Journal of Animal Research: v.5 n.2, p. 353-357. June 2015

DOI Number: 10.5958/2277-940X.2015.00061.3

Evaluation of Immune response to Enterotoxaemia Vaccine in Sheep reared under Experimental and Field conditions using ELISA

Nagendra Reddy Thopireddy[1]*, Sreenivasulu Daggupati[2] and Eswara Prasad Pagadala[3]

[1]Department of Veterinary Microbiology, College of Veterinary Science, Sri Venkatewara Veterinary University, Proddatur, Andhra Pradesh, INDIA.

[2]Department of Veterinary Microbiology and [3]Department of Veterinary Biochemistry, College of Veterinary Science, Sri Venkatewara Veterinary University, Tirupati, Andhra Pradesh, INDIA.

**Corresponding author: NR Thopireddy; Email: tnreddy32@gmail.com*

Received: 30 November, 2014 **Accepted:** 28 May, 2015

ABSTRACT

Immune response to enterotoxaemia vaccine (ET) was evaluated in sheep reared under laboratory and field conditions using enzyme-linked immunosorbent assay (ELISA). Under experimental condition, six sheep were vaccinated with booster dose on 14th day and evaluated for immune response; however in field conditions, serum samples from 386 ET vaccinated sheep (139 from organized farms and 247 from unorganized sector of Andhra Pradesh) were evaluated for protective antibody titre using ELISA. Highest protective titre was recorded in sheep reared under experimental condition; on day 30 after vaccination followed by gradual decrease up to day 90. Immune responses of sheep maintained under rural conditions were found to be low when compared with sheep maintained under experimental/laboratory conditions. The protective titers were maintained up to 3 months in sheep maintained under village conditions whereas up to 4 months in those maintained in experimental conditions. So, it can be concluded that good managemental practices along with booster vaccination of ET in farms could evoke better immune response in sheep against ET.

Keywords: Enterotoxaemia vaccine, Immune response, ELISA, Field conditions, Experimental conditions

Sheep farming plays a pivotal role in socioeconomic life of rural areas. The sheep are important economic species, contributing greatly to the agrarian Indian economy especially in arid/semi-arid and mountainous areas. The major reasons for low productivity are inadequate grazing resources and diseases, causing high mortality and morbidity. Enterotoxaemia is one of the important bacterial diseases of sheep inflicting significant economic losses to sheep farmers. The disease is caused by *Clostridium perfringens* type D, a normal inhabitant of intestines. Epsilon toxin is the major lethal toxin produced by the organism and is responsible for acute toxemia (Markey *et al.,* 2013).Alum precipitated epsilon toxoid vaccine is used in field conditions for developing humoral immune response (Urguev and Ataev, 1977; Kennedy *et al.,* 1977; Srinivasan *et al.,* 2001). However, enterotoxaemia outbreaks were reported in spite of vaccination (NADRES reports, 2012). So, investigating the immune responses among the vaccinated animals by assessing the duration of protective titers will give us an idea to take necessary steps in the prevention of outbreaks. Usually the immune responses among the vaccinates are assessed by using mouse neutralization test (MNT) which is cumbersome, time consuming and requires use of large numbers of mice (Rosskopt-Streicher *et al.,* 2004). To overcome these difficulties, ELISA has been already standardized (Sharma, 2001) and validated (Vijaya Lakshmi, 2003) to study the immune responses, among the vaccinated animals. The correlation coefficient between indirect ELISA and MNT is 0.99 (Uzal *et al.,*2003). The present investigation was therefore aimed to evaluate immune responses in vaccinated sheep maintained under experimental (controlled) and field conditions.

MATERIALS AND METHODS

The present study was conducted to evaluate immune response in ET vaccinated sheep reared under experimental controlled conditions and at field conditions.

Experimental group

Six Nellore sheep of one year age group were randomly selected to use them as an experimental group and are vaccinated with enterotoxaemia toxoid vaccine procured from Veterinary Biological Research Institute (VBRI), Hyderabad. The vaccine was administered @ 3 ml / animal, subcutaneously on the inner aspect of thigh following all aseptic precautions. The booster dose was given after 14 days. The serum samples were collected on day Zero, 30^{th}, 60^{th}, 90^{th} and 120^{th} day after vaccination.

Field group

Serum samples from 386 ET vaccinated sheep (139 from organized farms and 247 from unorganized sector of Andhra Pradesh) were collected for comparative study.

