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MATERNAL IMMUNOGLOBULIN IN THE SERUM OF NEWBORN LAMBS AND ITS RELATION WITH NEONATAL MORTALITY

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Supporting Information

ABSTRACT: The study was conducted on 153 neonatal lambs of one of the highland breeds of sheep, locally called "Menz sheep" in North-Eastern part of Ethiopia, with the aim of assessing the relationship of total serum immunoglobulin level and neonatal lamb mortality in the first one month of life. The overall mortality in neonates was 8.5%. Surviving lambs (2.43±0.35 kg) were significantly heavier than those that died during the neonatal period (2.21±0.55 kg). Males (2.45±0.31 kg) were significantly heavier than females (2.37±0.43 kg). The lambs that survived the neonatal period had a significantly higher level of immunoglobulin (31.71±12.88 Zinc Sulphate Turbidity units) than those that died (12.77±5.25 Zinc Sulphate Turbidity units). Neonatal lambs with total serum immunoglobulin levels below 12 Zinc Sulphate Turbidity units may be considered as an indication of failure of passive transfer of colostrum immunoglobulins and consequently increased the susceptibility of lambs to diseases and subsequent deaths. The neonatal lambs with lower average birth weight (2.21 kg) and lower average total serum immunoglobulin level (12.77 Zinc Sulphate Turbidity units) had found dead before the first 30 days of their age. Most deaths of lambs occur in the first few days of birth that are typically associated with lower birth weight which also led to weakness, taking longer time to stand up and reduced chance of survival than lambs of heavier weight. Hence, several works have to be done to further improving the birth weight of newborn lambs as well as the nutritional status of the dam so that lambs may receive sufficient and good quality amount of colostrum from the first few hours of birth.

Keywords: Colostrum, Immunoglobulins, Neonatal lamb, Mortality.

INTRODUCTION

Neonatal lambs are extremely vulnerable to infectious diseases as they are born immunologically nave. A major factor affecting neonatal sensibility to pathogens is the permeable immature gut. This permeability of the gut allows the initial immunoglobulin passage, but increases also the risk for pathogens to enter (Fischer et al., 2019). However, colostrum ingestion itself accelerates the process of intestinal closure; thereby it is also preventing the route of neonatal infection (Dwyer, 2008).

The maternal immunoglobulins acquired through the colostrum play a pivotal role in the defense mechanism of lambs against neonatal diseases until their own immune system is primed and produces a protective amount of antibodies. Immunoglobulins are not detected in the serum of lambs before the first intake of colostrum (Klobasa and Werhahn, 1989). The absorption of immunoglobulins from the intestine is maximum during first six hours of life and no absorption occurs 24-36 hours postpartum. Hence, the peak lg levels are obtained in the serum of neonatal ruminants around these times in the immediate postpartum period (Tizard, 1992). Ideally, the maternal immunity should be transferred in utero to their fetuses so that they are brought into the world protected against the microorganisms (Fisher, 1980; Godden et al., 2019). However, placental barriers in ruminants do not allow the passage of immunoglobulins from dams to neonates, and therefore the lamb has to be dependent entirely on antibodies received via colostrum (Tizard, 1992). Colostrum is not only rich in immunoglobulins as compared to milk but is also an excellent source of energy, vitamin A and essential minerals (Khan and Khan, 1996).

Globulin proteins are serum proteins that are classified into three groups in ruminants; α -, β -, and γ - globulins (Tizard, 1987). The γ -globulin fraction contains mainly immunoglobulins, which are proteins with antibody activity (Tizard, 1987). According to Tizard (1987), sheep have four different types of immunoglobulins; IgG, IgA, IgM and IgE and IgG is the immunoglobulin found in highest concentration in serum. 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 (Nowak and Poindron, 2006). Immunoglobulin-synthesis is initiated at approximately 3 weeks of age in neonatal lambs (Klobasa et al., 1985).

Intestinal closure happens approximately 24 hours after birth, meaning that the passive absorption of immunoglobulins in the intestine seize. 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 (Nowak and Poindron, 2006). Shubber et al, (1979), concluded that larger volumes of colostrum correlate with larger amounts of immunoglobulins. 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). The intestine is unselectively permeable, therefore, all immunoglobulins types can be absorbed (Sawyer et al., 1977). The permeability remains highest immediately after birth to 6 hours of life. Then immature fetal type of cells capable of transfer of intact immunoglobulins is gradually replaced by a digestive type of cells (Tizard, 1992). Smeaton and Simpson-Morgan (1985) also observed that the layer of cells responsible for absorption of colostral antibodies progressively disappears from the villi, resulting in closure which usually completes 24-36 hours after birth (Khan and Khan, 1991 b). So, lambs absorb intact immunoglobulins from ingested colostrum only during the first day of life (Klobasa et al., 1986). Immunoglobulins reach peak level on day I of lamb life, then decline during the next 3 weeks (Smith et al., 1976).

