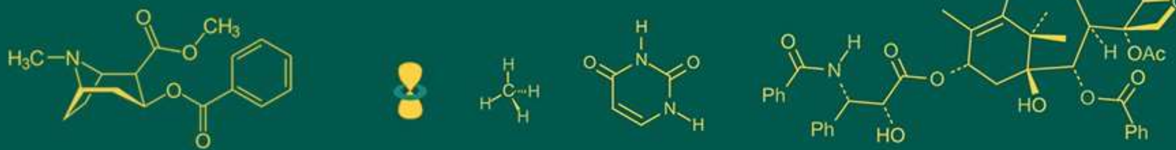

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Prevalence of enterotoxaemia in sheep in Bikaner district of Rajasthan

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Abstract
Enterotoxaemia is a well-known disease in sheep caused by gram-positive, spore-forming, anaerobic bacteria called *Clostridium perfringens*. For this study, we screened 100 sheep of different age groups to determine the prevalence of enterotoxaemia. Out of these, 68 sheep with a history of diarrhoea and distended intestine with dark fluid were screened for the prevalence of epsilon toxins in enterotoxaemia through intestinal content samples. Clinical samples for this study were collected from veterinary hospitals, local abattoirs and farms in Bikaner City. The prevalence of enterotoxaemia in sheep was found to be 22.05% by using the sandwich ELISA test for direct detection of epsilon toxins. Based on the results, we concluded that adopting an all-year-round surveillance system is necessary to enable early detection of the disease among sheep. Some livestock owners keep different animal species in one flock/herd, which increases the inter-species transmission of the disease.

Keywords: Enterotoxaemia, sero-prevalence, sandwich ELISA

Introduction

Enterotoxaemia is a common economically important death causing disease of sheep and goats all around the world (Niilo, 1980; Kriek *et al*., 1994) [9, 7], and is the most important cause of sudden death in different age groups of sheep (Veschi *et al*., 2008) [15]. Enterotoxaemia is caused by the bacteria *Clostridium perfringens*, anaerobic spore-forming Gram-positive bacilli. Bacteria can produce different kind of toxins including lethal toxins in sheep that increases mortality rates causing considerable losses to the farmer's economy (Rood and Cole, 1991) [11]. Besides of continuous vaccination programs, outbreaks of enterotoxaemia in animals are reported regularly during monsoon season every year. *C. perfringens* is a normal inhabitant of the intestines of animals but due to a sudden change in diet, it proliferates in large numbers and produces several potent toxins in the intestinal lumen (Ujal *et al*., 2014) [13]. Depending on the production of four toxins, Alpha, Beta, Epsilon, and Iota, *C. perfringens* is classified into five types A, B, C, D and E. As a result of these toxins, lambs can develop necrotic enteritis, as is the case with beta toxin; or they can be absorbed into the general circulation, producing systemic effects (for example, lambs can develop cerebral microangiopathy from epsilon toxin); or they can act both locally and systemically (for example, epsilon toxin can cause diphtheritic colitis and microangiopathy in goats that are not vaccinated) (Nilo, 1980) [9].

An evaluation of the history, clinical signs, and postmortem findings of sheep and goats is an essential tool for the presumptive diagnosis of enterotoxaemia, even though there is no way to make a conclusive diagnosis of these diseases without laboratory confirmation. Isolation, culturing, and typing of *C. perfringens* by conventional methods like the mouse neutralization tests is a time-consuming, expensive process that involves it being used on live animals in the process (Kumar *et al*., 2014) [18]. Rapid and easy-to-use *in-vitro* techniques like ELISA are used to demonstrate the toxins in the intestinal contents of diseased animals (Hassanien *et al*., 2014) [16] with limited options for subtyping.

The present study is aimed to standardize PCR for epsilon toxin of *C. perfringens* and apply ELISA to investigate the sero-prevalence of *C. perfringens* epsilon toxin in suspected cases.