ELISA

Sera samples collected after vaccination were subjected to ELISA. Previously standardized (Sharma, 2001) and validated (Vijaya Lakshmi, 2003) procedure of ELISA was used to assess protective antibody titre. Epsilon prototoxin was used to coat the ELISA plates. *Clostridium perfringens* type D cultures were grown in the laboratory for production of *epsilon* toxin. The *Epsilon* prototoxin from *Clostridium perfringens* type D was produced in protein free medium with pH 7.5. ELISA plates coated with 12 µg/ml of epsilon toxin, serum dilution at 1:100, with conjugate dilution of 1:10,000 were used to detect anti-epsilon antibodies. Percent positivity was calculated using the mean Optical Density (OD) values of positive and negative controls, using the formulae given below.

$$\text{Present Positivity Value} = \frac{\left(\text{Test sample OD mean Negative control OD mean}\right)\times 100}{\left(\text{Positive control OD mean-Negative control OD mean}\right)}$$

Determination of cutoff point

The anti-epsilon antibody titers ranging from 0.15 to 0.2 International Units (I.U) were found to have protective effect against enterotoxaemia (Hepple *et al*., 1959 ; Farzan *et al*., 1996). Therefore the upper limit of 0.2 I.U has been considered to determine cutoff value to differentiate protective and non-protective titres among the vaccinates. The percent positive (PP) value was noted as 30 for the serum samples containing antibody titre of 0.2 I.U. Hence the PP value 30 was considered as the cutoff point to determine the protective levels of immunity in vaccinated animals.

Statistical analysis

The data obtained was systematically tabulated and statistical analysis (two way ANOVA) was carried out as per the procedure described by Snedecor & Cochran (1994).

RESULTS AND DISCUSSION

All the sheep under experimental conditions showed highest titers at day 30 of post vaccination indicating the booster vaccination effect, which are in agreement with results reported by Bentancor *et al.* (2009). The titers were gradually decreased till 120 days of post-vaccination (Table 1). There was a significant ($P<0.05$) decrease in protective titers (PP values) from 30 days of post vaccination to 90 days of post vaccination period. This observation was in accordance with the report of Gogoi *et al.* (2004). Present investigation illuminated the thought that a revaccination after 90 days would evoke satisfactory immunity to ET in sheep to effectively combat the natural ET out breaks. However the decrease in protective titer was not much significant ($P>0.05$) from 90 days to 120 days post vaccination period.

Table 1. Immune responses in sheep vaccinated against Enterotoxaemia under experimental conditions.

Sheep No.	Percent positive values of ELISA Post vaccination period				
	0 day	30 days	60 days	90 days	120 days
M1	0.51	76.72	58.65	52.13	43.40
M2	3.01	66.29	54.48	33.49	33.12
M3	1.99	56.21	37.80	35.98	22.50
M4	7.26	71.00	60.17	36.61	34.74
M5	5.56	78.36	56.27	45.27	35.76
M6	1.67	78.42	60.10	29.47	22.16
Mean±SEM	3.33^{d}±1.05	71.17^{a}±3.57	54.57^{b}±3.48	38.83^{c}±3.40	31.95^{c}±3.37

Mean P.P Values in a row with different superscripts differs significantly (P<0.05)

The results of vaccine responses in the 139 sheep maintained under organized farms indicated that one year age group were responded significantly higher (P < 0.05) than the other age groups (Table 2). Similar type of age related changes in immunity were reported in wild sheep by Nussey *et al.*, (2012). Non-significant (P>0.05) variations were seen among the sheep aged between three to five years in response to ET vaccination. The mean percent positive values observed at three different farms were 44.85±3.67 (Sheep unit, College of Veterinary Science, Turupati), 47.35±1.88 (Penukonda sheep farm) and 39.47±1.87 (Sidderampuram farm) (Table 2). The immune responses of the sheep maintained at three different farms showed variation due to differences in management practices, nutrition and deworming among different farms.

Table 3. Mean PP values of serum samples collected from sheep vaccinated against Enterotoxaemia at different villages.

