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Screening of sheep for brucellosis by indirect ELISA

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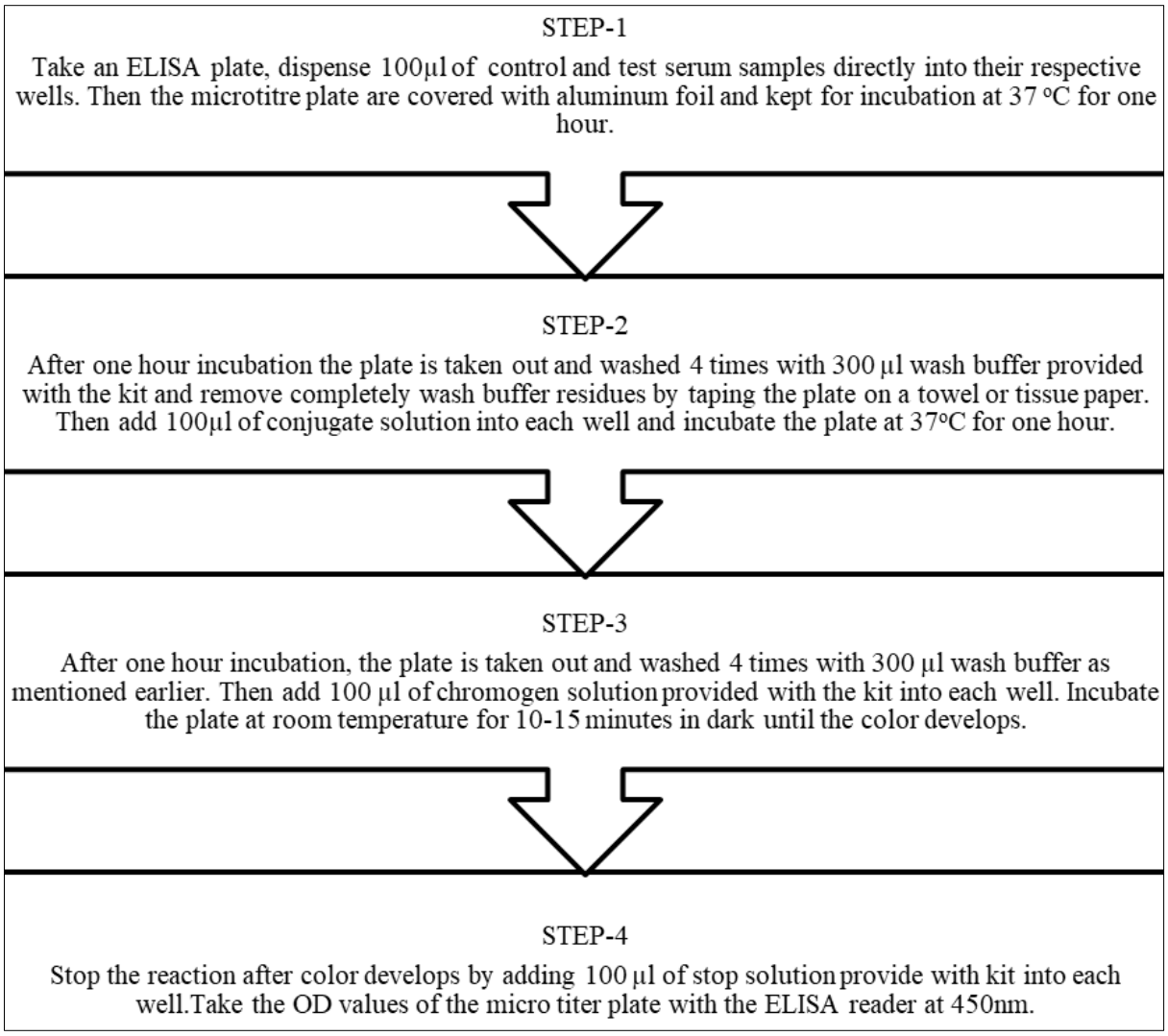
Abstract
To know the incidence of brucellosis in sheep of by Indirect ELISA. A total of 180 bloodsamples collected from apparently healthy sheep of Visakhapatnam District, Andhra Pradesh. Antibodies against *Brucella* were found by Indirect ELISA in 9 sera samples (5%) out of 180 sera samples tested. The results indicate that the incidence of brucellosis is lowsheep and the disease is more in ewes when compared with sheep, but regular screening should be done to minimize economic loss to the farmers and to avoid spread of disease to humans as it is a Zoonotic in nature.

