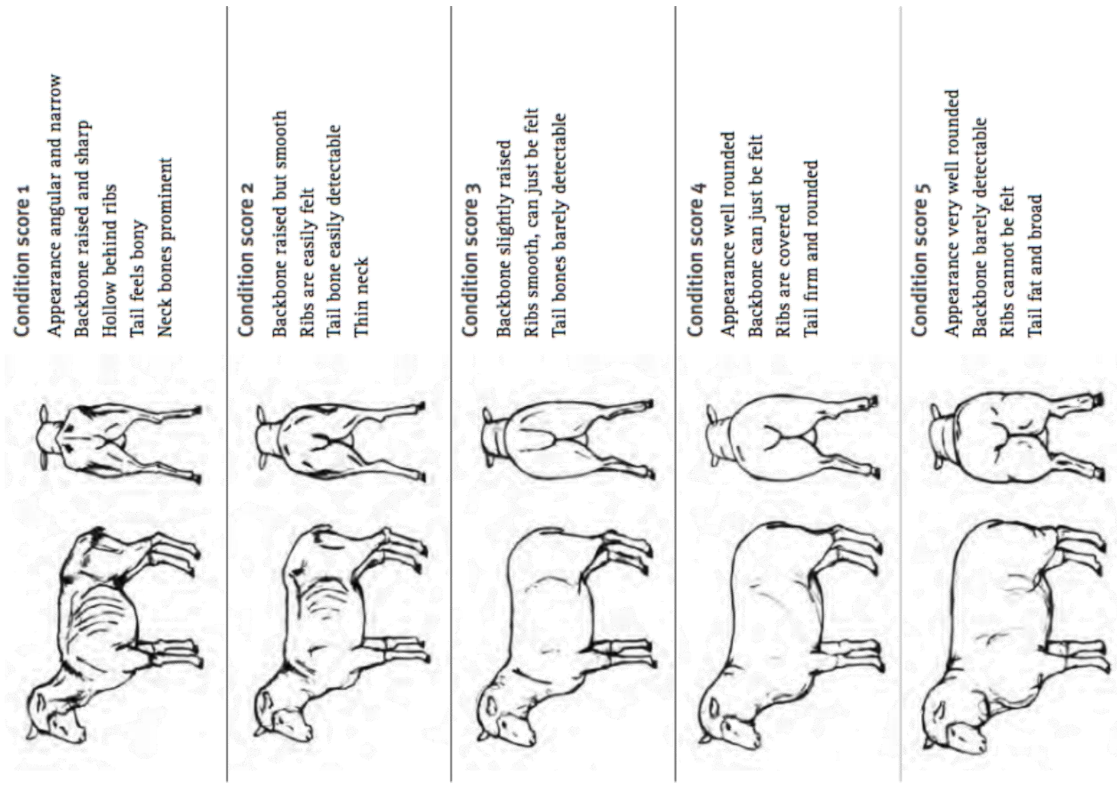
4. Dark back ground were used for best visualization of wells during deliver specimen and reference serum

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.

