OCCURRENCE OF ANTIBODIES AGAINST *CLOSTRIDIUM PERFRINGENS* TYPES B AND D IN GOATS

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Five hundred serum samples collected from Faisalabad abattoir, Livestock Production Research Institute, Bahadurnagar (Okara) and private livestock farms were processed for antibody detection to differentiate enterotoxaemia caused by *Clostridium perfringens* types B and D using agar-gel precipitation test (AGPT). The results showed 56% serum samples positive for type B and 54% for type D. It was concluded that AGPT may be a better serological test to screen out positive cases of enterotoxaemia in goats in field conditions.

INTRODUCTION

Goat population in Pakistan is continuously threatened by different infectious diseases amongst which enterotoxaemia, caused by *Clostridium perfringens* types A, B, C, D, E and F is of prime importance with relation to toxin elaboration and disease production. *Clostridium perfringens,* type D is widespread and more pathogenic than any other type (Seddon and Edgar, 1930).

Serodiagnosis of the enterotoxaemia has been accomplished through serum neutralisation test, indirect haemagglutination test (IHA) (Guo *et al.,* 1988), passive haemagglutination test (Biktimirov, 1979) and agar gel precipitation test (AGPT) (Ellner and Bohan, 1962). Bychenko (1965) observed for the first time a serological affinity between the somatic antigens of *C. perfringens* types B and E while Biktimirov (1979) reported no cross reaction between types A, B, C and D through serum neutralisation test.

The study under report was designed to differentiate *C. perfringens* types B and D and determine the correlation between antibody response mounted by these two types through AGPT.

MATERIALS AND METHODS

A total of 500 serum samples from vaccinated and non-vaccinated goats were collected from Faisalabad abattoir, Livestock Production Research Institute, Bahadurnagar (Okara) and some private livestock farms. A hyperimmune serum against *C. perfringens* types B and D (courtesy Veterinary Research Institute, Lahore) was separately prepared in white New Zealand rabbits following Wickhan (1956).

AGPT was performed following Ellner and Bohan (1962) as modified by Grist *et al.* (1974). Briefly, 20 ml of 1% borate buffer was poured into sterilised petriplates and held at 4°C for 24 hours prior to use. Twenty wells were punched per petriplate at four various places in the periphery such that each group had its five wells spread over an area of nearly 10 mm diameter. In each group of five wells, four surrounding well equidistant encircled the fifth central one so that the edge of every surrounding well was 5.0 mm distant the near edge of the central well. The diameter of individual well was 4.0 mm. The bottom of each well was sealed with melted agar in order to avoid seepage of the biologics.

The central well was filled with 2-3 drops of soluble antigens of *C. perfringens* types B and D separately and four different serum samples were dispensed (2-3 drops) separately using different Pasteur pipettes in each of the four wells. In the negative and positive control wells, flanking the central antigen well right at the centre of the petriplate, borate buffer saline (pH 9.0) and hyperimmune sera were added, respectively. In this way, all the serum samples were tested against *C. perfringens* types B and D antigens separately.

RESULTS AND DISCUSSION

Soluble (sonicated) antigens of both types of *Clostridia* accomplished better response through AGPT with clear precipitation band formation within 24 hours using homologous system. Cross reactions between heterologous system were also found between *C. perfringens* type D antigen with respect to hyperimmune sera with single spur formation. The test serum samples showed comparatively high positive percentage against the maximum of 56% samples were positive for *C. perfringens* type B while 54% samples were positive for type D (Table 1). These results are in consonance with Ellner and Bohan (1962) who also reported the strain variation using AGPT.

Table 1. Positive percentage of serum samples against *Clostridium perfringens* types B and D antibodies using agar gel precipitation test

Number of samples	Number of positive samples against			
	C. perfringens type B		*C. perfringens* type D	
	Positive	Per cent	Positive	Per cent
300 (Unknown)	31	10.33	65	21.66
100 (Non-vaccinated)	6	6	15	15
100	56	56	54	54
Total	93	18.6	134	26.8

A wet filter paper piece was placed inside of each lid cover so as to avoid too much drying of the agar medium during incubation. The plates were left under refrigeration (4°C) for overnight and later on shifted to incubator (37°C) and examined up to 72 hours for the presence of precipitation band under an oblique transmitted light initially with the unaided eye and afterwards under 2.4 x.

The results on comparison with IHA (Anjum *et al.*, 1992) revealed that highest percentage (37.8%) of serum samples showed IHA antibody titre from 1:8 to 1:16 whereas 12.6% serum samples showed positive response to AGPT as IHA antibody

Table 2. IHA and AGPT titres using *Clostridium perfringens* types B antigen

IHA antibody	Number of positive samples for IHA	Per cent	Number of positive samples for AGPT	Per cent
1:2 - 1:4	146	29.2	0	0
1:8 - 1:16	189	37.8	28	5.6
1:32 - 1:64	65	13	63	12.6

Table 3. IHA and AGPT titres using *Clostridium perfringens* type D antigen

IHA antibody	Number of positive samples for IHA	Per cent	Number of positive samples for AGPT	Per cent
1:2 - 1:4	124	24.8	0	0
1:8 - 1:16	207	41.4	32	6.4
1:32 - 1:64	104	20.8	99	19.8

titre ranged from 1:32 to 1:64 (Table 2). Comparison of the two tests i.e. IHA and AGPT indicated that as the antibody titre goes high, the percentage of AGPT increases whereas the maximum percentage of IHA antibody titre (41.4%) existed at the IHA antibody titre 1: to 1:16 (Table 3).

Bychenko (1965) recommended that AGPT is a rapid diagnostic test but Anjum *et al.* (1992) reported that IHA is more sensitive than AGPT and better able to detect antibody titres in vaccinated, non-vaccinated and randomly selected animals which is not otherwise possible through AGPT. This has indicated the high sensitivity of IHA than AGPT as described by Thrushfield (1986).

In conclusion, AGPT may be a better serological test to screen out the positive cases of enterotoxaemia in goats in field conditions, preferably in vaccinated flocks where more chances of high antibody titres have been found to exist. This test is simple and easy to perform on various livestock farms and even in veterinary hospitals for the diagnosis of the malady.

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