The Journal of Animal & Plant Sciences, 28(1): 2018, Page: 341-347
ISSN: 1018-7081

Short Communication

EFFECTS OF IMMUNIZING PREGNANT EWES AND DOES ON THE HUMORAL IMMUNE RESPONSE OF SECRETED COLOSTRUM

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ABSTRACT

The main objective of the study was to produce hyperimmune colostrum of ewes and does by vaccinating them during their pregnancy and to examine the performance of their lambs/ kids receiving the colostrum from the dams. Animals were divided as control (T-1) without vaccination and 3 treatments (vaccinated flocks) (T2-T4). The vaccines used were; *Clostridia sp*., *Pasteurella sp*., and *Sheep pox*. Study variables were concentration of immunoglobulins (Igs) in both serums' of ewes/ does as well as quantifying the Ig classes of colostrum of both species. Results showed that vaccination of pregnant ewes and does were improved by doubling of serum' Igs in vaccinated dams compared to the control flock. Likewise, a significant increase of colostrum' Igs of ewes and does by 20 and 23%, while serums' Igs of the dams increased by 48 % of both species. The studies focused on challenging pregnant dams with individual vaccines of *Clostridia sp*., *Pasteurella sp.,* and *Sheep pox*. Morbidity and mortality rates during the early age of offspring were almost none in vaccinated flocks compared to those without vaccination.

Key words: Hyperimmune colostrum, immunoglobulin, vaccination, mortality, lambs, kids.

INTRODUCTION

Colostrum contains immunoglobulins, enzymes, hormones, nutrients, in addition to unidentified growth factors. It is the only source of Igs for newborn lambs/kids. Feeding colostrum to lambs/ kids is vital for their immunity and survival. Igs cannot pass through the placental barrier to fetus due to their large size (Ocak' *et al.,* 2005). After suckling, the concentration of Igs of serum of offspring increased rapidly during the first few hours with peak level at about 24 hours after birth (Cortese, 2009; Castro *et al.,* 2011). The concentration of Igs in colostrum will be elevated due to the pinocytosis process, and then Igs will be moved from the blood to the colostrum (Guedes *et al*., 2010). Small ruminants in hot arid zone, especially Kuwait, where animals are raised in intensive system (zero-grazing) due to desertification, the mortality rate of lambs/ kids is high and could be as high as 30-40%. In such management, dams are not challenged by the prevalent diseases, therefore, their colostrum was found to be low in immune bodies Burezq *et al.* (2016). The present study addresses this issue of immunizing dams and producing hyperimmune colostrum for their offspring.

MATERIALS AND METHODS

Animals: A total of 50 animals (24 ewes, 24 does, a ram, and a buck), were housed in a closed steel shed of size 60m X 20m, the space allocated for each animal was $3m^2$ with an access to outside fence of the total $900m^2$ shed. Six ewes/ does were grouped in fenced pens having feeding managers and water troughs. Animals were randomly distributed to four flocks as a control (T1) without vaccination during pregnancy, and treatment flocks (T2-T4) were vaccinated against *Clostridia sp., Pasteuralla sp.,* and *sheep pox*, respectively. Vaccination was carried out twice at the beginning of their pregnancy and 4 weeks before lambing/kidding. Naeemi fat-tailed (Awassi) ewes and the (Shami) does, were of 1-2 years old, and were housed at Kuwait institute for scientific research (KISR) research center.

Feeding and Management: Rams/ bucks were provided with 1.0 kg/head/day mixed feed. Two weeks before joining ewes/ does, and during pregnancy the amount of feed was increased to 1.25 kg/head/day according to NRC, (2007) recommendation, and KISR's feedlot feeding and nutritional studies (Razzaque, 1995). Pregnant ewes/ does were provided with 70% concentrate and 30% roughages. Weaned lambs were provided with 80% concentrate and 20% roughages. Dry ewes/ does were fed 0.8 kg/head/day of the same feed given to the rams/ bucks.

Mating and Pregnancy Diagnosis: A straight breeding programs of mating Naeemi ewes X Naeemi rams, and Shami does X Shami bucks were used. Ewes and does were synchronized for estrus induction (Razzaque, 1995).

