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# Safety and immunogenicity of Bluetongue polyvalent vaccine in sheep and goats

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***Abstract:*** *Bluetongue (BT) is a non-contagious, arthropod transmitted viral disease of domestic and wild ruminants caused by bluetongue virus (BTV), the prototype member of the genusOrbivirusin the family Reoviridae. In India, the disease was first reported in Maharashtra in 1964. In South India, bluetongue disease occurs annually while sporadic outbreaks occur elsewhere in India. The immunization of susceptible sheep is the most effective and practical control measure against BT. In the present study, 25 sheep and 25 goats of either sex aged more than 4 months which tested negative for BTV antibodies were enrolled. In each species, twenty animals were vaccinated subcutaneously each with bluetongue polyvalent vaccine and five animals acted as controls. Vaccine group animals were administered a booster dose of vaccine on 28th day, while the control group animals received normal saline. Animals were observed for local or systemic reactions after vaccination as well as during the study period. Blood samples were collected on 28th day and 60th day post-vaccination for antibody response. The results revealed that the bluetongue polyvalent vaccine is safe with good immune response in both sheep and goats.*

***Keywords:*** *Immune response, Bluetongue polyvalent vaccine, cELISA, Serum Neutralization Test (SNT), sheep, goats*

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## I. Introduction

Bluetongue (BT) is a non-contagious, arthropod transmitted viral disease of domestic and wild ruminants caused by bluetongue virus (BTV), the prototype member of the genus*Orbivirus*in the family *Reoviridae*. The disease spreads through blood sucking midges of the genus *Culicoides*. Multiple serotypes circulate in each vector season with the occurrence of different serotypes depending largely on herd-immunity. It is basically a disease of sheep and young sheep within the age group of one year are more prone to infection. Goats, cattle and wild ruminants exhibit milder symptoms and may act as non-clinical carriers. The immunization of susceptible sheep remains the most effective and practical control measure against BT (Peter Coetzee *et al* 2012).[1]In India, the disease was first reported in Maharashtra (Sapre, 1964).[2] Later it was reported from different states. In South India, bluetongue (BT) disease occurs annually while sporadic outbreaks occur elsewhere in India. The losses are mainly due to high morbidity, mortality, abortions, stillbirths, fetal abnormalities and loss of milk, meat and fleece production. Because of the wide host range of BTV and its biological transmission by insects, control of BT in an endemic region is based primarily on the active immunization of susceptible animals (sheep) and amplifying hosts (goats) as well as on the prevention or limitation of contact between the susceptible host and insect vectors. Bluetongue polyvalent vaccine was developed by Indian Immunologicals Limited (IIL) in technical collaboration with Indian Council of Agricultural Research (ICAR) & Tamil Nadu Veterinary and Animal Sciences University (TANUVAS). ICAR funded All India Network Program on Bluetongue (AINPBT) has recommended bluetongue polyvalent inactivated vaccine by incorporating five BTV serotypes 1,2,10,16 and 23 which were isolated from India. Therefore, the present study was designed to evaluate safety and immunogenicity for the newly developed Bluetongue polyvalent vaccine in sheep and goats.

## II. Material And Methods

**Study Centre and Study Design:**

This study was conducted at College of Veterinary Science, P.V. Narasimha Rao Telangana Veterinary University (PVNRTVU), Hyderabad, India. A total of 25 healthy sheep and 25 healthy goats 25 in the age group of more than 4 months which were found seronegative for BT antibody by competitive ELISA were enrolled for the present study.

**Animal groups and vaccination protocols:**
The animals were grouped as follows:
Vaccine group sheep (group 1):
A Total of 25 sheep, negative for bluetongue antibody, of either sex aged more than 4 months were selected as per the inclusion and exclusion criteria. Twenty were vaccinated subcutaneously each with 2 ml of the vaccine.
Control group sheep (group 2):
Five sheep were given 2 ml of sterile normal saline subcutaneously and acted as controls.
Vaccine group goats (group 3):
Similarly 20 goats were used for vaccination. Each was given 2 ml vaccines subcutaneously.
Control group goats (group 4):
Five goats were given 2 ml of sterile normal saline to act as controls.
Prior to the study, all the experimental animals were dewormed as per the recommended doses.

**Administration of vaccine and placebo (normal saline):**

Bluetongue polyvalent vaccine manufactured by Indian Immunologicals Limited (IIL) was used for the study. The vaccine contains 5 inactivated BT virus serotypes - 1, 2, 10, 16 and 23.
The vaccine was administered in 2 ml volume by subcutaneous route to each of the 20 sheep in group 1 and 20 goats in group 3.