Keywords: Screening, brucellosis, ELISA, serum samples, sheep

Introduction
India stands 3rd in world sheepproduction[1], which is reared as primary source of meat and wool. Andhra Pradesh stands first in sheep population in India. One of the important contagious diseases of sheep is brucellosis. Sheep brucellosis is mainly caused by *brucella melitensis* and rarely by *B. abortus* (Luchsinger and Anderson, 1979; Garin-Bastuji*et al.*, 1994) or *B. suis* (Paolicchi *et al.*, 1993) [2]. Sheep brucellosis is a zoonotic disease except brucellosis caused by *B. ovis*.. *Brucella* is a gram negative facultative intracellular organism.It occurs in small ruminants in Latin America, Southern Europe, Middle-east, Central Asia and Africa. Human can acquire brucellosis with close contact with infected animal secretions and carcasses or consumption of their milk and meat products [3, 4, 5].
In sheep, brucella causes abortions in last trimester [6], stillbirth, reduced fertility, decreased milk production and in humans it causes undulant fever, malaise, insomnia, arthralgia, sexual impotence, nervousness and depression [7]. Human brucellosis is also known for multiple organ involvement causing encephalitis, meningitis, endocarditis, arthritis and it can induce spontaneous abortions in pregnant women [8, 9]. Hence early screening, isolation of infected animals from flock is important to control the spread of the disease to humans. The specific confirmation of brucellosis requires laboratory diagnosis. There are many tests for screening of brucellosis which include RBPT, STAT and ELISA. Now a days ELISA is extensively used for screening of antibodies in milk and serum samples of small ruminants because of its economy, sensitivity, specificity, rapidity, reproducibility, and easy interpretation through colorimetric end product [10, 11, 12, 13, 14]. In the view of above, the present study was undertaken to screen the suspected sheep for presence of brucella antibodies in the serum samples by using Indirect ELISA.

Materials and Methods
Sample collection
The blood samples were carefully collected and packed, avoiding and possibility of leakage or cross-contamination. Individually identified containers were placed in large and strong outer containers and packed with enough absorbent material to protect from damage and packed in a cooler bag with ice packs and kept cool during transport from the place of collection to the laboratory as recommended in the OIE Manual (2000) [16]. About 2 ml of blood was aseptically collected from the sheep into vacutainer tubes (AcCuvet, Quantum Biologicals Pvt Ltd, Chennai) with Heparin. Further, 5 ml of blood was collected in a vacuette with serum clot activator (BD). The vacuettes were kept in upright position at room temperature for about 2 h. The separated sera was collected in a screw capped plastic vials and transported to the laboratory. The serum samples were heat inactivated at 56 ^{0}C for 30 min and merthiolate (1:10,000) was added in all vials as preservative. The sera and blood samples with anticoagulant were stored at -20 ^{0}C till further use [15].

Elisa procedure



Interpretation of result

Test results are based on the antibodies concentration present in the test serum samples. The antibody concentration of unknown sample is calculated by using a formula.

$$\text{SP RATIO} = \frac{\text{Sample OD} - \text{Negative OD}}{\text{Positive OD} - \text{Negative OD}}$$

The obtained SP ratios of 180 samples are compared with Established Standards.

Established Standards

Negative SP Ratio: 0 to 1, Equivocal SP Ratio: 0.1 to 0.24 and Positive SP Ratio is 0.25 and above.

Result	Interpretation
Negative	Indicates the absence of *Brucella* specific antibody detected in the test specimen
Equivocal	These samples should be repeated. Samples which are stand equivocal after repeating should be tested with other method or another sample should be collected and tested.
Positive	Indicates the presence of *Brucella*specific antibodies in the test specimen.

Statistical analysis

The data was analyzed using by using chi square test, significance of difference was determined and value of $p< 0.05$ was considered statistically significant in analysis of sex wise prevalence.

Results and Discussion

Screening of 180 sera samples by Indirect ELISA revealed the sero-prevelance of brucellosis was 5 % in the examined sheep (Table No.1). The chi square statistic is 5.9157; thep value is 0.015007, significant at $P< 0.05$.

Table 1: Presence of antibodies against *Brucella* in sheep

	Number of sera tested	Positive	Negative
Male	9	2	7
Female	171	7	164

The results of the present study are in accordance with the results of Shome *et al* (2015) [17] Table No.1: Presence of antibodies against *Brucella* in sheep [16], and reported the seroprevalence of brucellosis in sheep as 8.1% in Andhra Pradesh. Rahman *et al* (2011) [18] reported 2.31 % positivity in sheep of Bangladesh which is slightly lower the results of the present study [17]. Dashrath *et al* (2015) [19] reported 11.75% positivity by indirect ELISA in sheep belong to Banaskantha district of North-Gujarat which is higher [18].

Conclusions

As per results of the present study, it can be concluded that the sero-positivity of brucellosis in sheep is though low, regular sero-surveillance programmes should be taken up to avoid the spread of disease not only among sheep but also to shepherds and other human beings.

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