Annex 5. Dam and newborn body condition score form



Annex 6. Selected pictures taken during field work

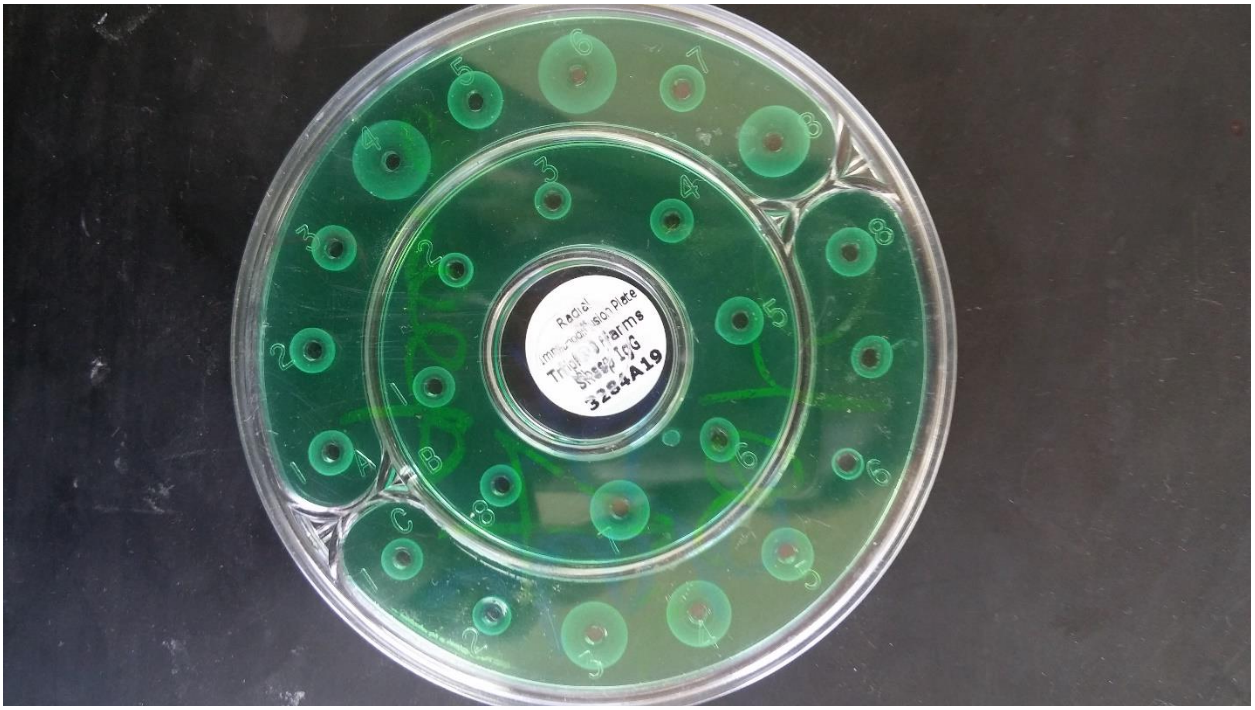
Photo 1: Lamb and goat kid newborns

Photo 2: Blood sample collection