Each of five sheep in sheep control group (group 2) and 5 goats in goats control group (group 4) received 2 ml of sterile normal saline by subcutaneous route.

Vaccine groups 1 and 3 were given a booster dose of vaccine on 28th day. Controls were administered normal saline on 28th day.

**Biological sample collection:**

Blood samples were collected through jugular venipuncture with the help of plain vacutainer tubes (Beckton Dickinson, USA) from vaccinated and control group sheep and goats on 0, 28 and 60 days. About 10 ml blood was collected and serum was separated from each of them as per standard protocols for estimation of antibodies. The serum samples were stored at -200celsius till further analysis.

**Safety evaluation:**

The animals were monitored daily for local reactions like pain, swelling, rashes, skin eruption, sloughing of mucous membrane and redness at administration site, etc. and systemic reactions like fever, anorexia, diarrhea and restlessness and also for any serious adverse reactions (reactions which result in death, are life-threatening, result in significant disability or incapacity or which result in permanent or prolonged signs in the animals) at the time of inoculation of the vaccine and in the follow up period.

**Serological testing procedures:**

Sera samples collected on day 0, 28 and 60 were tested for immune response by group specific ELISA (Bluetongue virus antibody test kit, cELISA by VMRD, Pullman USA USDA Product Code 5010.20) as per the manufacturer's instructions.

Test sera were considered positive if they produced optical density less than 50% of the mean of the Negative controls. Test sera that produced an optical density greater than or equal to 50 % of the mean of the Negative controls were read as negative. When negative control mean OD value was between 0.3 to 2.0; the mean of the positive control must produce an OD less than or equal to 50% of the mean of the negative control.

The sera were also tested by Serum Neutralization Test (SNT) against all the 5 serotypes incorporated in the vaccine. SNT was conducted in BHK-21 cells. Neutralization was tested against 100 TCID50 of each of the serotypes (OIE 2012).[3] Serum titre of <6 was considered seronegative. Serum neutralization titres were expressed as SN50 values.

## *III.* Result& Discussion

**Safety evaluation:**

None of the experimental sheep showed any local or systemic reactions during the study period. However, two goats showed swelling at the site of inoculation: one for two days and another for five days. It may be because of individual variation in response to vaccine components like adjuvant and cell culture material. This is similar to the finding of Savini*et al.* (2007) who also found no adverse events except transient inflammatory reaction at the injection site in vaccinated sheep.[4]No serious adverse events were noticed in any of the experimental animals. This supports the use of vaccine safely in sheep and goats.

**Immunogenicity:**

**Immunogenicity evaluation by cELISA :**In the vaccinated sheep group (group 1), out of a total of 20 sheep one animal died before 28th day after first dose due to other causes. Of the remaining, the vaccine induced immune response in 18 out of 19 animals (95 %) on day 28 and 18 out of 19 animals (95 %) on day 60 i.e. after booster which was evident by cELISA results (Table 1). Percentage of seroconversion observed in vaccinated sheep is shown in Figure 1.

All the five control sheep group (group 2), animals remained seronegative till the end of the experiment. In the vaccinated goats group (group 3), out of a total of 20 goats two animals died before 28th day after first dose due to causes unrelated to vaccination. Of the remaining, the vaccine induced immune response in 94 % of the animals (17 out of 18) on day 28 and 94 % of animals (17 out of 18) on day 60PV, i.e. after booster which was evident by cELISA results (Table 1) and percentage of seroconversion observed in the vaccinated goats (Figure 1). All the five goats of control group (group 4) remained seronegative till the end of the experiment.

One sheep and 1 goat did not respond to the vaccination. It may be due to genetic variation of the individual animals and they may be poor responders (Hari Babu, 2003).[5]

**Table 1. cELISA results of the 28 and 60 days post vaccination (DPV) serum samples collected from sheep and goats vaccinated with two injections of bluetongue multivalent inactivated vaccine.**

| | **0th day** | **28DPV** | **60 DPV** |
|---|---|---|---|
| No. of sera Positive / No. of sera tested by cELISA (percentage) in sheep | 0/20 (0) | 18/19 (95) | 18/19 (95) |
| No. of sera Positive / No. of sera tested by cELISA (percentage) in goats | 0/20(0) | 17/18 (94) | 17/18 (94) |

