

Immune Profile of Enterotoxaemia in Sheep – A Field Study

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Abstract

Enterotoxaemia is one of the economically important epidemic diseases of sheep caused due to the absorption of epsilon toxin released by *Clostridium perfringens* type D. Several factors have been cited as predisposing to the occurrence of pulpy kidney disease, with the most important including sudden dietary changes and a reduction in intestinal transit of ingested food.

Two measures are emphasized as being of great importance in the prophylaxis against enterotoxaemia: vaccination of all animals and adequate nutritional management. Every year vaccination of sheep is being carried out extensively in Andhra Pradesh in pre-monsoon season to prevent the disease occurrence. In the present study, to evaluate the duration of immunity after vaccination under field conditions, Indirect ELISA kits were prepared, validated and used to detect the protective antibody levels in sheep vaccinated against ET.

Keywords: Immune; Disease; Vaccination

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Introduction

Enterotoxaemia is one of the economically important epidemic diseases of sheep caused due to the absorption of epsilon toxin released by *Clostridium perfringens* type D. Several factors have been cited as predisposing to the occurrence of pulpy kidney disease, with the most important including sudden dietary changes and a reduction in intestinal transit of ingested food [1].

Materials and Methods

Selection of districts for the study

Based on the number of OBR's reported during the past five years, Nellore, Prakasham, West Godavari, Krishna, Kurnool, Chittoor, Nizamabad and Mahaboobnagar districts of Andhra Pradesh were selected. Two villages from each district were identified to collect sera samples periodically.

Vaccination

Vaccinations were carried out for the sheep of the above districts in the months of May and June with Enterotoxaemia vaccine (Brew No. 1, 2, 3, 4, 5, 6 and 7 with a mouse Minimum Lethal Dose (MLD) ranging from 5,000-8,000) produced at VBRI with fermentor technology in new cGMP vaccine production facility. The efficacy of the vaccine was evaluated experimentally in

the Standardization of Biological Products (SDBP) Division, Rajendranagar before sending to the field institutions. All the batches were safe, sterile and found to produce not less than 5.0 IU of epsilon antitoxin per ml.

Collection of sera samples

Five sera samples were collected from each of the identified villages of the selected districts from same sheep at 0, 90, 135, 180, 225 and 270 days post vaccination and transported to VBRI in cold chain. The samples were preserved at -20°C until use.

Preparation of indirect ELISA kits

Antigen (Pure epsilon prototoxin) for ELISA was prepared, concentrated and purified as per the method described by Pavan Kumar et al. Briefly, 18 hours old *C. Perfringens* type D culture grown in thioglycolate broth was centrifuged at 10,000 rpm for 30 min. The supernatant containing epsilon prototoxin was concentrated by precipitating with Ammonium Sulphate. Ammonium Sulphate was removed by dialyzing in distilled water over night at +4°C. The crude toxin was purified by Ion exchange Chromatography using DEAE Cellulose column. Purified toxin was used as the antigen in indirect ELISA to increase the sensitivity and specificity of the test [2]. The eluted fractions having highest optical density at 280 nm were pooled together, MLD was determined in mice and stored at -20°C in aliquots until use.

Serum with antibody levels 0.01 IU/ml and 20 IU/ml were used as negative and positive controls in the test, which showed the OD values of 0.09-0.1 and 0.9-1.1 respectively.

Cut-off determination

As 0.2 IU of epsilon anti-toxin per ml and above gives protection against ET at field level. It is possible to determine the cut-off value separating positive from negative sera. The sera samples showing 20-50 percent positivity (PP) values were subjected to toxin neutralization test. One ml of each (pooled) sera sample was mixed with 30 MLD of epsilon toxin and kept at room temperature for half an hour because, sera containing one IU anti-toxin per ml will be able to neutralize 150 mouse MLD of toxin. Five mice weighing not less than 18g are each given 0.2 ml of mixture intra-venously and the control mouse each were given with 0.2 ml of 30 MLD of epsilon toxin and normal saline mixture. The test was repeated for 3 times to get consistent results.

ELISA

A total number of 480 samples were screened for protective antibodies against enterotoxaemia by indirect ELISA in duplicate. Antigen and test sera were used at 1:100 dilution in the test. Commercially available Anti-sheep IgG conjugated with Horseradish Peroxidase (Sigma cat. No A3415) was used in 1:10,000 dilution to detect sheep immunoglobulins. 3,3',5,5'-Tetramethyl Benzidine (Sigma cat. No T2885) at 0.01% level was used as substrate. The reaction was stopped with 1 M H₂SO₄ (Qualigens 29995) and the colour development was measured at 450 nm.