Materials and Methods

In the present study, 100 sheep were selected and screened for prevalence enterotoxaemia. Out of these 68 sheep were screened for prevalence of epsilon toxins in enterotoxaemia by intestinal content samples.

Collection of intestinal content samples

Clinical Samples for the present study were collected from veterinary hospitals, local abattoirs, and farms in Bikaner City. Intestinal content samples of sheep of different age groups with a history of pale mucous membranes, and dark fluid in the severely distended intestine were collected. many ulcers (1 to 2 mm diameter) were seen in oedematous intestinal mucosa with dark red borders. For detection of epsilon toxins about 20 ml of intestinal content samples (ileocecal junction) from local abattoir was collected in sterilized test tubes, the supernatant was separated by centrifugation and transported to the laboratory in the ice box for further analysis. Collected clinical samples are subjected to PCR and ELISA tests.

DNA extraction and PCR application-

To extract DNA from clear supernatants, we followed the triazole method described by Kalender *et al.* in 2005. PCR amplification was conducted using a thermocycler, with a total reaction volume of 25 microlitres containing 12.5 microlitres of Taq PCR buffer, 10 pmol of forward and reverse ETX primers, 2.5 microlitres of isolated DNA from clinical samples, and the remainder filled up with nucleus-free water. For amplification, we ran 35 cycles, starting with a denaturation step at 94 °C for 3 minutes, followed by denaturation at 94 °C for 1 minute, annealing at 53 °C for 1 minute, extension at 72 °C for 1 minute, and the final extension step at 72 °C for 10 minutes. Finally, we carried out electrophoresis of the amplified products on a 1.5% agarose gel and visualized and photographed the bands under UV illumination following the PCR.

Table 1: Primer details used in PCR for epsilon toxins

Gene	Primer	Sequence	Product size
Epsilon etx	Forward	F - 5'-GCG GTG ATA TCC ATC TAT TC-3'	655 bp
	Reverse	R - 5'-CCA CTT ACT TGT CCT ACT AAC-3	

Sandwich ELISA

The seroprevalence of enterotoxaemia among sheep was investigated by subjecting the supernatant of intestinal content to sandwich ELISA. Anti-ETX antibody-coated microtitre plates were provided and can be used directly after blocking. To begin the assay, all reagents in the kit were mixed, and 100 µL of 1:10 diluted unknown antigen (diluted in reagent No.3) was added to respective wells in duplicates. The plate was then covered and incubated at 37 °C for 1 hour. Wells were washed 3 times with 1x PBST before adding 100 µL of 1:10 diluted binding antibody (diluted in reagent 3) to respective wells in duplicates. The plate was then covered and incubated at 37 °C for another hour before washing the wells again with 1x PBST. Next, 100 µL of reagent No. 4 (1:100 diluted HRP conjugate) was added to each well, and the plate was covered and incubated at 37 °C for 50-60 min. Wells were washed 4 times with 1x PBST before adding 100 µL of substrate mix freshly spiked with H_2O_2 dissolved in substrate buffer to each well. The plate was then developed at room temperature for 15 minutes before reading the absorbance of each well at 450 nm (Alieiki *et al*., 2020) [1].

