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Determination of antibodies titer in enterotoxaemia in sheep in Bikaner

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Abstract
An epidemiological study was conducted in Bikaner, Rajasthan to determine the prevalence of Enterotoxaemia in sheep. 100 sheep serum samples were collected from various locations within the district, and an indirect ELISA test revealed an overall seroprevalence of 20 percent. The study's results demonstrate that Enterotoxaemia is widespread in Bikaner district. To enable early detection among sheep and other vulnerable animal species, it is necessary to implement a year-round surveillance system. This is particularly important as some livestock owners tend to keep different animal species in one flock or herd, which can increase the risk of inter-species transmission of the disease.

Keywords: Enterotoxaemia, species, livestock

Introduction
Enterotoxaemia is a fatal intestinal disease that affects small ruminants such as sheep and goats, causing sudden death at different ages (Veschi *et al*., 2008) [13]. It is caused by *Clostridium perfringens*, a ubiquitous organism found in the gastrointestinal tract of humans and animals, as well as in soil. *C. perfringens* is a spore-forming, non-motile, and Gram-positive rod (0.6-0.8×2-4 µm) that is relatively aero-tolerant (Uzal, F.A. *et al*., 2016) [12]. The bacteria are divided into five toxins types, namely A, B, C, D, and E, based on the four essential toxins it produces, namely, alpha (CPA), beta (CPB), epsilon (ETX), and iota (ITX) (Siqueira, *et al*., 2012; Milton, A.A.P *et al*., 2017) [6, 4].
Enterotoxaemia is a deadly disease that could have a significant impact on the farm economy if an outbreak occurs. The clinical and pathological characteristics of the infection differ between sheep and goats (Uzal FA and Songer JG, 2008) [11]. In sheep, the infection is mostly characterized by systemic changes such as brain and lung edema and hydropericardium, with only minor and inconsistent intestinal changes (Uzal FA and Songer JG, 2008) [11]. In severe and subacute forms of the disease, systemic adjustments similar to those seen in sheep can sometimes be found.

Materials and Methods
For the present study, 100 vaccinated sheep were selected to study antibody titer and seroprevalence of enterotoxaemia.

Collection of blood samples
For seroprevalence and antibody titer studies, about 10 ml of blood was collected in a test tube without anticoagulant for separation of serum. The serum was then separated. The serum samples were transferred to sterilized Pyrex tubes with the help of a pasture pipette and were stored at -20 ºC till analysis. Separated serum is subjected to indirect ELISA for determination of antibody titer of enterotoxaemia in sheep.

Indirect ELISA for ETX peptide
To detect antibody titer for enterotoxaemia, the separated serum undergoes testing using an indirect ELISA kit (Developed by CIRG, Makhdoom).

The procedure involves incubating the serum in a well with different samples. Positive and negative control serum are included among the 96 samples being tested. The process begins by using 10 ng of ETX peptide (Stock conc. 10 ug/µl) in coating buffer for coating. 100 µl of this is added to each well and kept at 37 ºC for 2-4 hours. Afterward, 100 µl of 3% skimmed milk is added as a blocking buffer and incubated at 4 ºC overnight. The next day, the wells are washed once and 1:500 of serum or primary antibody in dilution buffer (3% skimmed milk in PBST) is added and incubated at 37 ºC for 60-80 min. The wells are washed 3-4 times with PBST and 100 µl of 1:750 of anti-sheep HRP conjugate in PBS is added and kept at 37 ºC for 1 hour. The wells are washed 4 times with PBST. Finally, 1 tablet of OPD (sigma) is dissolved in 10 ml of substrate buffer and 10 µl of 30% H_2O_2 is added. 100 µl of this solution is then added to each well and left for 2-5 minutes to develop the color. Finally, the absorbance at 450 nm is taken.

Results and Discussions

As part of our investigation, we conducted a screening of sheep to determine the seroprevalence of enterotoxaemia. To do so, we detected sero-reactors using 100 collected blood samples from sheep in the study area. The serum samples were tested using indirect ELISA to detect antibody titers for enterotoxaemia. We collected samples from various locations, including veterinary hospitals, local abattoirs, and in and around Bikaner city.

Sero-prevalence of antibody titter of previously vaccinated sheep against enterotoxaemia in the sheep of the selected area

Out of 100 sheep serum samples collected from the studies area, 20 percent (20/100) were found to have protected antibody titer (Table-1), for enterotoxaemia based on an indirect ELISA kit (Developed by CIRG, Makhdoom).

Table 1: Antibody titer of previously vaccinated sheep against enterotoxaemia in the sheep

S. No.	Disease	Prevalence
1.	Enterotoxaemia	20% (20/100)

Perusal of available literature revealed a lot of variation in the incidence of *Clostridium perfringens* infection in sheep in different countries. Taj *et al*. (2018) [10] found out of 204 analyzed fecal samples of sheep and goats, 89.18 percent were found positive while 10.82 percent were negative for *C. perfringens* type D. Sheep were more affected (54.78 percent) than goats (34.4 percent). Soren *et al*. (2019) [9] Collected 209 samples of sheep and goats and total of 19 samples were found positive indicating the prevalence of *Clostridium perfringens* to be 9.09 percent in small ruminant populations.
The variation in the rates of prevalence might be attributed to the age, and route of migration with lack of medicines and veterinary facilities, immune status of the host species (Songer, 1996 and Vinod Kumar *et al*., 2014) [8, 14].