Serum and Colostrum Collection: Blood samples were collected (a) two weeks after 1^{st} and 2^{nd} vaccination (b) after lambing. Serum separation according to technique of (Stevanovic' *et al.,* 2015). Colostrum samples were collected from dams after parturition at 0 hrs, 12 hrs, and 24 hrs, centrifuged at 100,000 x g, and then whey stored at −20°C, for ELISA test.

Analytical Procedure

Total Protein Analysis (TP) of Colostrum: using Kjeldahl method (AOAC, 2012) for total protein content.

Ig Analysis of Serum/ colostrum: ELISA kits were used to measure Igs in colostrum/ serum samples, as per manufacturer instructions (Alfa Diagnostic International, USA).

RESULTS

TP of Colostrum of Ewes/ Does: Total TP content of ewes' colostrum of T1 was 5.32 %, while TP content of ewes' colostrum from vaccinated flocks (T2-T4) was increased significantly ($P<0.01$) to 10.75-10.88%. TP content of does' colostrum of control flock was 4.50 %. Vaccination of flocks T2-T4, in both species, resulted in a significant increase of TP ($P<0.01$) to 9.63-9.81% (Fig. 1).

Ig's of Ewes' Serum during Gestation: Table 1 showed a significant increase ($P<0.01$) after the 1st vaccination with *Clostridia sp*, *Pasteurella sp.*, and *Sheep pox*. Total Igs was increased by 68-88 %, as compared to T1 flock. Igs continued to increase significantly ($P<0.01$) after the 2nd immunization by 19-24 % (Gilbert *et al*., 2014; Hashemi *et al*., 2008).

Igs in Serum of Lambs Fed with Colostrum: Lambs were fed with 180-290 ml of colostrum/kg of live weight. The concentration of IgG of lambs' serum after feeding these animals with colostrum collected from T1 flock was (22.0±2.0 mg/ml). A significant increase was noticed reaches 66%, after feeding lambs with hyperimmune colostrum (Table 1).

Ig Classes (IgG, IgM, and IgA) of Ewes' Colostrum: Total IgG of ewes' colostrum of T1 flock was (54.3±4.3 mg/ml). A significant increase was noticed reached 20-23% after vaccination (Sanglid, 2003). No significant increase in IgM and IgA of ewe's colostrum was observed as compared to T1, after immunizing dams during pregnancy (Fig. 2a).

IgG of Does' Serum during Gestation: Total IgG in does' serum of T1 was (19.25±0.22 mg/ml). IgG concentration started to increase significantly ($P<0.01$) after the 1st vaccination with *Clostridia sp.*, *Pasteurella sp.*, and *Goat pox*. Total IgG was increased by 57-67 % compared to T-1. IgG continued to increase significantly ($P<0.01$) after the 2nd vaccination by 29-40 % compared to 1st vaccination (Table 1).

IgG in Kids Serum Fed with Colostrum: Total IgG in kids' serum after feeding colostrum of T1 was (19.12±0.91mg/ml). IgG increased significantly ($P<0.01$) by 73-89% after feeding kids' with hyperimmune colostrum collected from the vaccinated flocks (Table 1).

Ig Classes (IgG, IgM, and IgA) of Does' Colostrum: Total IgG of does' colostrum of T1 was (50.2 ± 2.33 mg/ml). A significant increase of IgG ($P<0.05$) in does' colostrum after vaccination by 48% during pregnancy as compared to T1. Igs in the colostrum started to decrease after 48 h and reached the control level (Fig. 2b) (Sanglid, 2003). No significant increase in the concentration of IgM and IgA of does' colostrum collected from T2-T4, (Fig. 2b).

Live Weight Changes and Body Conditions: After mating, the mean live weights (MLW) of ewes of T1-T4 were similar, with some individual variations (data not shown). However, MLW of ewes steadily increased as expected till the end of pregnancy period. The MLW of does before mating, ranged between 37.6 and 39.3 Kg with large individual variations (data not shown). During late gestation period, pregnant does gained live weight as expected almost in a similar pattern as that of ewes. The mean BCS for ewes ranged from 2.7-2.9 (1-5 scale) during gestation without showing any clear trends of changes. Furthermore, the BCS for does showed a lower scores during pregnancy from 2.3 to 3.0 (Corner-Thomas, 2015).