Age of the animals in years	Post Vaccination period				
	1 month	2 month	3 month	4 month	Mean*± SEM
1 year	52.54	49.29	35.15	26.92	42.23^{a}±2.34
2 year	49.11	45.06	35.15	27.24	38.02^{a}±2.07
3 year	47.27	41.53	33.86	27.71	37.58^{a}±2.36
4 year	44.61	40.39	31.66	21.17	30.48^{b}±2.30
5 year	43.77	37.79	30.34	17.23	31.72^{b}±2.54
Mean**± SEM	48.05^{A} ± 2.06	42.07^{A} ± 1.48	33.49^{B} ± 2.97	23.95^{C} ± 1.47	

*Mean P.P Values in a row with different superscripts differ significantly (P<0.01)
**Mean P.P Values in a column with different superscripts differs significantly (P<0.05)

Table 2: Percent positivity values of serum samples collected after two months of Enterotoxaemia vaccination in organized farms.

Name of the Farm	Age of Sheep in Years					
	1	2	3	4	5	Mean*± SEM
Sheep unit, College of Veterinary Science., Tirupati	55.19	44.09	39.62	37.11	30.59	44.85^{A}±3.67
Penukonda Sheep Farm	56.22	49.05	44.12	43.83	43.81	47.35^{A}±1.88
Sidderampuram Sheep Farm (RMF)	51.23	42.66	38.04	37.17	31.15	39.47^{B}±1.87
Mean**±SEM	54.61^{a}±2.25	45.16^{b}±2.59	40.16^{c}±2.52	39.56^{c}±4.77	38.79^{c}±3.62	

*Mean P.P Values in a row with different superscripts differs significantly (P<0.05)
**Mean P.P Values in a column with different superscripts differs significantly (P<0.05)

Table 4. Immune response of sheep vaccinated against ET under field conditions (different villages)

Village	Age of the animal in years					
	1	2	3	4	5	Mean± SEM
PUDIPATLA (1)*	52.54	49.10	47.28	44.61	43.77	48.44^{A}±2.09
PULIKUNTA (2)*	53.42	46.34	47.26	41.51	41.37	45.11^{AB}±2.74
TURAKA VANDLA PALLI (2)*	47.52	42.32	37.48	37.06	36.59	39.59^{BC}±2.54
KAMALAPURAM (2)*	48.37	45.27	43.89	42.32	36.23	42.03^{B}±2.93
ARAVEEDU (3)*	35.15	35.15	33.87	31.66	30.34	33.49^{CD}±3.01
A.RANGAMPETA (4)*	31.43	30.04	29.73	23.94	19.98	26.86^{D}±1.91
CHERLO PALLI (4)*	26.00	22.00	21.62	17.97	13.71	19.64^{E}±2.49
Mean SEM	42.03^{a}±2.33	38.60^{a}±2.10	37.30^{a}±2.34	30.15^{b}±2.28	31.71^{b}±2.54	

*Values in parenthesis against village name indicate the months of post vaccination
Mean P.P Values in a row with different superscripts differssignificantly (P<0.01)
Mean P.P Values in a column with different superscripts differs significantly (P<0.05)

The PP values of different age group of sheep varied between 42.23±2.34 and 31.72±2.54 (Table 3). Immune responses of sheep of different age groups maintained at different villages in unorganized groups showed a significantly higher PP values (P<0.05) in 1-3 years age group than that of 4-5 years group. The immune responses were gradually decreased with increase of age of animal.

The significant variations in immune responses were observed in sheep maintained at different villages (P<0.05) (Table 4). The protective titres were maintained only up to three months in different villages. In the two villages where serum samples were evaluated 4 months after vaccination; the titres were detected below the protective level. The low titers might be attributed to ill managemental practices, lack of concentrates in nutrition, improper deworming (Blackwell *et al*., 1983; Gogoi *et al*., 2004).

The immune responses to ET vaccine of sheep maintained under controlled/ experimental conditions and village conditions could be compared. It was observed that the protective titers were maintained up to three months post vaccination period under village conditions as compared to experimental conditions where the protective titers were maintained up to four months. This could be attributed to the effect of booster dose in controlled conditions which evoked a higher response than single dose vaccination. Similar type of results were reported by Naz *et al.* (2012) who observed differences in booster and single dose vaccination titres. On contrary, Bernath *et al.* (2004) suggested revaccination on 8 weeks after the primary dose. The decrease in the duration of immunity could be attributed to the managemental conditions prevailing in the rural sectors.

From this study it can be concluded that booster vaccination after 90 days would evoke satisfactory immunity to ET in sheep so as to effectively combat the natural ET outbreaks. However, better immune titers against the vaccination could be obtained by good management practices, nutrition and regular deworming irrespective of the age of animal. To elicit more accurate results the study need to be conducted taking large number of samples considering different parameters like antigen content in the vaccine, revaccination schedules, nutritional status, age and deworming schedules.