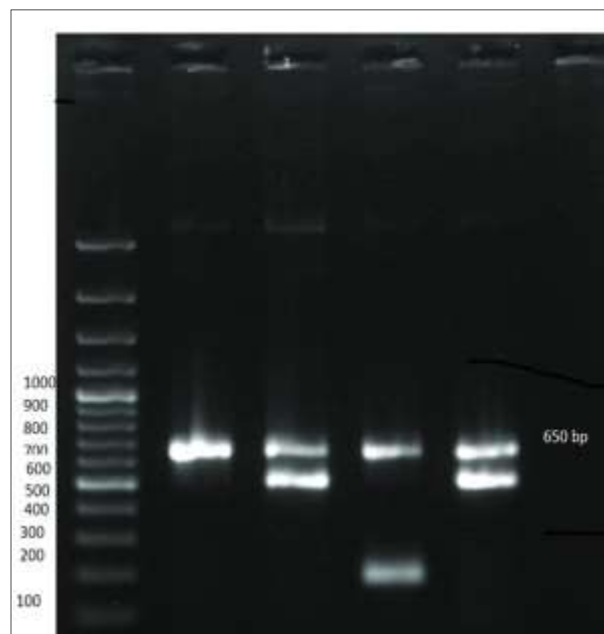

Fig 1: Presence of *C. perfringens* toxin

Result and Discussion

Results of the PCR test for detection of toxin genes of *C. perfringens* in intestinal content revealed simultaneous amplification of epsilon etx (655 bp) toxin genes in various combinations indicating the presence of *C. perfringens* toxin (Figure-1).

Sero-prevalence of epsilon toxins in ET suspected sheep based on Sandwich ELISA

Out of 68 sheep intestinal content samples collected from the studies area, 22.05 percent (15/68) (Table-2) sheep were detected positive (Table-2), for epsilon toxins based on sandwich ELISA kit (Developed by CIRG, Makhdoom).

Table 2: Overall seroprevalence of epsilon toxins in ET suspected sheep by Sandwich ELISA

S. No.	Disease	Prevalence
1.	Enterotoxaemia	22.05 percent (15/68)

According to a previous study conducted by Iqbal *et al.* in 2017 [4], the prevalence of *C. perfringens* was found to be 31 percent. In our present findings, we discovered that the overall prevalence of *C. perfringens* in sheep was 22.05 percent (15/68), indicating a lower prevalence than what was found in the previous study. Vaikosen and Ikhatua conducted research in Nigeria in 2004, where they used ELISA to investigate the presence of *C. perfringens* toxins in fecal samples of sheep and goats. They found that 26.90 percent (92/342) of the samples were positive for the

lecithinase enzyme of the *C. perfringens*. Additionally, Rahman *et al*. (2014) [10] evaluated the intestinal content of 46 samples from sheep and goats suspected of enterotoxaemia using culture and ELISA methods. They concluded that ELISA was found to be a convenient, precise, and cost-effective method for diagnosing enterotoxaemia.

Age-wise seroprevalence of epsilon toxins by Sandwich ELISA

As part of our current study, we divided the sampled 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 68 sheep sampled, 15 were in the first group, 25 were in the second group, and 28 were in the third group (as shown in Table 3). Our aim was to determine whether there is a correlation between Enterotoxaemia and age in sheep. Our analysis of the data indicates that sheep in age group II (6-12 months) had a higher seroprevalence of epsilon toxins, with 32.00% (08/25) testing positive. This is followed by 20% (03/15) in group-I (<6 months) and 14.28% (04/28) in group-III (>12 months) (as shown in Fig. 2).

Table 3: Age-wise seroprevalence of epsilon toxins in ET suspected sheep based on Sandwich ELISA

S. No.	Age (Months)	No. of positive for epsilon toxins in each group
1.	<6	20.00 percent (3/15)
2.	6-12	32.00 percent (8/25)
3.	>12	14.28 percent (4/28)

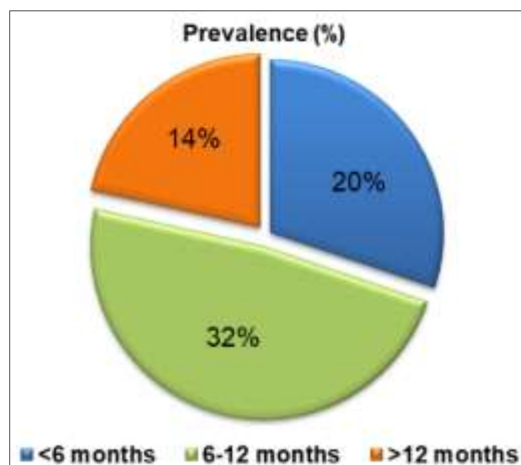

Fig 2: Age wise sero-prevalence of epsilon toxins by Sandwich ELISA

The young animals are highly susceptible for enterotoxaemia (Scholes *et al*. 2007) [12]. Findings of the present study were in accordance with previous study done by Gangwar, (2018) [2].

