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Comparison of Immune Responses Following the Administration of *Enterotoxaemia* Vaccine in Sheep and Goats

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ABSTRACT

Antibody responses in sixty clinically healthy pregnant sheep and goats were compared following the administration of enterotoxaemia vaccine. The thirty animals of each species were randomly divided into three groups. Antibody titers against *C. perfringens type D* epsilon toxin were detected in blood samples on day 15, 30, 45, 60 and 75 post vaccination. In unvaccinated control groups, S-I (sheep) and G-I (goat), no antibodies against C. *perfringens* epsilon toxin were observed. In the animals of groups S-II and G-II administered once with enterotoxaemia-cum-lamb dysentery vaccine, geometric means titers (GMT) were 6.5 and 2.64, respectively on day 15 post vaccination. In groups S- III and G-III administered with the same enterotoxaemia vaccine twice, GMTs were 59.7 and 12.12, respectively on day 30 post booster dose of vaccine. In conclusion, immune response of goats against enterotoxaemia vaccine was of short duration compared with the sheep indicating frequent use of vaccine for better immunity.

Key words: *Enterotoxaemia, Clostridium perfringens,* vaccine, sheep, goats

INTRODUCTION

Enterotoxemia (Pulpy Kidney Disease), caused by *Clostridium (C.) perfringens* type D, is a disease of great economic importance in sheep and goat farming worldwide (Niilo, 1980). The organism is the normal inhabitant of the alimentary tract of sheep, goat and other ruminants (McClane et al., 2006), but is usually found in low numbers. These organisms produce minute amounts of toxins under normal conditions which are removed either by normal gut movements or inactivated by circulating antibodies. Epsilon toxin produced by *C. perfringens* type D is the cause of enterotoxemia in sheep and goats. If the environment in the intestine is altered by sudden change in diet or other factors, that are not completely understood, *C. perfringens* type D proliferates rapidly and generates large amount of epsilon toxin, producing the disease (Nilo, 1986; Smith and Sherman, 1994).

Enterotoxemia in sheep involves a different pathophysiological mechanism than that of goats (Blackwell and Butler, 1992). In sheep, enterotoxemia may cause sudden death but peracute, acute and chronic forms of enterotoxemia have been reported in goats (Fernandez and Uzal, 2003). In sheep, the disease is mainly characterized by respiratory and neurological lesions while in goats, the lesions are confined mainly to the intestine (Uzal et al., 2008; Radad and Khalil, 2011).

Vaccination is recommended in the prophylaxis against enterotoxemia (Dela Rosa et al., 1997; Uzal, 1997; Uzal and Kelly, 1999; Bernath et al., 2004). Immunity against enterotoxemia in sheep has been reported to be readily produced by

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vaccination (Jansen, 1967). Since no commercial enterotoxemia vaccine is available for goats, polyvalent vaccines specifically produced for sheep have been considered effective in preventing enterotoxemia in goats (Blackwell et al., 1983). There is no data available in Pakistan on immunological responses in sheep and goats following the administration of enterotoxemia vaccine. Therefore, the present study was planned to assess the efficacy of enterotoxemia vaccine on the humoral immune responses in sheep and goats.

MATERIALS AND METHODS

Experiment Design

Sixty clinically healthy pregnant animals (thirty sheep and thirty goats), ranging from 1-1.5 years in age, raised at a private livestock farm in Lahore, Pakistan were included in the study. The selected animals of each species were randomly divided into three groups of ten animal in each group. The animals from group S-1 (sheep) and G-1 (goats) were not vaccinated and served as control. Animals in group S-II and G-II were administered once with 3.0 ml of enterotoxemia-cum-lamb dysentery vaccine (Veterinary Research Institute Lahore, Pakistan). It is an alum precipitated formalin inactivated bivalent bacterin toxoid prepared from *Clostridium perfringens* types B and D cultures. The animals were administered vaccine subcutaneously (S/C) 8 weeks prior to the expected date of lambing. The animals in groups S-III and G-III were administered same vaccine twice. First dose of vaccine was given on day 1 of study and booster dose one month after first dose. All the animals were kept under similar feeding, housing and management conditions.

Serology

Sera were obtained from blood samples collected from the test and control animals before vaccination. Sera samples were also collected on day 15, 30, 45, 60 and 75 post vaccinations. Sera samples were heat inactivated to 56ºC for 30 minutes in water bath and stored at -20°C. Antibody titers against *C. perfringens* type D epsilon toxin in serum were measured by an indirect haemagglutination test as described by Rehman et al. (2005). Briefly, the Epsilon toxins were separated by centrifugation from 24 cultures of *C. perfringens* type D grown in bullock heart medium anaerobically at 37ºC. Supernatants were trypsinized and then centrifuged at 13,000 rpm for 30 minutes. After configuration, the supernatant containing the epsilon toxin was dialyzed against 50mM phosphate buffered saline at 4 ºC over night. After the completion of dialysis, protein was determined as described by Lowry et al. (1951) using bovine serum albumin as a standard. Epsilon antigen with a protein concentration adjusted to 1 mg/mL was then used for coating 20% formalized tanned sheep red blood cells (SRBCs). All the sera samples were serially diluted as 1:2 through 1:2048 in micro-titration plates containing 96 U shaped wells, leaving the wells of last two columns for positive and negative controls, respectively. Epsilon toxin sensitized sheep red blood cells were then added to wells containing serum samples. Positive control wells were coated with hyperimmune serum against *C. perfringens* type D prepared in white New Zealand rabbits as described by Yamagishi et al., (1971) along with epsilon coated sensitized sheep RBCs. Negative control wells contained only suspension of epsilon coated sensitized sheep RBCs.

