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Maternal immunoglobulin in the serum of newborn lambs and its relation with neonatal survival

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ABSTRACT

The study was conducted on 153 neonatal lambs of one of the highland breeds of sheep, locally called "Menz sheep" at Debre Birhan Agricultural Research Center, Ethiopia, with the aim of assessing the relationship between total serum immunoglobulin level and neonatal lamb mortality in the first one month of life using Zinc Sulphate Turbidity. For this study, an observational study was conducted from August 2019 to October 2019. Blood samples without anticoagulant were collected from the jugular vein of these newborn lambs at the age of 24–48 hours post partem. All the data were analyzed using univariate analysis method of the General Linear Model in SPSS version 20. The overall mortality of neonatal lambs was 8.5%. Surviving lambs (2.43±0.35 kg) were significantly ($P<0.05$) heavier than those that died during the neonatal period (2.21±0.55 kg). Males (2.45±0.31 kg) were significantly ($P<0.05$) heavier than females (2.37±0.43 kg). The lambs that survived the neonatal period had a significantly ($P<0.05$) higher level of immunoglobulins (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. High proportion of neonatal lambs with history of lower average birth weight (2.21 kg) and lower average total serum immunoglobulin level (12.77 Zinc Sulphate Turbidity units) were 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 immune and 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.

1. INTRODUCTION

Sheep contributes significantly to the economy of Ethiopia. However, it is imperative to increase their productive performance to meet the ever-increasing demand for animal proteins. This objective can be achieved by increasing the number of lambs successfully reared per ewe in a given season (Khan et al 2006). However, lamb mortality, especially during the neonatal period, is a major dilemma that makes this goal difficult (Khan et al 2002). Mortality in the neonate lambs appears in both intensive and extensive systems and remains approximately 15-25% worldwide (Anna 2015). According to Anna (2015), the occurrence of neonatal deaths is even highest, 21%, during the first three days of life and 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 period in the survival of neonatal lambs (Boucher 2014).

In lambs, neonatal period is the period from birth to the first 28 days of life and it is the most critical (Gokcea et al 2014). Profound metabolic and morphological mechanisms, such as thermoregulatory system, cardiovascular system, respiratory system and metabolic homeostasis, complete maturation during the neonatal period (Piccione et al 2007; Piccione et al 2008; Piccione et al 2009; Piccione et al 2011). This period of time is also called an adaptive period which 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 2007; Piccione et al 2008; Piccione et al 2009; Piccione et al 2011). This extreme vulnerability to infectious diseases occurs because of the fact that placental barriers in ruminants do not allow the passage of immunoglobulins from dams to fetus and hence, lambs are born immunologically naïve (Dwyer 2008). Therefore, the lamb has to be dependent entirely on antibodies received via colostrum (Tizard 1992) since colostrum is the sole source of acquired immunity in ruminants (Stelwagen et al 2009).

Absorption of immunoglobulins from colostrum is a time bounded mechanism and it is maximum during first 6 hours of life and no absorption occurs 24-36 hours postpartum (Khan and Ahmad 1997). 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). Failure to acquire passive immune transfer (FPIT) for the neonatal lambs 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). Failure of passive transfer (FPT) predisposes the neonate to disease (Brujeni et al 2010; Vandeputte et al 2011) 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 diarrhoea, enteritis, septicaemia, arthritis, pneumonia (Thompson et al 2013) and high risk of mortality (Ahmad et al 2000; Wereme et al 2001; Zarrilli et al 2003a; 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).

The variation in neonatal serum immunoglobulin concentration is often associated with morbidity and mortality and inversely related to disease prevalence (Yilmaz et al 2011). The concentration of immunoglobulins in the serum of the newborn is lowest 1h post-partum; however, after successful transfer of passive immunity in young animals, it increases significantly 24 h post-partum (Hashemi et al 2008). Hence, 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

Several direct and indirect methods have been developed for determination of serum immunoglobulin status of neonates. Direct measurement of serum concentrations of immunoglobulins is usually accomplished using radial immunodiffusion while indirect measurements are accomplished using methods that include zinc sulfate turbidity, sodium sulphite precipitation, glutaraldehyde coagulation and serum refractometry (Perino et al 1993; Hogan et al 2015). The direct method is the most accurate means to determine concentrations of serum immunoglobulin (Perino et al 1993). However, the value of this test is limited by the high cost involved and the technical expertise required (Perino et al 1993). Due to these reasons, indirect measurements which are simple to execute in the laboratory like zinc sulphate turbidity test are usually used to estimate concentration of serum immunoglobulins in the neonatal ruminants (Khan and Ahmad 1997).