Sex wise sero-prevalence of epsilon toxins in ET suspected sheep on the basis of Sandwich ELISA

Out of 68 sampled sheep there were 43 male and 25 female (Table 4). Out of 15 positive sheep from the studies area, 14.70 percent (10/68) male and 07.35 percent (05/68) female were found positive (Fig. 3).

Table 4: Sex wise sero-prevalence of epsilon toxins by Sandwich ELISA

S. No.	Sex	Prevalence (percent)
1.	Male	14.70 percent (10/68)
2.	Female	07.35percent (05/68)

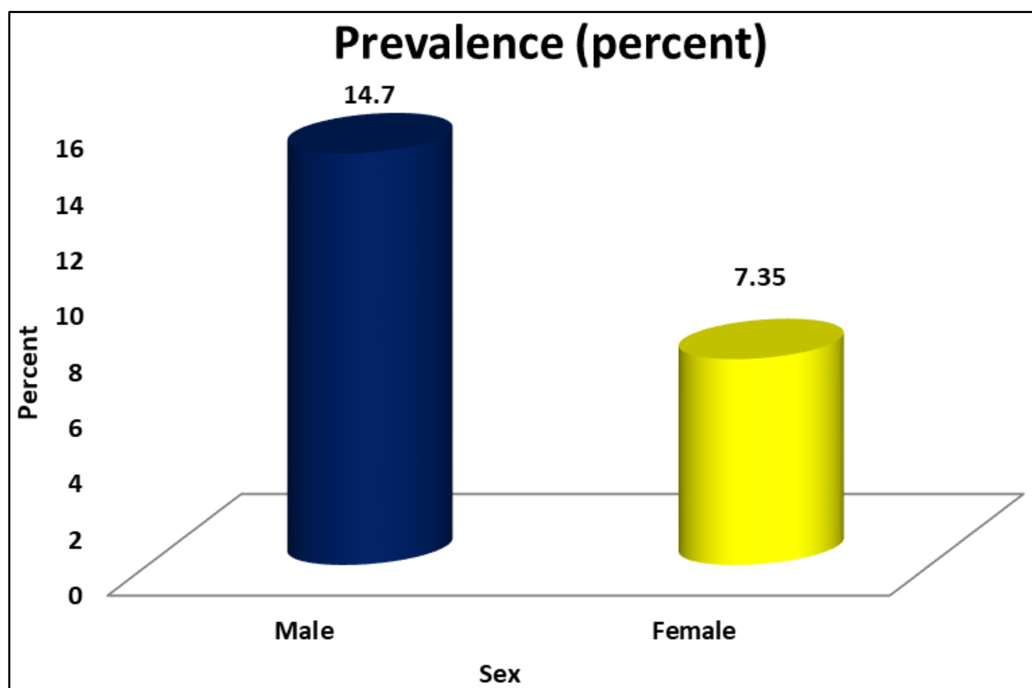

Fig 3: Sex wise sero-prevalence of epsilon toxins by Sandwich ELISA

The findings of the present study were by Islam *et al.* (2010) [5] 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) [5].

Conclusion

From the current study, we concluded that Seroprevalence for enterotoxaemia was 22.50 percent by using sandwich ELISA. The current findings prove that Enterotoxaemia in sheep is prevalent in the Bikaner district. However, adopting a year-round surveillance system is needed to enable early detection among sheep and other susceptible animal species because some livestock owners used to keep different animal species in one flock/herd which increases the inter-species transmission of the disease.

