

## Field level serodiagnosis of acute phase ovine brucellosis by OIE prescribed tests

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**Abstract:** Brucellosis in sheep caused by bacterium, *Brucella ovis* is characterized by decreased reproductive efficiency and abortions. Farmers of a village from YSR district of Andhra Pradesh, India, reported the history of extensive abortions in Nellore ewes, uni-orchitis in breeding rams with joint infections, swelling of male genital tracts after crossing, and reluctance to cross. Blood samples were collected from affected animals for diagnosis by using world organisation for animal health (OIE) listed field level tests like Rose Bengal Plate Agglutination Test (RBT), and Standard Tube Agglutination Test (SAT) which are suitable for acute phase infection. Screening of all flocks and other species in contact including farmers in the village by using RBT, which was negative revealed the non-transmissible ovine brucellosis caused by *Brucella ovis*. The positive samples from affected sheep for RBT were subjected to SAT for confirmation. Those sheep, which were positive for brucellosis based on serological tests, were culled immediately as a part of standard control measures. It can be concluded that during the acute phase, ovine brucellosis can be preliminarily diagnosed by screening test RBT at field level and further can be confirmed by SAT in the field level laboratory.

**Key words;** Ovine brucellosis, *Brucella ovis*, OIE tests, RBT, SAT

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### I. Introduction

Ovine brucellosis which is characterized by abortions in ewes, and epididymitis, orchitis and reduced fertility in rams is due to the infection of *Brucella ovis*, a gram-negative bacterium which is non-spore forming, and non-motile coccobacilli [1]. *Brucella* organisms get colonize in the udder and supramammary lymph nodes in non-pregnant animals and invade placenta when the animal becomes pregnant, causing lesions in the organ walls leading to endometriosis, ulcerosa in the inter cotyledonary space and destruction of villi resulting in death and expulsion of the foetus [2]. The main route of entry for *brucella* is oral, by the ingestion of feed and water, which is contaminated with secretions or aborted foetal remains from infected sheep or by licking the vaginal secretions, genitals, aborted foetus or new born lambs of infected ewes. A ten-year sero-epidemiology study of brucellosis in India indicates, seropositivity of 18% in swine, 13% in cattle and buffaloes and 8% in sheep and goats. The same study also reported the statewide incidence of brucellosis with highest prevalence (21%) recorded in Orissa and Andhra Pradesh [3]. Diagnosis is based upon the isolation of microbial organism by various bacteriological methods from aborted material, milk or necropsy material [4], which require a biosafety laboratory. As an alternative, specific cell mediated or serological responses to *Brucella* antigens can be demonstrated [5]. The field level efficient diagnostic test must be cost-effective and rapid which is sensitive to detect infected animals. The present study deals with the diagnosis of brucellosis in affected ewes and rams using OIE listed tests like Rose Bengal Plate Agglutination Test (RBT), and Standard Tube Agglutination Test (SAT) which are highly sensitive during acute phase infection and are cost effective [4].

### II. Materials And Methods

#### 2.1 Geographical data

The affected flocks were from Tripuravarm village of Khajipet mandal of YSR District, Andhra Pradesh, India (geographical coordinates - 14°36'45.8"N 78°42'26.1"E)

#### 2.2 History of the infection

Farmers from eleven flocks of the village reported the history of abortions in ewes and orchitis with bloody discharges from genital organs after crossing and overall reluctance to cross in rams. The gross clinical investigation of the affected rams revealed epididymitis along with orchitis and abortions with retention of foetal membranes in ewes.

### 2.3 Sample collection

Serum samples were collected from all the affected animals after 21 days (Table 1). Samples were also collected from healthy rams, ewes and other species like bovines, caprines and humans those were in contact with the affected animals.

### 2.4 Processing and testing of sera samples

The serum samples were subjected to OIE prescribed tests of RBT and SAT for diagnosis, which are highly sensitive in acute phase of infection. The required antigens, rose bengal plate agglutination antigen and brucella plain antigen were procured from Indian Veterinary Research Institute (IVRI), Izatnagar, Uttar Pradesh, India.