As one of the institutions with responsibility of supporting the agricultural sector in general and the livestock sub-sector in particular, Debre Birhan Agricultural Research Center has been conducting encouraging works on sheep breed improvement for mutton purpose. However, the data collected from the sheep health record book of the center from the years 2015-2019 indicated that the issues of neonatal morbidity and mortality are among the biggest problems encountered in the research center. According to these data, neonatal lambs in the center are usually found diseased with diarrhoea, enteritis, septicaemia, arthritis and pneumonia and mortality of about 8-12% has been recorded every year in the past 5 years. With these signs and diseases, the attending veterinarians in the center suspected that lack of sufficient absorption of colostral immunoglobulins in the first 24 hours of birth could be the cause for this neonatal mortality (Ahmad et al 2000). Therefore, the objective of this study was to evaluate the presence of relationship between the level of maternal immunoglobulin transfer in the serum of newborn lambs and mortality of these lambs in the first 30 days of life.

2. MATERIALS AND METHODS

2.1. 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 2, 828 meters above sea level. It has an average annual rain fall of about 897.8 mm and mean annual temperature of about 19.9°C (DBARC 2014).

2.2. 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 like albendazole, tetraclozash, tetramisole, ivermectin and triclabendazole were administered against internal and external parasites. The drugs were applied in four rounds per year in September, December, March and June following the manufacturers’ recommendationsand based on the laboratory findings. Moreover, vaccines against major infectious diseases which include *ovine pasteurellosis* (two times per year), *sheep and goat pox* (once a year) and *peste des petits ruminants* (once a year) were provided for sheep in the study area.

2.3. Study Type, Sample Size Determination and Sampling Method

An observational study was conducted from August 2019 to October 2019 to evaluate the impact of maternal immunoglobulin transfer on survival 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.

2.4. Experimental Design

Longitudinal study design was used for this study. Hence, 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).

2.5. Principle of the test

Zinc sulphate at a specific concentration precipitated the gammaglobulin. This creates turbidity which is proportional to the quantity of immunoglobulin in the sample and can be quantified in a calorimeter at 525 nm/ Spectrophotometer at 460nm.

2.6. Examination procedure

About 250 mg $ZnSO_4 \cdot 7H_2O$ was diluted in 1 L freshly boiled water (to remove CO_2) and 6-mL of the zinc sulphate solution was placed into sealed 7–10 mL vacutainer 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. The test was conducted in the

microbiology laboratory of Debre Birhan Agricultural Research Center.

2.7. Methods of Data Analyses

All the data that were collected based on the above procedures were analyzed using univariate analysis method of the General Linear Model in SPSS version 20. Statistically significant difference is established when P value < 5%.

3. RESULTS AND DISCUSSION

The study considered 153 neonatal lambs and assessed for the level of serum immunoglobulins with in the first 48 hours of age after birth. From these 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% (5/75), respectively. The average serum immunoglobulins level of surviving lambs was found 31.71±12.88 zinc sulphate turbidity (ZST) units while, the average serum immunoglobulins level of lambs that had died was found 12.77±5.25 ZST units)

(Table 1). 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 their 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) (Table 2).

Table 1: Mean ± Standard Deviation of serum immunoglobulin level of lambs in relation to survival and mortality during the neonatal period in Debre Birhan ARC.

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

Different letters (a and b) bearing different superscripts in a column show significant differences (p<0.05); N = number of animals in each category; ZST = Zinc Sulphate Turbidity

Table 2. Mean ± Standard Deviation of birth weight of lambs in relation to survival and Mortality during the neonatal period in Debre Birhan ARC.

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

Different letters (a and b) bearing different superscripts in a column show significant differences (p<0.05); N = number of animals in each category

Based on the result, the average serum immunoglobulins level of surviving lambs (31.71±12.88 ZST units) were significantly (P<0.05) higher than those that died during the neonatal period (12.77±5.25 ZST units. This result is similar with the findings recorded by Boucher (2014) who reported a highly significant relationship (P < 0.01) between serum immunoglobulin concentrations and lamb survival. According to Boucher (2014), the average serum immunoglobulin concentration of the lambs that survived was 10.99 ± 0.69 mg/mL and the average serum concentration of the lambs that died was 2.77 ± 2.08 mg/ml. Despite the differences in the units of measurements between Boucher (2014) and this study (ZST units), both findings indicate that the death of neonatal lambs becomes higher when their serum immunoglobulin drops to a very lower level.