According to Bekele et al. (1992), failure and partial failure of Ig transfer from dam to lambs are observed in 1.8 and 15.3 per cent lambs, respectively. Passive transfer failure was observed in 14 per cent of apparently healthy lambs and in 46 per cent of lambs dying of natural causes between 24 hours and 5 weeks of age (Sawyer et al., 1977). The results of Logan and Irwin (1977) showed that about 20.2 % of lambs born were hypogammaglobulinaemic and were more susceptible to neonatal diseases. Findlay (1973) also observed that all lambs with immunoglobulins less than 20 ZST units die during first week of life, mortality in lambs with 20-40 ZST units was very low and no mortality in lambs with 50 ZST units.

Failure of passive transfer of immunoglobulins to neonatal lambs has a significant effect on neonatal mortality, and losses due to infectious causes are positively correlated with low concentrations of serum immunoglobulins (Sallam, 2019; Ibrahim et al., 2020). According to Hodgson et al. (1992), morbidity and mortality rates are higher in colostrumdeprived lambs (80 and 67%) than colostrum fed lambs (20 and 13%) and 20% of colostrum-deprived lambs die within the first week of life. The concentration of these maternal immunoglobulins in the circulation at 24 hours after birth can be used as an indication of sufficient immunity for the survival of neonatal lambs or susceptibility of lambs to neonatal diseases (Reid, 1972). There are several simple tests that can be used to verify whether or not neonates have received adequate colostrum. The most popular test is the zinc sulphate turbidity test (Roy, 1990). This test has been used by different authors in different species of animals and has been found to be in good agreement with immunoglobulins values determined by other laboratory techniques and it is also simple to use in the laboratory (Ahmad et al., 2000). Hence, the objective of this study is to measure the influence of failure of maternal immunoglobulin transfer on mortality of lambs in the first 30 days of life.

MATERIALS AND METHODS

Study area

The study was conducted in Debre Birhan Agricultural research Center (DBARC). DBARC is found in North Shewa Administrative Zone of the Amhara National Regional State, North eastern part of Ethiopia. It is located in the central part of the Nation, at a road distance of about 120 kilometers from Addis Ababa, the capital city of the country. Geographically, the area lies between 09 0 35'45" to 09 0 36'45" north latitude and 39 0 29'40" to 39 0 31'30" east longitude with an average elevation of about 2828 meters above sea level. It has an average annual rain fall of about 897.8mm and mean annual temperature of about 19.9 °C.

Study population

The lambs which were born from indigenous breeds of sheep (locally named as Menz sheep) were included in the study. The study animals were sourced from the dams which were kept in semi-intensive management system in DBARC. These animals were provided harvested hay and commercial concentrate feed in addition to the morning and afternoon pasture grazing. Both broad and narrow-spectrum anthelmintic drugs were administered against internal parasites based on the laboratory findings and the sheep were also vaccinated against major infectious diseases which include pasteurellosis, sheep and goat pox and peste des petits ruminants (PPR).

Study design and sampling method

An observational longitudinal study design study was conducted from August 2019 to October 2019 to evaluate the impact of level of maternal immunoglobulin transfer on mortality of neonatal lambs in the first 28 days of life. For this study, all the 153 lambs that were born during the activity season were included. Blood samples without anticoagulant were collected from the jugular vein of these new born lambs at the age of 24-48 hours post partem. Serum was separated and stored at -20 °C for further processing and birth weight of lambs was recorded. The health of all lambs under study was monitored daily during the neonatal period. All the lambs that were included in the study were followed up starting from the date of sampling up to the first 28 days of age. By this, the lambs that died were recorded and their level of serum immunoglobulin was measured using zinc sulphate turbidity (ZST) test based on the principle of McEwan et al. (1970).

Zinc sulphate turbidity test (ZST)

The principle of the test is that Zinc sulphate at a specific concentration precipitated the gamma globulin. This creates turbidity which is proportional to the quantity of gamma globulin in the sample and can be quantified in a calorimeter at 525 nm/Spectrophotometer 460 nm.