**Table 1: Flock wise Animal details showing affected and apparently normal animals. Values in the parentheses indicate the number of sero-positive animals by RBT followed by SAT.**

Flock no.	Flock size	No. of affected animals (sero-positive)		No. of apparently normal animals (sero-positive)	
		Rams	Ewes	Rams	Ewes
1	152	8 (8)	77 (76)	5 (0)	62 (2)
2	110	5 (5)	49 (44)	4 (1)	52 (1)
3	148	7 (7)	42 (41)	5 (1)	94 (2)
4	115	4 (3)	46 (45)	3 (0)	62 (3)
5	145	5 (5)	51 (50)	6 (1)	83 (2)
6	155	6 (5)	35 (35)	7 (0)	107 (5)
7	123	5 (4)	21 (21)	3 (0)	94 (2)
8	165	8 (8)	73 (71)	6 (0)	78 (3)
9	105	4 (4)	34 (33)	2 (0)	65 (1)
10	110	5 (5)	25 (25)	6 (0)	74 (0)
11	110	4 (4)	27 (27)	8 (1)	71 (0)
<b>Total</b>	<b>1438</b>	<b>61(59)</b>	<b>480(468)</b>	<b>55(4)</b>	<b>842(21)</b>

### 2.5 Endpoints

The agglutination reaction in RBT was preliminarily considered positive for brucellosis. Samples positive for RBT were subjected to SAT for confirmatory diagnosis. The titre of more than 1 in 40 in SAT was considered a confirmatory field level diagnosis in sheep (Table 1).

## III. Results And Discussion

The clinical history of abortions, retention of foetal membranes, swollen testicles and upon physical examination, observations like epididymitis and orchitis were indicative of infection by *Brucella ovis* [1], which is substantiated by no evidence of infection in other species like caprines, bovines and humans indicating its non-zoonotic feature [1]. A total of 1438 sheep were screened. There were 116 rams of which 61 were affected and 55 were apparently healthy. Out of 1302 ewes, 480 were affected and 842 were apparently healthy. The initial screening test RBT was positive for all affected animals and few apparently healthy animals (4 out of 55 rams and 21 out of 842 ewes). All positive samples were re-examined using a more specific serological test like SAT. All the RBT positive samples were showing the SAT titres above 1 in 40 indicating a confirmatory positive result. The negative results of the other species including humans who were in contact with the affected sheep indicates non transmissible, non-zoonotic ovine brucellosis possibly caused by *Brucella ovis*. Healthy animals were separated from the diagnosed animals, which were culled according to the standard procedures. And disinfection of flock premises with hypochlorite was done to prevent further spread with in the species.

## IV. Conclusion

Testing and culling of breeding stock is essential to ensure brucella carriers are not present in the flock as antibiotic therapy is expensive, prolonged and ineffective. To detect positive cases in rams, palpation of testis is suggestive but not definitive. Clinical histories along with serological tests such as RBT & SAT are feasible and more reliable in field to screen sheep flocks to confirm brucellosis. In the present study a preliminary diagnosis was made by the clinical history and symptoms as Ovine Brucellosis in affected sheep flocks. The screening tests were positive in all the affected sheep and very few in contact sheep, whereas the humans and goats in contact were found to be negative indicating the infection by *Brucella ovis*. Acute phase ovine

brucellosis can be diagnosed at field level by using initial screening test, RBT and further can be confirmed by SAT, a more specific serological method.

### References

- [1]. OIE, *Terrestrial Manual, Chapter 2.7.9*, 2015, Ovine epididymitis.
- [2]. M.N. Xavier, T.A. Paixao, F.P. Poester, A.P. Lage, and R.L. Santos, Pathological, immunohistochemical and bacteriological study of tissues and milk of cows and fetuses experimentally infected with *Brucella abortus*, *Journal of Comparative Pathology*, 140 (2-3), 2009, 149-157.
- [3]. PD-ADMAS (Project Directorate on Animal Disease Monitoring and Surveillance), Hebbal, Bangalore, *Annual report*, 2011-12.
- [4]. WHO, WHO Library Cataloguing-in-Publication Data, *Brucellosis in humans and animals*, (WHO Press, Geneva, Switzerland, 2006)
- [5]. A. Robinson, *Guidelines for coordinated human and animal brucellosis surveillance*, (FAO, Agriculture department, Rome, 2003).