Annex 7. Selected pictures taken during laboratory activities

Photo 3: Sheep and goat RID kit and collected blood serum samples

Photo 4: A ready-to-use RID kit to fill plates



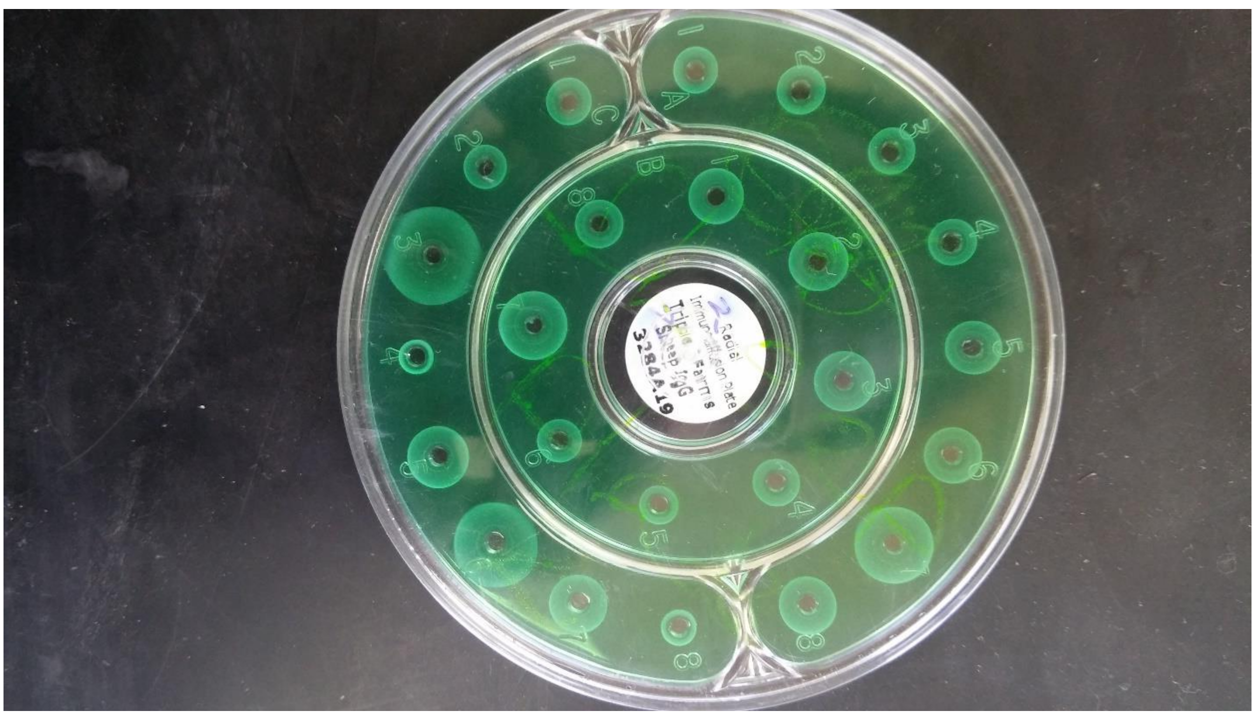
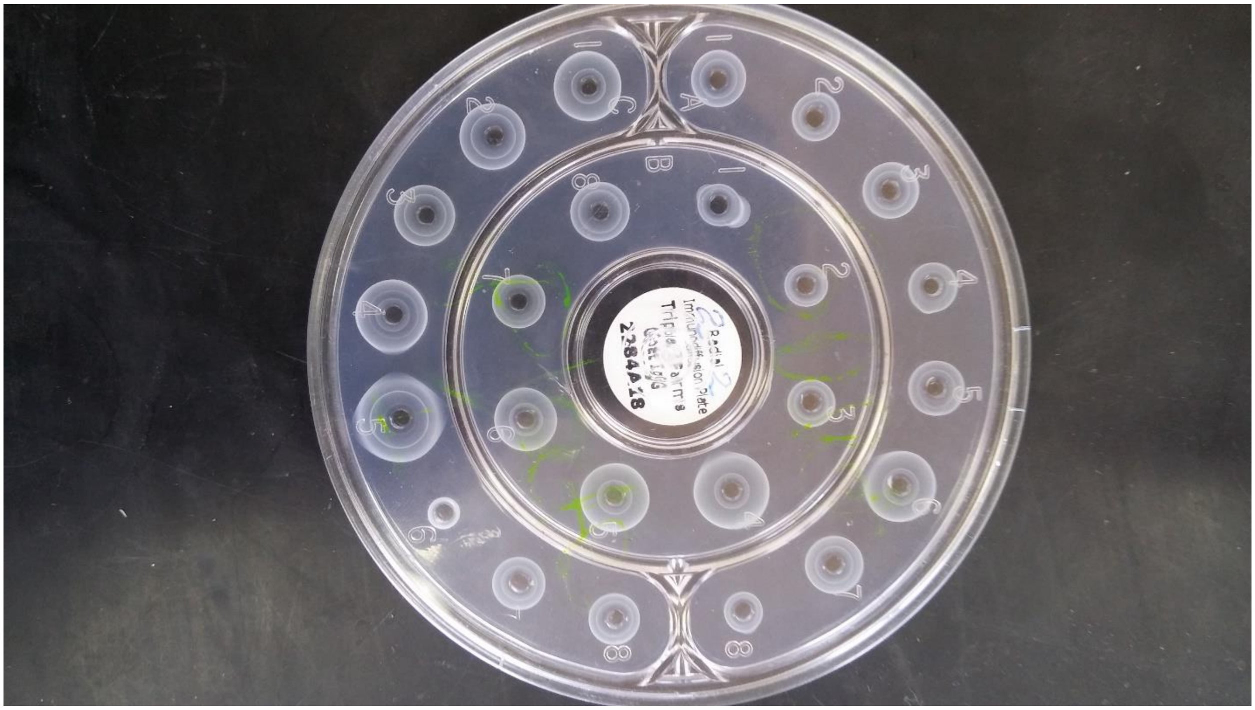