Mortality Rate of Lambs and Kids: Mortality rate in pre-weaned lambs and kids were 28.0 and 33.3%, respectively. In contrast, there was no mortality between lambs/ kids receiving hyperimmune colostrum (T2, T3, and T4) in the first seven days of age. Vaccination of pregnant ewes/does resulted in improving immunity of lambs/ kids. Therefore, morbidity and mortality of young animals from birth to weaning was reduced to zero (Fig. 5).

DISCUSSION

IgG in Serum of Ewes/Does during Gestation: A significant elevation of Igs in dam's serum was observed during pregnancy and before lambing/kidding (Figs. 3 and 4), reaching about 43.45 to 47.13 % in ewes and 39.5 to 45.2 % in does over T1 flock. IgG started to drop sharply ante-partum until it reached control level at parturition as shown in figures 3 and 4, and then started to rise again during the next four weeks after parturition. These findings agreed with results of Saucedo *et al*. (2011) and Vatankhah (2013). The sharp loss of immunity after lambing/kidding is due to hormonal changes, related to lambing and lactation. These changes are the main source of the supply of protein and energy, to maintain immunity. The energy and protein requirement increases 2-3 fold, respectively, in the 4 weeks period between lambing and milk production (www.wormboss.com.au/news/articles/nonchemical-management/why-are-lambing-ewes-susceptible-to-worm-infection.php).

Igs in Colostrum of Ewes and Does: Vaccination of dams during pregnancy in housed flocks was very effective in boosting IgG levels significantly (*P*<0.5) in their blood and colostrum (Nikbakht *et al*., 2010; Rudovsky *et al*., 2008; Weaver *et al*. 2000). The mechanism of boosting serum IgG and production of hyperimmune colostrum by vaccinating pregnant dams is an interesting topic of study, especially the biochemical process. However, it was found that IgG were synthesized in mammary gland epithelium during the later 5 wks of pregnancy due to hormonal influence, mainly by estrogen (Cortese, 2009; Castro *et al*. 2011).

Immunity of Lambs and Kids: Three different classes of Igs including IgG, IgM, and IgA are usually synthesized during the last few weeks of pregnancy by the plasma cells of the mammary gland (Yilmaz and Kasikci, 2013). 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 regulated by hormonal changes (Yilmaz *et al.* 2011; Tabatabaei *et al*. 2013). Among the three classes, only IgG was found to affect passive immunity functions in small ruminants (Table 1). Fc receptor plays important role for IgG metabolism and help in preventing the degradation of IgG in maternal circulation (Brujeni *et al.* 2010; Lerias *et al*. 2014).

Anti-mortem and Post-mortem Symptoms of Lambs and Kids: Young lambs showed sever diarrhea and dehydration from day 0 to 10 days. Usually most lambs died within the first week of life, similar symptoms were observed in kids. No interventions such as fluid therapy were provided to the animals of control and treatment flocks. The postmortem examination showed hemorrhage in the intestine, and the entire respiratory tract. Diarrheas appeared to be associated with septicemia and in some cases death especially within 24 hrs.

Our findings should be treated with caution because of the small size of lambs/ kids and their different time of birth. In Kuwait, intensive management of calves were observed to face serious losses due to high rate of morbidity and mortality of diary calves, especially during the first seven days of their lives (Razzaque *et al.* 2009a) due to the following causative antigens including; *E. Coli, Pasteurella sp*., and *Salmonella sp.* (Razzaque *et al*., 2009b).

Vaccination of pregnant ewes/ does boosted the production of hyperimmune colostrum for both lambs and kids. Thus, we managed to decrease the mortality rate significantly in newborn lamb/kids by vaccinating the pregnant dams with single pathogen (i.e., *Clostridia sp.,* alone, *Pasteurella sp.,* alone, and *sheep pox* or *goat pox* alone). Gilbert *et al*. (2014) and Hashemi *et al*. (2008) indicated positive effects of immunizing dams during the gestation period, yet the mortality rate of treated flocks was about 8-10% after the first week of life, which was mainly due to the single vaccination. Combined vaccines against multi-pathogens would help in controlling the infection caused by *Clostridia sp.,* and *Pasteurella sp.,* and *sheep pox* or *goat pox* in newborn lambs/kids for longer period of time.