REFERENCES

Bentancor, A.B., P.Halperin, P., M. Flores, M. and F, Iribarren, F. 2009. Antibody response to epsilon toxin of *Clostridium perfringens*following vaccination of Lama glama Crias. *J. Infect. Dev. Ctries.,* **3**: 624-627.

Bernath, S., Fabian, K., Kadar., Szita, G. and Barna, T. 2004. Optimum time interval between first vaccination and booster of sheep for *Clostridium perfringens* type D. *Acta Vet.*, **73**: 473-475.

Blackwell, T.E., Butter, D.G. and Bell, J.A. 1983. Enterotoxaemia in the goat. The humoral response and local tissue reaction following vaccination with two different bacterin toxoids. *Canadian. J. Comp. Med*., **47**: 127-132.

Farzan, A.V., Madani, R., Mousawi, M., Pilechian, R., Golchinfar, F. and Mansoorbakht, M. 1996. Measurement of epsilon antitoxin in sheep sera by ELISA, *Archives – de-Institut-Razi Inst*., 4**6/-47**:75-79.

Gogoi, M., Phukan, A. and Sarma, D.K. 2004. Immuneoresponse of goats vaccinated simultaneously with FMD and enterotoxaemia vaccines and with (or) withoutLevamisol injection. *Indian J. Anim. Sci.*, **74**:357-359.

Hepple, J.R., Chodnik, K.S. and Price, E.K. 1959. Immunization of lambs against *Clostridium welchii* type D enterotoxaemia (Pulpy kidney disease) with purified aluminium treated toxoid. *Vet. Rec*., **71**:201-206.

Kennedy K. K., Norris, S. J., Beckenhauer, W. H. and White, R. G. 1977. Vaccination of cattle and sheep with a combined *Clostridium perfringenes*type C and D toxoid. *Am. J. Vet. Res.,* **38**: 1515-1517.

Markey, B.K., Leonard, F.C., Archambault, M., Cullinane, A. and Maguire, D. 2013. Clinical veterinary microbiology. 2nd Ed., Mosby Elsevier Publishing, 215-237.

NADRES reports. 2012. Project Directorate on Animal Disease Monitoring and Surevillance (PDADMAS), ICAR. Status of Enterotoxaemia in Andhra Pradesh, December 2012: 2-3

Naz, S., Ghuhan, M.A., Anjum, A.A., Manzoor, A.W., Rana, W. and Akther, R. 2012. Comparison of immune response following the administration of Enterotoxaemia vaccine in sheep and goats. *J. Vet. Anim. Sci.,* **2**: 89-94.

Nussey, D. H., Watt, K., Pilkington, J. G., Zamoyska, R. and McNeilly, T. N. 2012. Age-related variation in immunity in a wild mammal population. *Aging. Cell.,* **11**: 178–180.

Rosskopt – Streicher, U., Volkers, P., Noeske, K., and Werner, E. 2004. Quality assurance of *Clostridium perfringens* epsilon toxoid vaccines – ELISA versus mouse neutralization tests. *ALTEX,* Suppl **3**: 65-69.

Sharma, A.K. 2001. Standardization of I-ELISA for the detection of epsilon antitoxin M.V.Sc. thesis submitted to A.N.G.R.A.U., Hyderabad.

Snedecor, G.W. and Cochran, W.G. 1994. Statistical methods. 8th Ed., Iowa State University Press, Ames, Iowa, USA.

Srinivasan, E. V. R., Rafeek, S. M., Anbu, K. A., MeignaSundar, V., Krishna Murthy, A. and Rajendran, M. P. 2001. Studies on the immunological efficacy of a toxoid vaccine angainnst enterotoxaemia in sheep. *Indian Vet. J.,* **78**: 579-582.

Urguev, K. R. and Ataev, U. I. 1977. Efficacy of toxoids prepared with various adjuvants against infectious enterotoxaemia of sheep. *Veterinariia,* **4**: 48-51.

Uzal, F.A., Kelly, W.R., Thomas, R., Hornitzky, M. and Gulea, F. 2003. Comparisons of four techniques for the detection of *Clostridium perfringens* type D epsilon toxin in intestinal contents and other body fluids of sheep and goats. *J. Vet. Diag. Invest*., **15**:94-99.

Vijaya Lakshmi, S. 2003. Evaluation of immunodiagnostic kits to enterotoxaemia. M.V.Sc. Thesis submitted to A.N.G.R.A.U, Hyderabad.