The plates were incubated at 37°C for 2 hours. Negative samples exhibiting no haemagglutination indicated central settling of erythrocytes. The indirect haemagglutination antibody titers of all samples were recorded. The geometric mean titers (GMT) were calculated using the procedure described by Thrusfield (1986).

RESULTS AND DISCUSSION

On day zero before vaccination, no antibody titer against epsilon toxin was observed in any of the groups of sheep and goat. The GMT was 6.50 and 2.64 for S-II (Sheep) and G-II (Goat), respectively on day 15 post enterotoxemia vaccination (Figure 3). In group S-II, maximum GMT (12.13) was observed on day 45 post vaccination (Figure 1). The results of the present study are in agreement with McClane et al. (2006). In group G-II, antibody titers were comparatively low compared with the group S-II (Figure 3) which declined very rapidly. (Figure 2). Finnie (2003) and McClane et al. (2006) reported that conventional enterotoxaemia vaccine produced low and short duration titers in goats than in sheep and the animals required booster doses every 3 or 4 months throughout their life after first vaccination (Uzal and Killy, 1999; Veschi et al., 2006).

In group S-III, antibody titers gradually increased and on 30 day post booster dose of enterotoxemia vaccine, GMT was 59.7 which was higher than the GMT observed in group S-II on day 60 post vaccination (Figure 4).

In group G-III of goats, GMT was comparatively higher than group G-II and remained detectable till 75 days post vaccination (Figure 2). Our results agree with those reported by Blackwell et al. (1983); Uzal and Kelly (1998a,b, 1999); Veschi et al. (2006) which demonstrated that immunization of goats with two initial vaccine doses yielded good results, in terms of higher antibody titers and lasted longer when compared to those obtained with simple immunization scheme.

Bentancor et al. (2009) recorded similar observations for llamas which failed to develop antibody titers after administration of a single dose of vaccine. However, a week following a second vaccination, mean antibody titers to *C. perjringcns* type epsilon toxin elevated significantly ($p<0.05$).

On day 75 post vaccination, the GMT declined in all the vaccinated groups. Butler (1974) and Tizard (1982) reported that transfer of immunoglobulins from serum to colostrum takes place after parturition.

In unvaccinated control sheep and goat groups, no naturally acquired antibodies against *C. perfringens* epsilon toxin were observed throughout study period which contradict the findings of Thomoson and Batty (1953) and Griner (1961) who detected anti-epsilon toxin serum antibodies in non-vaccinated sheep beyond the age they would carry a level of maternal immunity. Similar observations have also been reported by (Blackwell et al., 1983; Veschi et al., 2008) in unvaccinated herds of goat without history of clinical disease resembling entrotoxemia. From this it can be hypothesized that no sub clinical form of entrotoxemia or other stimulus exists in the study area which could lead to production of antibodies against *C. perfringens* epsilon toxin in unvaccinated sheep and goats.

Table 1: Post-vaccination antibody titres (GMT) in sheep and goats

Days Post Vaccination	Sheep			Goat		
	S-I	S-II	S-III	G-I	G-II	G-III
15	-	6.5	6.06	-	2.64	2.46
30	-	9.85	11.3	-	2.64	2.8
45	-	12.13	32	-	1.52	7.46
60	-	10.6	59.7	-	-	12.12
75	-	6.46	32	-	-	6.5

In conclusion, the immune response observed in goats was only of short duration indicating that vaccine would need to be given frequently. Alternatively, adjuvant may also be added in vaccine formulations to induce high antibody titers and long term immune responses.

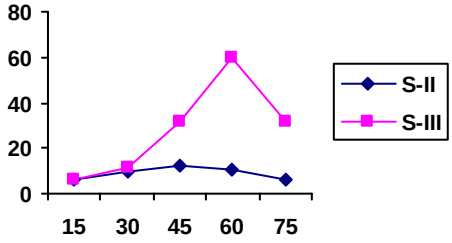

Figure 1. Post-vaccination antibody titres in vaccinated groups of sheep

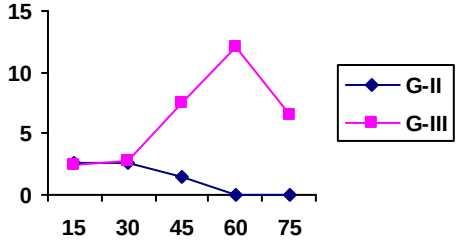

Figure 2. Post-vaccination antibody titres in vaccinated groups of goats

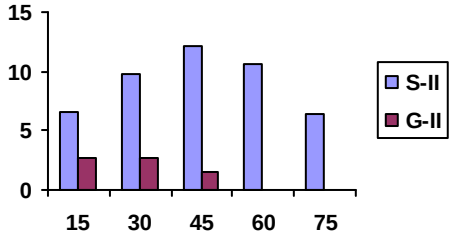

Figure 3 Immune response in sheep and goats after single dose of vaccination

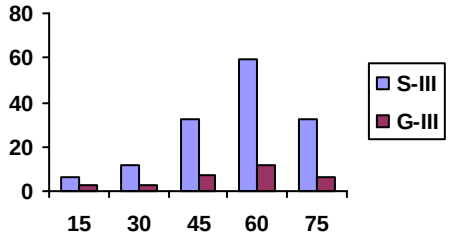

Figure 4 Immune response in sheep and goat after booster dose of vaccination

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