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The level of immunoglobulins in relation to neonatal lamb mortality in Pak-Karakul sheep

Rehan Ahmad[1], Ahrar Khan[1]*, Muhammad Tariq Javed[1], and Iftikhar Hussain[2]

[1]*Department of Veterinary Pathology, University of Agriculture, Faisalabad, Pakistan*
[2]*Department of Veterinary Microbiology, University of Agriculture, Faisalabad, Pakistan*

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ABSTRACT

The study was conducted on 85 neonatals of Pak-Karakul sheep at the Sheep and Goat Development Centre, Rakh Khairewala, District Layyah, Pakistan, with the aim of assessing the relationship of immunoglobulins to neonatal lamb mortality. Overall mortality in neonates was 8.24%. Surviving lambs (3.77±0.07 kg) were significantly ($P<0.001$) heavier than those that died during the neonatal period (2.79±0.018 kg). Males were significantly ($P<0.05$) heavier than females (3.84±0.11 vs. 3.52±0.09 kg). The lambs that survived the neonatal period had a significantly ($P<0.001$) higher level of immunoglobulins (30.89±0.87 ZST units) than those that died (7.08±1.99 ZST units). The mean total serum protein values for surviving and dead lambs, using a refractometer, were 78.51±1.86 and 47.14±4.84 g/l, respectively. The turbidity values below 10 ZST units and total serum protein level below 50 g/l may be considered as an indication of hypogammaglobulinemia and consequently an increased susceptibility to diseases and subsequent deaths.

Key words: neonatal lamb mortality, Pak-Karakul sheep, immunoglobulins, birth mass, hypogammaglobulinemia

Introduction

Maternal immunoglobulins play a pivotal role in the defence mechanism of lambs against neonatal diseases, until its own immune system is primed and produces a protective level of immunity. Ideally, maternal immunity should be transferred *in utero* to their foetuses so that

* Contact address:
Dr. Ahrar Khan, Department of Veterinary Pathology, University of Agriculture, Faisalabad-38040, Pakistan, Phone: 92 41 30281-89; Fax: 92 41 647846; E-mail: ahrar@fsd.paknet.com.pk

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they are brought into the world protected against microorganisms. However, placental barriers in ruminants do not allow the passage of immunoglobulins from dams to neonates, and therefore the lamb has be to depend entirely on antibodies received via colostrum (TIZARD, 1992).

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. 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%). According to VIHAN (1986), 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; HALLIDAY, 1976). The turbidity and index values that may be considered indicative of immune deficiency, lead to an increased susceptibility of neonatal diseases. In the present study, zinc sulphate turbidity test and refractometer was used to determine hypogammaglobulinemia in neonatal lambs. This paper also describes the relationship of birth mass and sex of lambs and parity of dams with the immune status of the neonatal lambs of Pak-Karakul sheep.

Materials and methods

Blood samples without anticoagulant were collected from the jugular vein of 85 lambs at the age of 24 hours during lambing season, i.e. February to April 1998, at the Sheep and Goat Development Centre, Rakh Khairewala, District Layyah, Pakistan. Serum was separated and stored at -20 °C for further processing. Sex and birth mass of lambs and parity of ewe was recorded. The birth mass of lambs was grouped into three categories: up to three kg, between three to four kg, and more than four kg. The health of all lambs under study was monitored daily during the neonatal period.

Immunoglobulin levels were estimated by the zinc sulphate turbidity (ZST) method, as described by McEWAN et al. (1970). Total serum proteins were measured by the biuret method (ANONYMOUS, 1984) and also by using field refractometer (Serum protein refractometer SPR-NE; ATAGO, Japan). Serum albumin was measured by the bromocresol green binding method (NORTHAM and WIDDOWSON, 1967).

Data on all the above-mentioned parameters in relation to neonatal lamb mortality were analysed by applying ANOVA, using Micro Computer program Minitab (ANONYMOUS, 1989).

Results and discussion

Overall mortality in Pak-Karakul neonates involved in the study was 8.24% (7/85). The mortality rates in male and female neonatal lambs were 9.09 (4/44) and 7.32% (8/41), respectively.