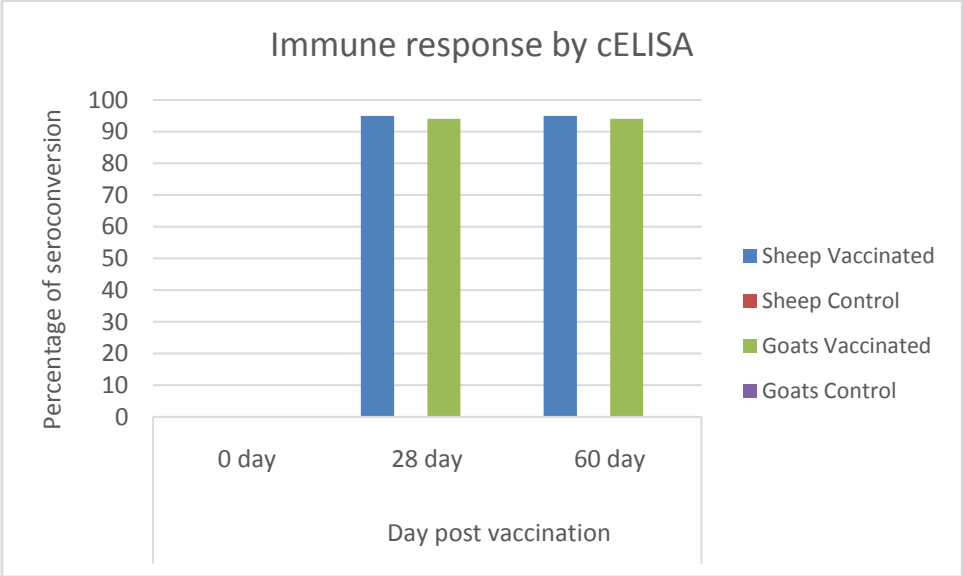


**Fig. 1. Percentage positivity for cELISA at different days after vaccination**

**Efficacy evaluation by SNT:**Mean $SN_{50}$titresin the vaccinated sheep rangedfrom 98.89 to 482.10 on day 28 and from 224.89 to 674.53 on day 60PV. All the sheep had shown good immune response to all the serotypes in the vaccine.Mean $SN_{50}$titres in the vaccinated goats ranged from 170 to 572.93 on 28 day PV and from 397.38 to 767.44 on 60 day PV. Controls remained seronegative. The vaccine induced good immune response in the goats against all the serotypes.Details are given in Table 2.

**Table 2. Immune response in experimental animals by SNT**

| Mean**SN Titres in vaccinated sheep against BTV serotype (GROUP 1: Vaccinated Sheep)** | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| BTV-1 | | | BTV-2 | | | BTV-10 | | BTV-16 | | | | BTV-23 | | |
| 0' day | 28th day | 60th day | 0' day | 28th day | 60th day | 0' day | 28th day | 60th day | 0' day | 28th day | 60th day | 0' day | 28th day | 60th day |
| 6 | 98.89 | 224.89 | 10.44 | 189.26 | 347.05 | 11.11 | 456.16 | 636.37 | 8.5 | 488.37 | 674.53 | 10 | 390.63 | 646.37 |

| **Mean SN Titres in vaccinated Goats against BTV serotype (GROUP 3: Vaccinated Goats)** | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| BTV-1 | | | BTV-2 | | | BTV-10 | | BTV-16 | | | | BTV-23 | | |
| 0' day | 28th day | 6oth day | 0' day | 28th day | 60th day | 0' day | 28th day | 60th day | 0' day | 28th day | 60th day | 0' day | 28th day | 60th day |
| 8.5 | 170 | 397.38 | 9.33 | 224.94 | 479.88 | 7.54 | 572.93 | 767.44 | 7.66 | 262.06 | 440.5 | 7.92 | 378.06 | 677.12 |

The results obtained are in agreement with the findings of Di Emidio*et al.* (2004) who found that all vaccinated animals developed virus neutralizing BT antibody titres 14 days after the booster dose which peaked on day 60 PV.[6] Similar finding was reported by Savini*et al.* (2007) who observed high titres of virus-specific neutralizing antibodies in sheep vaccinated with inactivated bluetongue vaccine.[4]

## IV. Conclusion

The inactivated polyvalent bluetongue vaccine manufactured by IIL was found to be safe as evidenced by absence of adverse reactions. It was also found to be immunogenic in eliciting adequate immune response in sheep and goats as indicated by immune responses both by cELISA and SNT assays in sheep and goats, hence can be advocated for immunizing sheep and goats in India to reduce mortality and morbidity due to bluetongue and improve productivity.

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