Screening of sheep for brucellosis by indirect ELISA

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Abstract
To know the incidence of brucellosis in sheep 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 sheep production1, 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) 15. 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 °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 °C till further use 16.

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**Elisa procedure**

| **STEP-1** | Take an ELISA plate, dispense 100µl of control and test serum samples directly into their respective wells. Then the microtitre plate are covered with aluminum foil and kept for incubation at 37 °C for one hour. |
| **STEP-2** | After one hour incubation the plate is taken out and washed 4 times with 300 µl wash buffer provided with the kit and remove completely wash buffer residues by taping the plate on a towel or tissue paper. Then add 100µl of conjugate solution into each well and incubate the plate at 37°C for one hour. |
| **STEP-3** | After one hour incubation, the plate is taken out and washed 4 times with 300 µl wash buffer as mentioned earlier. Then add 100 µl of chromogen solution provided with the kit into each well. Incubate the plate at room temperature for 10-15 minutes in dark until the color develops. |
| **STEP-4** | Stop the reaction after color develops by adding 100 µl of stop solution provide with kit into each well. Take the OD values of the micro titer plate with the ELISA reader at 450nm. |

**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.

\[
SP\ RATIO = \frac{Sample\ OD - Negative\ OD}{Positive\ OD - Negative\ OD}
\]

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
- Positive SP Ratio: 0.25 and above

**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-prevalence of brucellosis was 5% in the examined sheep (Table No.1). The chi square statistic is 5.9157; the p value is 0.015007, significant at P<0.05.

| Number of sera tested | Positive | Negative |
|-----------------------|----------|----------|
| Male                  | 2        | 7        |
| Female                | 171      | 7        |

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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