Birth mass

The mean birth mass was 3.68±0.07 kg with a range of two to five kg. The males (3.84±0.11 kg) were significantly ($P<0.05$) heavier than females (3.51±0.09 kg) (Table 1). The surviving lambs (3.77±0.07 kg) were significantly ($P<0.001$) heavier than those that died during the neonatal period (2.79±0.18 kg) (Table 2). Being physically weak, the lambs with low birth mass were unable to suckle a sufficient amount of

Table 1. Mean±SE of birth mass, total serum proteins, albumin, globulin and immunoglobulins of neonatal lambs in relation to sex

Parameters		Male (N=44)	Female (N=41)	Overall (N=85)
Birth mass (kg)		3.84±0.11[a]	3.51±0.09[b]	3.68±0.07
Immunoglobulins (ZST units)		29.07±1.63	28.73±1.44	28.90±1.09
Total serum proteins (g/l)	Refractometer	76.83±2.97	74.21±2.74	75.62±2.03
	Biuret method	76.93±2.81	75.17±2.79	76.05±1.97
Albumin (g/l)		39.25±0.99	38.53±1.04	38.91±0.71
Globulin (g/dl)		37.36±2.58	36.53±2.29	36.95±1.72

Figure bearing different superscripts in a row differ significantly ($P<0.05$).

colostrum, and as a result the immunoglobulins level in their serum was low (Table 2). The physical weakness and low immunoglobulins led to increased mortality in lambs with a low birth mass. Similar findings were reported by PURSER and YOUNG (1983), WOOLLIAMS et al. (1983), DUCROT et al. (1989), TADICH et al. (1990) and OTESILE and ODUYE (1991). Parity of ewe did not affect the birth mass of neonatal lambs (Table 4). All lambs with mass of more than 3.5 kg at birth 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.

It is abundantly clear from the above discussion that lambs having a higher birth mass survive well. In this regard, OSAER et al. (1999) reported that nutritional supplementation to dams ensured a better erythropoietic response and better offspring survival. Therefore, strategies should be opted to enhance the birth mass of lambs for their better survival. According to CLARKE et al. (1997), maternal body mass bears a direct influence on size, conformation and survival of newborn lambs.

Table 2. Mean±SE of birth mass, immunoglobulins, total serum proteins, albumin and globulin of lambs in relation to survival and mortality during the neonatal period

Parameters	Male		Female		Overall	
	Survived	Died	Survived	Died	Survived	Died
Birth mass (kg)	3.95±0.01[a]	2.75±0.32[b]	3.57±0.10[a]	2.83±0.17[c]	3.77±0.07[a]	2.79±0.18[b]
Immunoglobulins (ZST units)	31.56±1.20[a]	4.78±1.43[b]	30.21±1.28[a]	10.14±3.93[b]	30.89±0.87[a]	7.08±1.99[b]
Serum total protein[A] (g/l)	80.32±2.68[a]	44.50±4.35[b]	76.41±2.45[a]	50.76±10.73[b]	78.51±1.85[a]	47.14±4.84[b]
Serum total protein[B] (g/l)	80.6±2.41[a]	44.18±4.48[b]	77.25±2.67[a]	49.67±9.37[b]	78.99±1.80[a]	46.50±4.41[b]
Albumin (g/l)	39.40±1.04	38.05±3.75	38.90±1.03	34.00±1.15	39.14±0.73	36.31±3.09
Globulin (g/l)	40.82±2.18[a]	6.13±1.96[b]	38.30±2.23[a]	15.60±4.39[b]	39.55±1.55[a]	10.20±2.74[b]

[a]=Total serum proteins determined by refractometer method; [b]= Total serum proteins determined by Biuret method; Figures bearing different letters in a row differ significantly ($P<0.001$)

Total lamb birth mass, placental mass and foetal cotyledonary mass were lower with light than with heavy ewes. Lambs born to light ewes had less perirenal adipose tissue and smaller liver, heart, kidneys, brain, adrenals and thyroid, although their heart, brain and pancreas represented a larger proportion of total body mass; pancreas mass was similar to that in lambs born to heavy ewes. Hence, maternal body mass critically influences placental mass and lamb size and survival after birth. Another factor which HODGSON et al. (1997) indicated is that maternal nutrition status influences gut permeability of immunoglobulin in newborn lambs: the healthier the dam is the greater will be the absorption of immunoglobulins in neonates, and the better will be their survival.