Percent Positivity (PP) was calculated by using the following formula:

PP (%) = Mean OD value of positive control sample - Mean OD value of Test sample X 100

Mean OD value of positive control sample- Mean OD value of Negative sample

Results and Discussion

PP value of 30% and above which is corresponding to 0.2 IU epsilon antitoxin/ml was adopted as the minimum protective threshold, as 0.2 IU antitoxin/ml and above gives protection against ET at field level [3]. Hence, in the present study sera samples showing the PP value of 30% and above (**Table 1**) were considered to have the protective antibody levels.

Based on the results it is observed that, on 0 day of vaccination 10.8% of animals showed protective antibody levels (**Tables 2 and 3**). There was 100% seroconversion in all the districts with an average PP value of 74.98% by 90 days post vaccination (PV). Gradual decline of PP value from 74.98% on 90 days PV to 42.55% on 270 days PV is observed and the percent animals showing protective level of antibodies also declined from 100% on 90 days PV to 81% on 270 days PV. The findings are in contrary with the observations [4] where the protective antibodies remained for 3 months only. The variation may be due to the vaccine produced at VBRI is improved and tested as per the new IP standards which

Table 1 Determination of cut-off PP value.

PP value of sera samples		Control	20%	25%	30%	35%	40%	45%	50%
No. of mice alive (Inoculated 5 mice/ group in all tests)	Test I	0	0	2	5	5	5	5	5
	Test II	0	0	1	5	5	5	5	5
	Test III	0	0	2	5	5	5	5	5

Table 2 Mean PP values of indirect ELISA.

S. No	Name of the District	Average PP values on days PV					
		0 day	90	135	180	225	270
1	Nellore	-3.7	73.45	59.9	57.1	53.2	34.1
2	Prakasham	14.82	69.53	56.5	52.7	52.4	33.2
3	WG	13.08	60.14	53.5	52.08	51.0	35.7
4	Krishna	24.09	79.7	74.1	62.6	61.7	45.7
5	Kurnool	-5.01	72.2	62.4	62.0	60.0	45.3
6	Chittoor	5.363	77.97	75.57	73.4	66.3	47.6
7	Nizamabad	34.31	79.26	76.5	70.3	62.7	46.9
8	Mahaboobnagar	44.95	87.66	81.41	79.86	75.23	51.9
Total		15.98	74.98	67.48	63.75	60.31	42.55

Table 3 Percent animals showing protective level of antibodies.

S. No	Name of the District	No. of samples/ each collection	% of animals showing protective PP value					
			0 day	90 days	135 days	180 days	225 days	270 days
1	Nellore	10	0	100	100	90	90	80
2	Prakasham	10	0	100	100	80	80	80
3	WG	10	10	100	100	90	80	80
4	Krishna	10	30	100	100	100	90	80
5	Kurnool	10	0	100	100	100	100	90
6	Chittoor	10	0	100	100	100	80	80
7	Nizamabad	10	30	100	100	100	100	90
8	Mahaboobnagar	10	60	100	100	100	80	70
Total		80X6=480	10.8	100	100	95	87.5	81.25

is able to produce not less than 5.0 IU of epsilon antitoxin per ml and hence, the protective levels are continued till the end of the study period.

At the same time, the protective levels of antibodies are present only in 81% animals by 270 days PV. The recommended schedule for ET vaccination is 3-4 weeks prior to lambing to the ewes, at the age of 4-6 weeks and booster dose within 4 weeks after first dose to the lambs and yearly vaccination to the adults [5]. In the present study, vaccination was carried out to all sheep during the months of May and June and there was no booster dose administered even to the lambs. Hence, the immunity might have lost early in case of lambs and also the immune response to any vaccine depends on the individual, age, breed and various managemental practices.

Basing on the above results, annual vaccination of sheep is recommended as the protective levels were maintained up to 9 months in 81% of animals. The remaining 3 months period i.e., from March to May is generally dry period and the availability

of lush grass pastures is very less. Since the causative bacteria proliferate in the intestine in response to ingestion of abnormally high levels of starch, sugar or protein, the disease may not occur during this period [6].

Further studies are indicated to confirm loss of immunity in lambs, effect of booster dose to lambs and vaccination to the ewes prior to lambing [7-9].

Conclusion

A field study was taken up to know the immunity profile against

ET in sheep vaccinated with Enterotoxaemia vaccine in Andhra Pradesh. A total number of 480 samples collected at different intervals were tested by indirect ELISA, developed at R & D section, VBRI. This study confirmed that there is 100% seroconversion by 90 day PV and 81% of animals showed protective level of antibodies till the end of the study period i.e., 270 day PV.

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