This is also consistent with other reports (Khalaf et al 1979; Gilbert et al 1988) of < 10 mg IgG/mL for failure of or inadequate transfer of passive immunity. Gilbert et al (1988) found that mortality was three to four times higher in lambs with serum IgG concentrations of < 10 mg/ml. Khalaf et al (1979) found lambs that survived beyond 10 days had a serum IgG concentration of > 20 mg/mL at 24 h of age, compared to lambs that died which had < 18 mg /mL at 24 h of age.

In addition to this finding, Tizard (1992) indicated that antibodies from ruminant colostrum 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. Tabatabaei et al (2013) also found that lambs that died had lower serum immunoglobulin concentrations than lambs that survived. Ahmad et al (2000) indicated that low absorption of immunoglobulins often results in high risk of mortality and Murphy et al (2014) reported that failure to acquire passive immunity can be directly responsible for half of all neonatal mortalities. Vatankhah (2013) reported mortality rates of 67% in lambs deprived of colostrum compared to 13% in lambs that consumed colostrum. However, based on his work on factors affecting morbidity and mortality of the Ethiopian highland sheep in Debre Birhan area, Bekele et al (1992) reported no significant differences between mortality during the neonatal period and immunoglobulins concentration.

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. This finding is similar with the studies conducted by Britti et al *(*2005), Brujeni et al (2010), Vandeputte et al *(*2011) and Mech et al *(*2011) and which reported that failure of passive immune transfer predisposes the neonates to diseases due to them being hypogammaglobulinaemic. According to Thompson et al *(*2013), low absorption of immunoglobulins often results in an increase in diseases such as diarrhoea, enteritis, septicaemia, arthritis and pneumonia.

When sex variation of lambs is considered, more male lambs have been died than their female members however, the relationship between sex of lambs and survival was not found statistically significant. Similarly, higher mortality in male lambs compared to female lambs has been reported in Vatankhah and Talebi (2009), Ahmed et al (2010) and Abdelqader et al (2017). Contrary to these findings, Turkson and Sualisu (2005) in Ghana reported higher mortality for female lambs and this difference has been attributed to sex linked determinants which according to Mandal et al (2007) are yet to be identified.

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 their 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) and the relationship between birth weight and survival was found statistically significant ($P<0.05$). This result might be due to the fact that vigorous lambs at birth are faster to stand up at their own strength, find the ewe's teat sooner and suckle adequate colostrum which is the determining factor to build up the serum immunoglobulin, an antibody which saves lambs from neonatal diseases.

This finding was similar with the reports of Ahmad et al (2000) and Hashemi et al *(*2008), which indicated that the birthweight of newborns can have a significant effect on their subsequent immunoglobulin concentrations. According to these authors, newborns that have a lower birth weight tend to be weaker and therefore unable to or have more trouble suckling adequate amounts of colostrum to provide sufficient levels of antibodies in their blood for initial immune protection. Nowak and Poindron (2006) also indicated that lamb birth weight can have a major influence on the survival of lambs at post-partum. In this study, all the lambs with birth weights higher than 2.43 kg survived the neonatal period. Chirstley et al (2003) found that there was a linear relationship between the birth weight of lambs and their serum immunoglobulin status. Therefore, mortality rates are generally higher in newborns with low birth weights. Brujeni et al (2010) also found that lambs with birth weights lower than 3 kg had significantly lower immunoglobulin concentrations than lambs that weighed greater than 3 kg at birth.

4. CONCLUSIONS AND RECOMMENDATIONS

From 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, which are obtained by using simple laboratory tests like zinc sulphate turbidity test had a good indication for the extent of the absorption of colostral antibodies from the dam. The neonatal lambs with history lower average birth weight (2.21 kg) and lower average total serum immunoglobulin level (12.77 ZST units) were found dead before the first 30 days of their age. Hence, several works have to be done to further improve the immune and nutritional status of the dam and birth weight of newborn lambs so that lambs can suckle starting from the first few hours of birth and receive sufficient amount good quality colostrum.

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