Immunoglobulins levels

The mean serum immunoglobulins level was 28.90±1.09 ZST units (Table 1). The mean serum immunoglobulins level recorded in the present study was almost similar to those reported by REID (1972) and AL-SALAMI and SINCLAIR (1977), i.e. 27.40±1.70 and 30.90 ZST units, respectively. In the present study, the majority of lambs (76.19%) had

ZST values between 20 and 40 units. Similar findings have been report by REID (1972) and LOGAN and IRWIN (1977) in lambs, and by PETRIE (1984) and KHAN and KHAN (1996) in calves. Seven lambs out of 85 (8.24%) were markedly hypogammaglobulinemic (<10 ZST units), and which died later. There was no significant difference between immunoglobulins levels of both sexes (Table 1). Similarly, CINPERCESCU (1977) and ESSER et al. (1989) also reported no difference in male or female immunoglobulins levels.

Table 3. Mean±SE immunoglobulins, total serum protein, albumin and globulin in relation to birth mass groups of lambs

Parameters		3 kg (N=32)	3-4 kg (N=42)	>4 kg (N=11)
Immunoglobulins (ZST units)		23.29±2.31[a]	31.55±1.28[b]	30.39±1.91[c]
Total serum proteins (g/l)	Refractometer	69.00±4.04	78.65±2.53	77.64±5.67
	Biuret method	68.72±3.61[a]	79.33±2.66[b]	80.15±4.23[c]
Albumin (g/l)		38.60±1.45	39.15±0.89	38.33±2.23
Globulin (g/dl)		30.01±3.17[a]	40.10±2.27[b]	40.41±3.83[c]

Figure bearing different superscripts in a row differ significantly (P<0.05).

Birth mass has a significant effect on immunoglobulins levels (P<0.05) (Table 3). However, parity of the ewe has no significant effect on immunoglobulin levels (Table 4). Mean immunoglobulins concentrations in lambs were 23.29±2.31 ZST units up to 3 kg birth mass, 31.55±1.28 in three to four kg, and 30.39±1.91 units in the group more than four kg birth mass (Table 3).

The lambs that survived the neonatal period had a significantly (P>0.001) higher level of immunoglobulins than those that died (Table 2). These findings supported the results of SAWYER et al. (1977), VILLAR and VULICH (1980), McGUIRE et al. (1983), VIHAN (1986), HODGSON et al. (1992) and OTESILE (1994). 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 non-pathogenic 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 defence mechanism of newborn lambs until their own immune systems are primed and produce a protective level of antibodies (TIZARD, 1992).

Total serum protein (TSP)

There was no significant difference between the readings of TSP taken by refractometer or with the biuret method. The readings obtained by biuret method (76.50±1.96 g/l) were slightly higher than those of refractometer (75.62±2.03 g/l) (Table 1). These values are comparatively higher as reported by HALLIDAY (1976), 62.0±0.04 g/l and lower than those reported by BEKELE et al. (1992), 82.0±0.62 g/l, but were similar to those reported by KEAY and DOXY (1984), and 76.0±1.17 g/l. However, according to CZARNECKI et al. (1991), TSP increases after colostrum feeding to 8.8 g/dl from 6.6 at birth and stabilizes at 82.0-83.0 g/l by the end of the second week. No significant difference was found between the TSP values of male and female neonatal lambs (Table 1), different birth mass groups (Table 3.) and lambs born to dams with different parity (Table 4). The level of serum total protein was significantly ($P<0.001$) higher in lambs that survived than those died during the neonatal period (Table 2).

Table 4. Mean±SE of birth mass, total serum proteins, albumin, globulin and immunoglobulins of neonatal lambs in relation to parity of ewes

Parity no.	Number of lams	Birth mass (kg)	Total serum protein (g/l)		Albumin (g/l)	Globulin (g/l)	Immuno-globulins (ZST units)
			Refractometer	Biuret method			
1	20	3.85±0.15	76.10±3.51	76.01±3.31	38.43±1.19	37.31±2.84	29.81±2.84
2	19	3.84±0.12	79.20±3.85	81.97±3.88	39.41±1.84	40.63±2.98	31.00±2.98
3	17	3.62±0.17	69.95±4.54	73.23±5.68	37.52±1.75	35.71±4.85	27.03±4.85
4	9	3.51±0.20	75.33±6.40	74.64±6.33	36.90±1.90	37.77±6.37	29.28±6.37
5	7	3.64±0.28	71.50±6.80	75.82±5.90	43.23±2.27	31.83±5.93	24.21±5.93
6	7	3.71±0.26	75.70±9.75	65.60±4.99	38.70±2.65	28.0±3.36	26.73±3.36
7	4	3.57±0.48	95.00±7.80	84.90±7.05	39.68±1.40	45.20±6.65	31.67±6.65

The mean value of serum albumin 38.91±0.71 g/l, measured by bromocresol green method was fairly constant, as has also been reported by MCEWAN et al. (1970) and REID and MARTINEZ (1975). KEAY and DOXY (1984) reported 26.7±0.27 g/dl concentration of albumin at the age of 24 hours.