Test procedure

About 250 mg ZnSO₄·7H₂O was diluted in 1L freshly boiled water (to remove CO₂) and 6-mL of the zinc sulphate solution was placed into sealed 7–10 mL plain blood collection tubes. Then, 0.1 mL serum was added to it and each tube was shaken by repeated inversion of the tube. After that, the mixture was kept for 1 hour at room temperature for the turbidity to develop. Finally, the turbidity developed in each tube was read in a spectrophotometer at a wavelength of 460 nm and the absorbance (optical density) of the turbid solution was determined and compared with control and percent turbidity calculated. Before taking the reading, null adjustment was made against the zinc sulphate solution and all the tubes were shaken further to make a uniform turbid solution.

Data analysis

All the data that were collected based on the above procedures were analyzed using multivariate analysis method of the General Linear Model in SPSS version 20.

RESULTS

The study considered 153 neonatal lambs and assessed for the level of serum immunoglobulins with in the first 48 hours of age after birth. 13 out of 153 lambs were died before 30 days of age with overall neonatal mortality of 8.5%. The mortality rates in male and female neonatal lambs were found 10.3 (8/78) and 6.7% (5/75), respectively.

Serum immunoglobulins levels

The mean serum immunoglobulins level recorded was 30.10 ± 13.49 ZST units. The average serum immunoglobulins level of surviving lambs was found 31.71 ± 12.88 ZST units while, the average serum immunoglobulins level of lambs that had died was found 12.77 ± 5.25 ZST units) (Table 1).

Birth weight

The mean birth weight was 2.41 ± 0.37 kg with a range of 1.2 to 3.6 kg. The males (2.45 ± 0.31 kg) were found heavier than the females (2.37 ± 0.43 kg). The surviving lambs (2.43 ± 0.35 kg) were also heavier than those that died during the neonatal period (2.21 ± 0.55 kg) (Table 2).

Parameter	Sex	Survival Status	Mean± Standard deviation	Ν
Immunoglobulins (ZST units)		Died	11.00±6.63ª	5
	F	Survived	31.17±13.85 ^b	70
		Total	29.83±14.38ª	75
	М	Died	13.88±4.29ª	8
		Survived	32.24±11.91 ^b	70
		Total	30.36±12.66ª	78
		Died	12.77±5.25ª	13
	Total	Survived	31.71±12.88 ^b	140
		Total	30.10±13.49	153

Table 2 - Mean ± Standard Deviation of birth mass of lambs in relation to survival and mortality during the neonatal period.

Parameter	Sex	Survival Status	Mean± Standard deviation	N
Birth Weight (kg)		Died	2.03±0.53ª	5
	F	Survived	2.40±0.41 ^b	70
		Total	2.37±0.43	75
	м	Died	2.32±0.57 ª	8
		Survived	2.46±0.27 ^b	70
		Total	2.45±0.31	78
	Total	Died	2.21±0.55 ^a	13
		Survived	2.43±0.35 ^b	140
		Total	2.41±0.37	153

Different letters (a,b,c) in a column show significant differences (p<0.05); N: number of animals in each category; ZST: zinc sulphate turbidity test

DISCUSSION

From 153 lambs studied, 13 of them died before 30 days of age with overall neonatal mortality of about 8.5%. According to this study, the mortality rates in male and female neonatal lambs were found 10.3 (8/78) and 6.7% (5/70), respectively. However, there was no significant difference (P>0.05) between immunoglobulins levels of both sexes. Similarly, Cinpercescu (1977) and Esser et al. (1989) also reported no difference in male or female immunoglobulins levels.

Serum immunoglobulins levels

The present study finding, indicates the average serum immunoglobulins level of surviving lambs $(31.71\pm12.88 \text{ ZST}$ units) were significantly (P<0.05) higher than those that died during the neonatal period $(12.77\pm5.25 \text{ ZST}$ units. The mean serum immunoglobulins level recorded in thisstudy was almost similar to the findings of Reid (1972) and AL salami and Sinclair (1977), who reported serum immunoglobulin levels of about 27.40 ± 1.70 and 30.90 ZST units, respectively. In the present study, the majority of lambs had ZST values between 20 and 40 units which are in accordance with findings recorded by Reid (1972) and Logan and Irwin (1977) in lambs. Based on this study, thirteen lambs out of 153 (8.5%) were found markedly deficient in serum immunoglobulin level (<13 ZST units), and which died later.