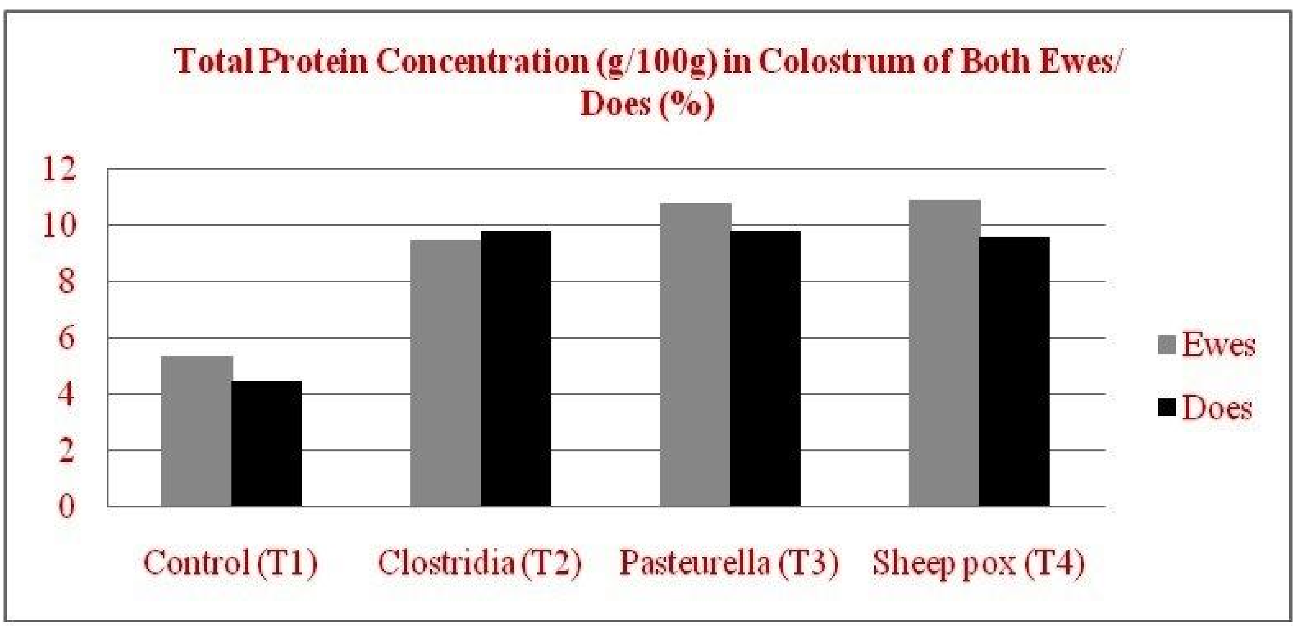

Figure 1. TP Concentration (g/100g) in Colostrum of Both Ewes and Does (%)

Table 1. Concentrations of IgG in Serum of Ewes and Newborn Lambs (Mean ± SD)

Treatment	IgG mg/ml					
	Pregnant Ewes		Newborn Lambs	Pregnant Does		Kids
	20.89 ± 1.22		22.0 ± 2.0	19.25±0.22		19.12 ± 0.91
	1st *	2nd **		1st *	2nd **	
Vaccinated *Clostridia*-T2	35.1 ± 0.23	43.45 ± 0.22	33.0 ± 0.32	36.0 ± 0.39	39.5 ± 0.5	36.0 ± 0.39
Vaccinated *Pasteurella*-T3	37.2 ± 0.13	46.22 ± 0.14	35.0 ± 1.20	33.0 ± 0.56	40.5± 1.3	33.0 ± 0.56
Vaccinated *Sheep pox*-T4	39.3 ± 0.33	47.13 ± 1.13	32.0 ± 0.87	35.0 ± 0.21	45.2 ± 2.3	35.0 ± 0.21

**The first vaccination was at the beginning of the pregnancy period. **The second vaccination was 4-6 weeks before lambing). All animals were vaccinated against Clostridia and Pasteurella two months before starting the experiment as per Public Authority for Agriculture Affairs and Fish Resources (PAAFR) vaccination protocol.*