Photo 5: Lamb serum result after fill of wells



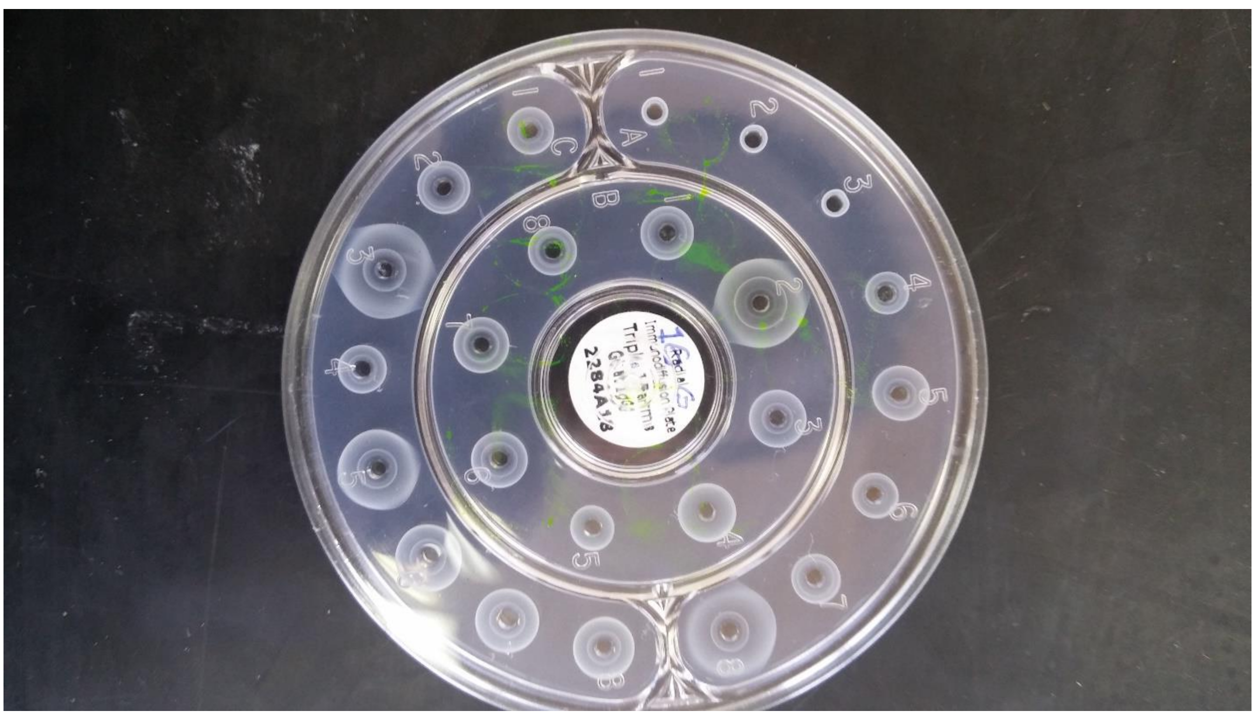


Photo 6: RID result picture of goat kid serum

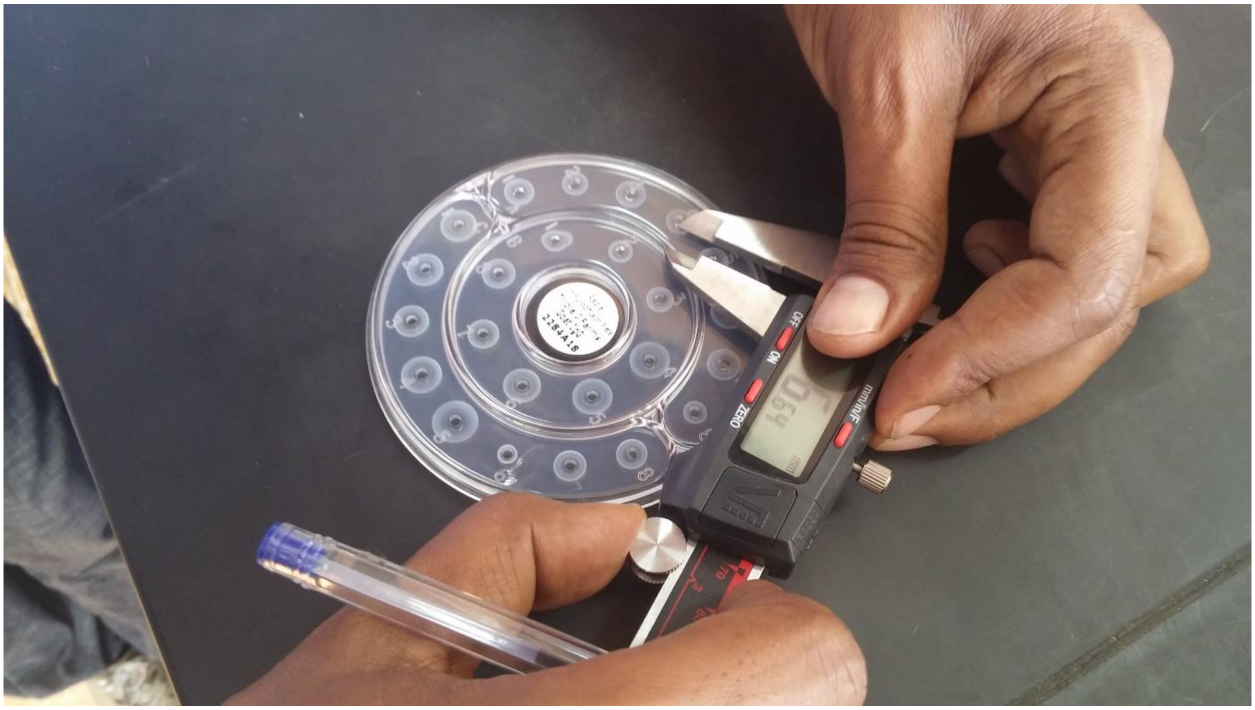


Photo 7: Ring precipitation diameter read by digital caliper (mm)