Age-related seroprevalence of antibody titer of previously vaccinated sheep against enterotoxaemia in the sheep

To analyze the data, we divided the sheep into three age groups: those less than 6 months old, those between 6 and 12 months old, and those over 12 months old. Out of the 100 sheep sampled, 16 were in the first group, 38 in the second, and 46 in the third. We wanted to see if there was a possible association between protected antibody titer of enterotoxaemia and age (Table 8).
Our analysis of the age-related data of protected antibody titer of enterotoxaemia sheep (n=20) showed that goats in age group-II (6-12 months) had the highest seroprevalence, with 26.31 percent (10/38) testing positive. This was followed by 25.00 percent (4/16) in group-I (<6 months) and 13.04 percent in group-III (06/46) (Fig. 1).

Table 2: Antibody titer of previously enterotoxaemia-vaccinated sheep according to age group

S. No.	Age (Months)	No. of positive for Enterotoxaemia in each group
1.	<6	25.00% (04/16)
2.	6-12	26.31% (10/38)
3.	>12	13.04% (06/46)

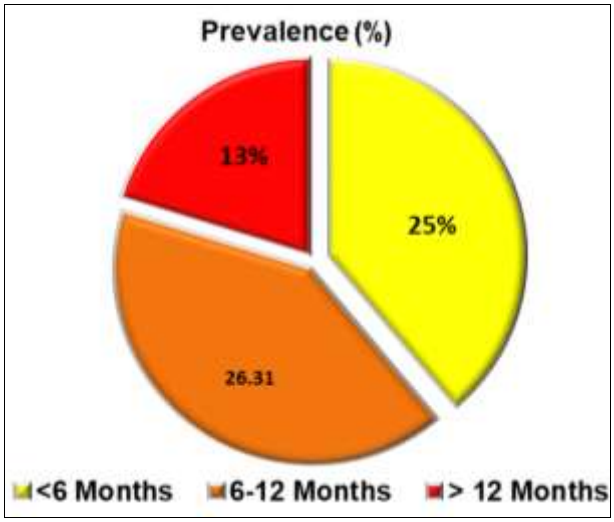

Fig 1: Age wise antibody titer of previously vaccinated sheep against enterotoxaemia

According to Scholes *et al*. (2007) [5], young goats are at a heightened risk of developing enterotoxaemia. This study's results align with previous research conducted by Islam (2008) [2], Singh (2017) [7], and Gangwar (2018) [1], all of whom noted a greater incidence of the disease in younger animals. One possible explanation for this correlation is that younger goats have lower immunity levels, as suggested by Islam *et al*. (2010) [3].

Sex-wise seroprevalence of antibody titer of previously vaccinated sheep against enterotoxaemia in the sheep

Out of a hundred sampled sheep, there have been fifty-nine males and forty-one females. Out of 20 positive sheep from the studies area, 12.00 percent (12/100) males and 08.00 percent (08/100) females were found positive (Fig. 2).

Table 3: Antibody titer of previously vaccinated sheep according to sex

S. No.	Sex	Prevalence (per cent)
1.	Male	12.00% (12/100)
2.	Female	08.00% (08/100)

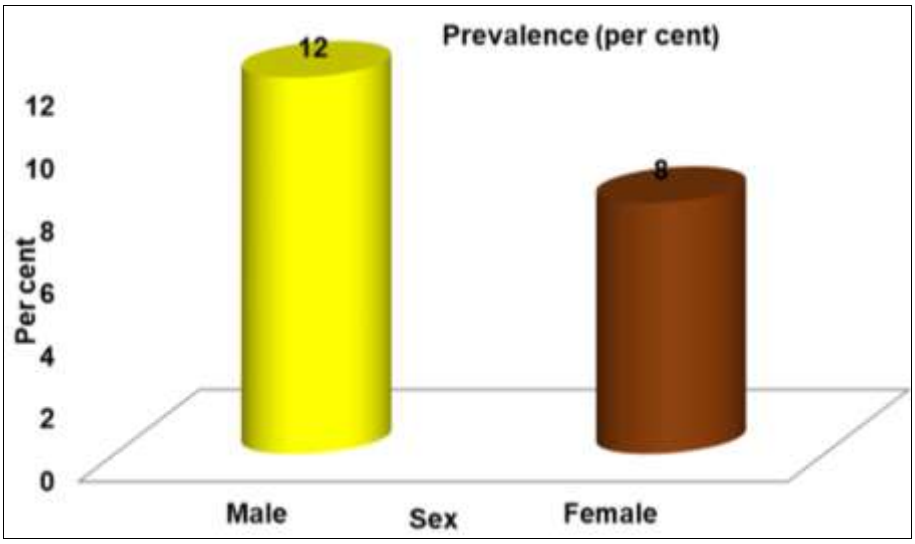

Fig 2: Sex wise sero-prevalence of antibody titer of previously vaccinated sheep against enterotoxaemia in the sheep

The findings of the present study were by Islam *et al.*, 2010 [3] who also reported a higher prevalence of enterotoxaemia in males. Males are usually stronger and eventually more vigorous than females so they can have more access to feed and consequently get affected (Islam *et al.*, 2010) [3].

Conclusion

According to the results of an indirect ELISA test, the Enterotoxaemia seroprevalence rate was found to be 20 percent. These findings confirm that Enterotoxaemia is widespread in the Bikaner district. To effectively combat this condition in small ruminants, a vaccination program targeting *C. perfringens* type A and type D would be a suitable preventative measure.

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