Male neonatal lambs had slightly higher values of albumin and globulin compared to female lambs (Table 1). Serum albumin values of different birth mass groups were not significant. However, birth mass had a significant ($P<0.05$) effect on globulin concentration (Table 3). Parity of the ewe showed no significant effect on serum concentrations of albumin and globulin in lambs (Table 4).

Health and mortality

In the present study, three lambs out of seven (42.86%) showed sign of diarrhoea, and later on died; while two (28.57%) died because of a respiratory disorder. According FISHER (1980), IgM was the class of immunoglobulin found to be deficient in neonates that died of septicaemic and bacteremic causes, whereas IgG was found to be deficient in neonates that died of diarrhoea. IgA seems to be re-excreted and somehow halts the diarrhoeic process. According to WELLS et al. (1975) and SMITH et al. (1975), 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.

The importance of colostrum in reducing the incidence of neonatal lamb mortality is obvious by the fact that the six out of seven lambs that died in the present study had an immunoglobulin level below 10 ZST units. The seventh lamb that died had a level of 17.85 units. These results were in concordance with findings of VILLAR and VULICH (1980) and BEKELE et al. (1992). All mortalities occurred during first week of life in the present study. SINGH et al. (1987), TREJO et al. (1988) JORDAN and LE-FEUVRE (1989) and OTESILE and ODUYO (1991) also reported maximum morbidity/mortality during the first week of life.

Determination of hypogammaglobulinemia

In the neonates, the level of circulating immunoglobulins reflects the extent of the absorption of colostral antibodies and is widely accepted as an indicator of immune status. Hence, the determination of immunoglobulin has been used to monitor the disease susceptibility of the stock (AL-SALAMI and SINCLAIR, 1977). 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 and has been found to be in good agreement with immunoglobulins values determined by other laboratory techniques (BAUER and BROOKS, 1990; GROUTIDES and MICHELL, 1990; SATAPATHY et al., 1992; HUDGENS et al., 1996).

According to the present study, a turbidity value below 10 ZST units can be considered indicative of an immunoglobulins deficiency. Such lambs would be at high risk of susceptibility to diseases, and subsequent death. Similar observations have also been 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 is an indication of high risk of subsequent death.

The alternative method used to determine hypogammaglobulinemia in the field is the use of a refractometer to measure the refractive index, which depends on the concentration of proteins in the serum. As albumin concentration is fairly constant, the refractive index can also be used to measure concentration of globulin (REID and CLIFFORD, 1974; REID and MARTINEZ, 1975).

From the present study it can be concluded that total serum proteins values obtained by refractometry had a good correlation with ZST units (r=0.819) and globulin concentration r=0.881). Total serum proteins concentration below 50 g/l is an indication of subsequent death, as is ZST units below 10.

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SAŽETAK

Istraživanje je provedeno na 85 novorođenčadi pak-karakul ovaca u Sheep and Goat Development Centru u Rakh Khairewala, u području Layyah u Pakistanu, s ciljem procjene odnosa imunoglobulina i neonatalne smrtnosti janjadi. Ukupna smrtnost novorođenčadi je bila 8,24%. Janjad koja je preživjela (3,77±0,07 kg) imala je značajno (P<0,001) veću masu od one koja je uginula u neonatalnom razdoblju (2,79±0,018 kg). Masa mužjaka bila je značajno (P<0,05) veća od mase ženki (3,84±0,11 prema 3,52±0,09 kg). Janjci koji su preživjeli neonatalno razdoblje imali su značajno (P<0,001) višu razinu imunoglobulina (30,89±0,87 ZST jedinica) od onih koji su uginuli (7,08±1,99 ZST jedinica). Prosječne vrijednosti ukupnih serumskih proteina preživjelih i uginulih janjaca, mjerene refraktometrom, bile su 78,51±1,86 odnosno 47,14±4,84 g/l. Vrijednosti za turbiditet ispod

10 ZST jedinica i za ukupne serumske proteine ispod 50 g/l mogu se smatrati indikacijom hipogamaglobulinemije i posljedične povećane prijemljivosti za bolesti i smrtnost.

Ključne riječi: neonatalna smrtnost janjadi, pak-karakul ovca, imunoglobulini, masa pri porodu, hipogamaglobulinemija