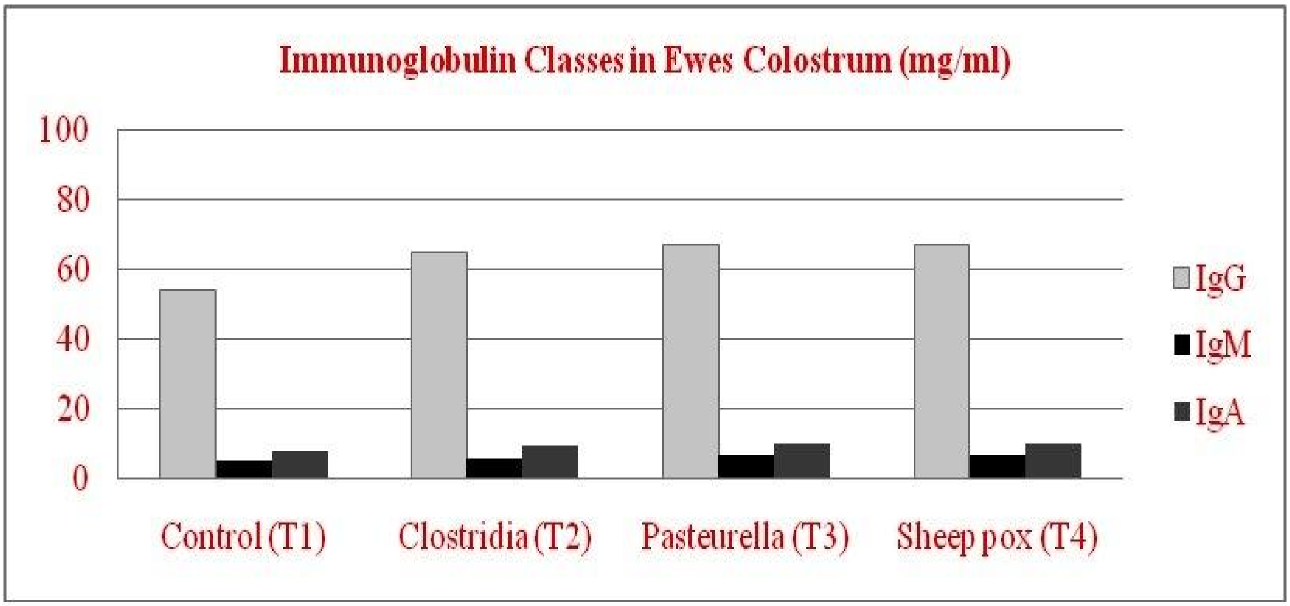

Figure 2a. Concentrations of Total Ig Classes in Ewes' Colostrum

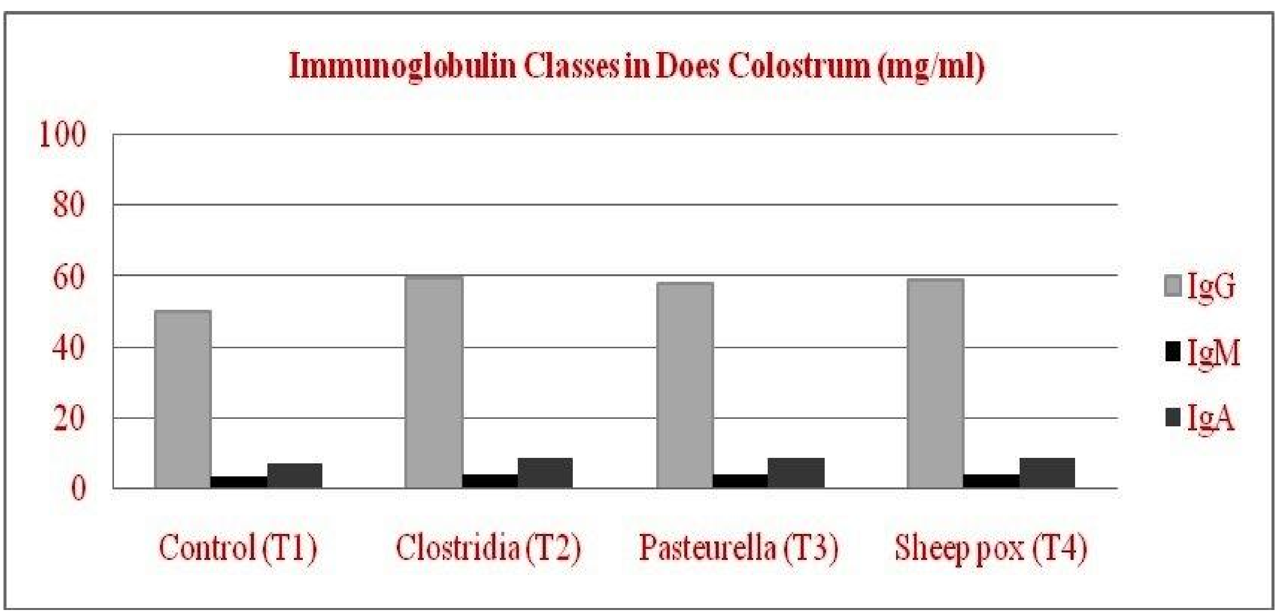

Figure 2b. Concentration of Ig in Does Colostrum

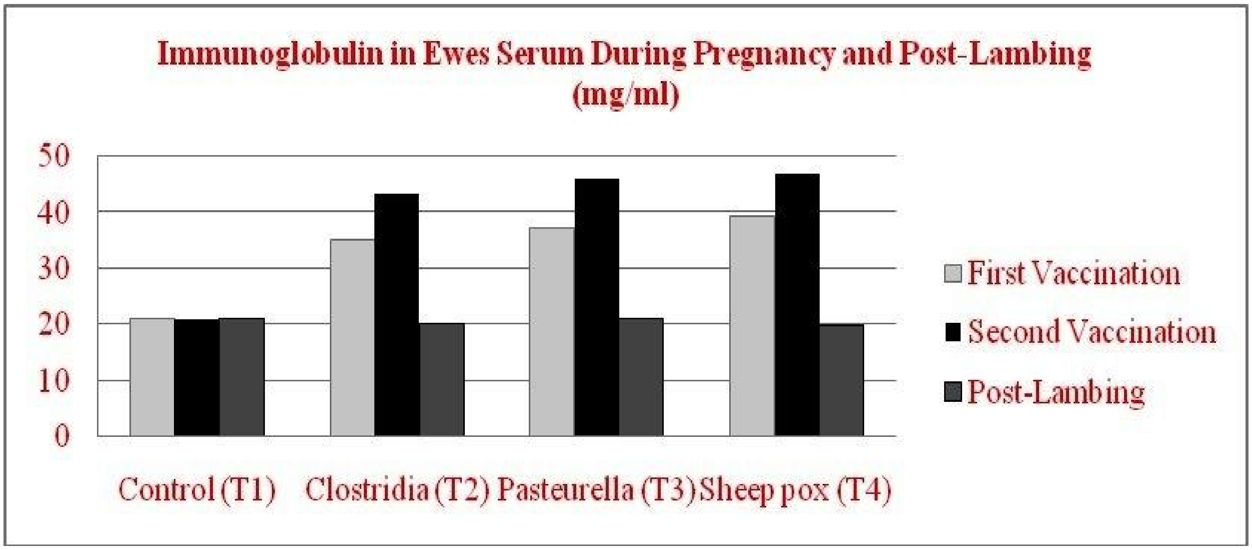

Figure 3. Concentrations of Total Igs in Serum of Ewes

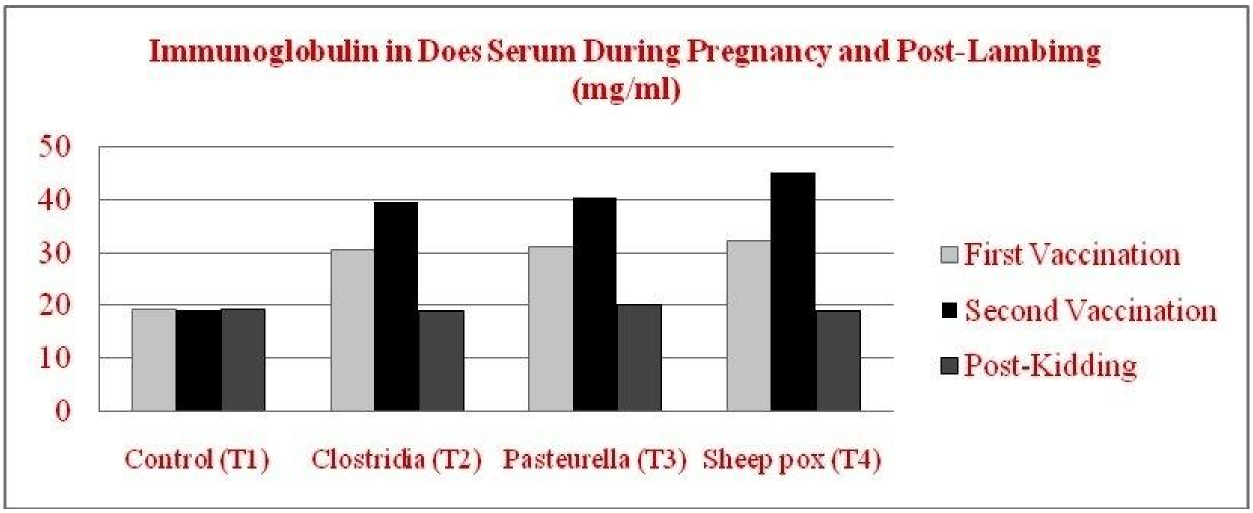

Figure 4. The Concentration of Total IgG in Serum of Does