Reference

1. Alibeiki M, Golchin M, Tabatabaei M. Development of a double-recombinant antibody sandwich ELISA for quantitative detection of epsilon toxoid concentration in inactivated *Clostridium perfringens* vaccines. BMC veterinary research. 2020;16(1):1-7.
2. Gangwar NK. Molecular Pathology and Transcriptional Profiling of Enterotoxaemia in Post-Weaned Goats. Ph.D. Thesis, U.P. Pandit Deen Dayal Upadhyaya Pashu Chikitsa Vigyan Vishwavidyalaya Evam Go Anusandhan Sansthan (DUVASU), Mathura; c2018.
3. Hassanein KM, Sayed MM, Hassan AM. Pathological and biochemical studies on enterotoxaemia in sheep. Comparative Clinical Pathology. 2017;26:513-518.
4. Iqbal KM, Maqbool B, Ijaz M, Aslam BH, Ahmad IH. Prevalence and Chemotherapy of Enterotoxaemia (*Clostridium perfringens*) in diarrheic Sheep and Goats. Journal of Integrative Bioinformatics-Research. 2017;1(1):1-8.
5. Islam SM, Rahman SM, Ershaduzzaman MD, Taimur AF, Jang KH. Detection of *Clostridium perfringens* and its toxinotypes by enzyme linked immunosorbent assay from enterotoxaemic goats in Bangladesh, Korean Journal of veterinary services. 2010;33(1):37-44.
6. Kalender H, Ertas HB, Cetinkaya B, Muz A, Arslan N,. Typing of isolates of *Clostridium perfringens* from healthy and diseased sheep by multiplex PCR. Veterinarni Medicina-Praha. 2005;50(10):439.
7. Kriek NPJ, Odendaal MW, Hunter P. *Clostridium perfringens* type D enterotoxaemia. In: Coetzer, J.A.W., Thomson, G.R., Tustin R.C. (Eds.), Infectious Disease of Livestock with Special Reference to Southern Africa. Oxford University Press, Cape Town; c1994. p. 1314-1344.
8. Kumar NV, Sreenivasulu D, Reddy YN. Prevalence of *Clostridium perfringens* toxin genotypes in enterotoxemia suspected sheep flocks of Andhra Pradesh. Vet World. 2014;7(12):1132-1136.
9. Niilo L. *Clostridium perfringens* in animal disease: A review of current knowledge. Can. Vet. J. 1980;21:141-148.
10. Rahman PA, Rahman L, Razieh H, Reza L, Shamsedin G. Detection and Determination of Various Type of *Clostridium perfringens* in Sheep and Goat by Culture and ELISA Method in Khuzestan Province. Research Journal of Pharmaceutical, Biological and Chemical Sciences. 2014;5(1):32.
11. Rood JI, Cole ST. Molecular genetics and pathogenesis of *Clostridium perfringens*. Microbiol. Rev. 1991;55(4):621-648.
12. Scholes SF, Welchman Dde B, Hutchinson JP, Edwards GT, Mitchell ES. *Clostridium perfringens* Type D Enterotoxaemia in Neonatal Lambs. Veterinary Recod. 2007;160:811-812.
13. Uzal FA, Vidal JE, McClane BA, Guraj AA. *Clostridium perfringens* toxins involved in mammalian veterinary diseases. Open Toxicol. J. 2014;2:24-42.
14. Vaikosen ES, Ikhatua UJ. Detection of high level of enterotoxin of *Clostridium perfringens* types C and D in small ruminants in Nigeria. Small Ruminant Research. 2004;58(3):287-290.
15. Veschi JLA, Bruzzone OA, Losada-Eaton DM, Dutra IS, Fernande Miyakawa ME. Naturally acquired antibodies against *Clostridium perfringens* epsilon toxin in goats. Vet. Immunol. Immunopathol. 2008;125:198-202.
16. Hassanien RH, Hou TZ, Li YF, Li BM. Advances in effects of sound waves on plants. Journal of Integrative Agriculture. 2014 Feb 1;13(2):335-348.