The importance of colostrum in reducing the incidence of neonatal lamb mortality is obvious by the fact that the nine out of thirteen lambs that died in the present study had an immunoglobulin level below 10 ZST units. Such lambs would be at high risk of susceptibility to diseases, and subsequent death as the observations reported by Reid (1972), Findlay (1973) and Logan and Irwin (1977). But according to Villar and Vulich (1980), ZST units in the range of 0-20 are indications of high risk of subsequent death. According to the present study, most of the mortalities were recorded during the first week of life. The finding is in agreement with Jordan and Le-Feuvre (1989), Otesile and Oduyo (1991) and Fentie et al. (2020) who reported maximum morbidity/mortality of lambs during the first week of life.

The lambs that survived the neonatal period had a significantly (P<0.05) higher level of immunoglobulins than those that died (Table 1). The result is similar with the findings recorded by Sawyer et al. (1977), Villar and Vulich (1980), Otesile (1994), and Kenyon et al. (2019). However, Bekele et al. (1992) reported no significant differences between mortality during the neonatal period and immunoglobulins concentration. The newborn leaves the sterile uterus to an environment containing many pathogens. The neonates are often overcome by infectious diseases, even by agents that are relatively nonpathogenic to adult animals (Banks, 1982). In the absence of specific immunity at birth due to of placental barriers (Tizard, 1992), ruminant neonates have to rely on antibodies received via colostrum (Khan and Khan, 1991). These antibodies play a significant role in the defense mechanism of newborn lambs until their own immune systems are primed and produce a protective level of antibodies (Tizard, 1992).

In the present study, six lambs out of thirteen (46.15%) showed sign of diarrhea, before death; while four (30.77%) died after signs of a respiratory disorder and the rest three died with no specific clinical signs. According Fisher (1980), IgM was the class of immunoglobulin found to be deficient in neonates that died of septicemic and bacteremic causes, whereas IgG was found to be deficient in neonates that died of diarrhea. IgA seems to be re-excreted and somehow halts the diarrheic process. According to Smith et al. (1976), a small amount of colostrum IgG, after being absorbed, is secreted in the nasal and lachrymal secretions of lambs and this plays a valuable role in preventing respiratory infections before local production of IgA and IgM at the age of 2-3 weeks.

Birth weight

The survival of neonatal lambs was also observed with respect to their birth weight. The mean birth weight was found 2.41 ± 0.37 kg and the males (2.45 ± 0.31 kg) were found heavier than females flock members (2.37 ± 0.43 kg). The surviving lambs (2.43 ± 0.35 kg) were also heavier than those that died during the neonatal period (2.21 ± 0.55 kg). Being physically weak, the lambs with low birth mass were unable to suckle sufficient amount of colostrum, and as a result, the immunoglobulins level in their serum was low. The physical weakness and low immunoglobulins led to increased mortality in lambs with a low birth mass. The finding of the study is similar with the works which were reported by Purser and Young (1983), Ducrot et al. (1989), Tadich et al. (1990) and Otesile and Oduye (1991). All lambs with higher birth weight survived the neonatal period. However, according to Poonia et al. (1983), as birth mass increases above 3.0 kg, the mortality also increases. Contrary to this finding, Dalton et al. (1980) reported that a lamb with a birth mass of from 3.5 to 5.5 kg had the lowest mortality. According to Hindson and Winter (2002), neonatal lambs with low birth weight often have poor suckling drive or they are unable to compete with stronger lambs for available milk. Hence, they are disadvantaged both from total milk intake and reduced immunoglobulin intake.

CONCLUSION

Based on the finding of the present study, it can be concluded that the total serum immunoglobulin levels in neonatal lambs within the first one to two days of age, had a good indication for the extent of the absorption of colostral antibodies from the dam. The neonatal lambs with lower average birth weight (2.21 kg) and lower average total serum immunoglobulin level (12.77 ZST units) had found dead before the first 30 days of their age. Hence, several works have to be done to further improve the birth weight of newborn lambs as well as the nutritional status of the dam so that lambs can suckle starting from the first few hours of birth and receive sufficient amount good quality colostrum.

DECLARATIONS

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Authors' contributions

Chekol D contributed to the research design, analysis, interpretation of the data and writing the manuscript. Derib A, Yeshitila W, Firdawok A, Enview A and Tadiwos A contributed to the blood sample collection and laboratory work.

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Conflict of interest

None of the authors have conflict of interest.

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