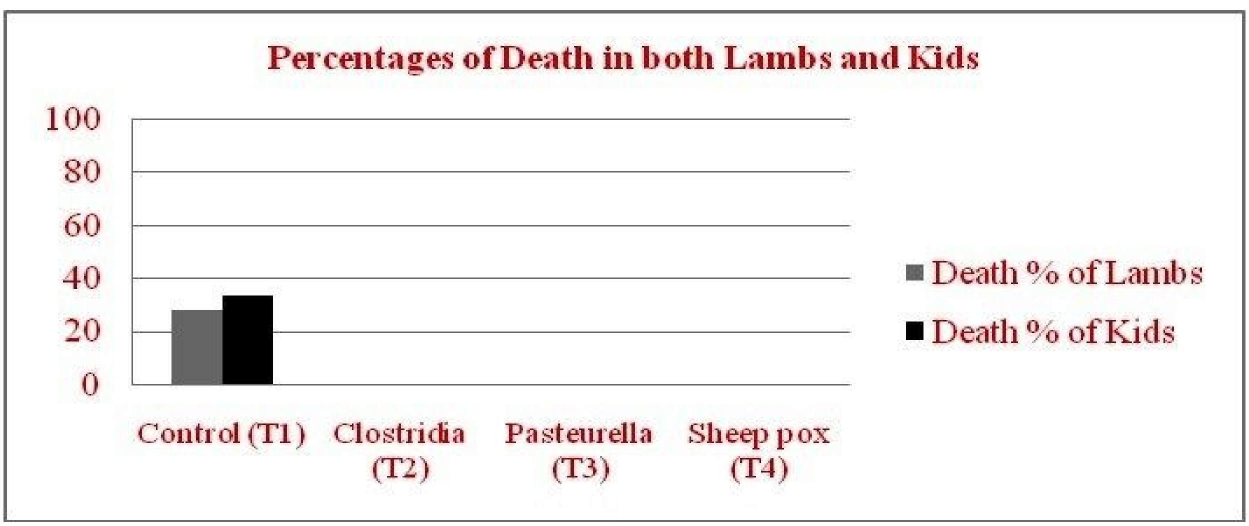

Figure 5. The Percentages of Lambs/ Kids Mortality Fed on Hyperimmune Colostrum

Conclusion: Vaccination of pregnant ewes/does was effective in producing hyperimmune colostrum for lambs/kids. Young lambs/kids receiving hyperimmune colostrum help in doubling IgG in offspring's serum. The results of vaccinating dams during pregnancy using a single pathogen vaccine showed significant positive impacts on the quality of colostrum produced and the survival of lambs/kids during their first seven days. The mechanism of action for raising the quality of colostrum by single species vaccination of pregnant animals is not known. Therefore, further studies are needed for using the vaccination protocols, i.e., single species vaccine.

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