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Serodiagnosis of brucellosis in sheep

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Abstract
Brucellosis is an important global zoonotic disease affecting both domestic and wild animals. In the present study paired serum samples (50 in no) were purposively collected from a sheep flock with a history of late abortions, retention of placenta in females and orchitis in breeding rams in Proddatur region of Y.S.R Kadapa District, Andhra Pradesh. Initially, Rose Bengal plate test (RBPT) was carried out for screening and later positive sera samples were subjected to standard tube agglutination test (SAT) to estimate the titre of infection. Among fifty, twenty-two (42%) were positive for brucellosis. As a preventive measure, all the infected sheep were isolated and strict quarantine measures were followed to control the spread of infection.

Keywords: RBPT, SAT, Zoonotic disease

Introduction
Brucellosis is caused by bacteria belonging to the genus *Brucella*. It includes many species namely *B.melitensis*, *B.abortus*, *B.ovis*, *B. Suis*, *B.canis*, affecting various species of animals. (Cutler and Whatmore, 2003) [1]. It is also known as Malta fever, undulant fever, Mediterranean feveContract teaching faculty, Dept of Veterinary Microbiology C.V.SC Proddatur SVVUr and it is characterised by low grade undulant fever, night sweating, early fatigue, orchitis and joint pains leading to spondylitis. It imposes a major threat to both animals and humans. Economic loss in females is due to late term abortions, retained placenta, still birth and subsequent infertility (Alton 1988) [2], while in males it causes orchitis, epididymitis and infection of the accessory sex glands and hygroma. Other economic losses are due to loss of wool, skin, meat and international trade. Aborted foetal contents, uterine discharges, and excretions from infected animals act as source of infection to healthy animals and humans. Brucellosis is transmitted through ingestion of contaminated food and water, contact with infected materials and artificial insemination. Brucellosis in humans is considered as occupational disease of stockyard, butchers, slaughter house workers, and veterinarians (Walker, 1999) [3]. Diagnosis of brucellosis is carried out by isolation and identification, amplification of nucleic acid and detection of brucella specific antibodies. (Solara *et al*., 1997) [4]. Among the different diagnostic tools, serological methods like Rose Bengal Plate Agglutination Test (RBPT), standard tube agglutination test (SAT), complement fixation test, ELISA, milk ring test (MRG) are commonly used tests in the diagnosis of brucellosis (Lucero *et al.*, 2003) [5]. Rose bengal plate agglutination test is a rapid screening test in high risk rural areas and serum agglutination test (SAT) is the most popular worldwide diagnostic tool for brucella diagnosis because it is easy to perform and does not requires any training and specialized equipments to conduct. Serum agglutination test measures the total quantity of agglutinating antibodies IgM and IgG (Young, 1991) [6].

Material and Methods
Fifty paired serum samples were purposively collected from a sheep flock with a history of late abortions, retention of placenta in females and orchitis in breeding rams in Proddatur region of Y.S.R Kadapa District, Andhra Pradesh. Serum samples were also collected from shepherdwith general illness in serum vaccutainers. Later the serum was separated; RBPT and SAT were carried out according to standard protocol (WHO 2010 and OIE manual, 2000c) [7, 8].

Rose Bengal plate test
Rose Bengal plate test (RBPT) was carried out according to standard protocol (Stemshorn *et al*., 1984) [9]. On a glass slide 40 µL of Rose Bengal antigen (I.V.R.I., Izatnagar) was taken

initially and later 40 µL of test serum was added, mixed thoroughly and results were read immediately with in 4 min. Test samples with visible agglutination are taken as positive and without agglutination are negative for brucellosis. Based on degree of agglutination, Serum samples with high agglutination are highly positive (+++), moderate agglutination taken as positive (++), and slight agglutination are weakly positive or suspected (+).

Serum tube agglutination test (SAT)

Serum samples which were positive on RBPT were tested by SAT. It is performed according to standard protocol (WHO 2010 and OIE manual, 2000c) [7, 8]. Seven test tubes were taken and placed in test tube rack. About 0.8 ml of phenol saline was taken in first test tube and 0.5ml in remaining tubes. Later 0.2 ml of test serum sample was added to first tube, mixed properly and after proper mixing 0.5 ml of mixed contents from first tube was transferred to second tube and same process was repeated up to last tube. Finally 0.5ml of Brucella plain antigen (I.V.R.I., Izatnagar) was added equally to all test tubes, mixed properly and incubated at 37°C for 24 hrs. This provided a final dilution of 1:10, 1:20, 1:40, 1:80 and 1:160 and so on by mixing 0.5 ml antigen with 1.5 ml of 0.5 % phenol saline in an agglutination tube. After incubation interpretation is done by comparing antigen control tube showing 50% agglutination (0.5 ml of antigen and 0.5% phenol saline). Dilution which matches exactly with 50% antigen control tube is considered as titre of serum (i.e., 50% clearing). The titre thus obtained was expressed in unit system by doubling the serum titre as International Unit (I.U.) per ml of serum. A titre of 1:40 IU and above was considered as a positive for brucellosis (OIE, 2000c) [8].

Results and discussion

In the present study, diagnosis of brucellosis is carried out using RBPT and SAT. Among the 50, 22 are positive as shown in Table-1, 2, 3. In the present study results of RBPT and SAT were correlated with previous studies. Deepthi. et., al (2017) [10] and Samadhi *et al.* (2010) [11] reported a seroprevalence of 100% (25/25) and 48% (51/100) in aborted goat and sheep respectively where as in present study 80% (8/10), 75.0% (6/8), 22.2% (6/22) and 66.66% (2/3) positivity was reported in pregnant ewes, aborted ewes, non-pregnant ewes and rams with orchitis respectively.

Table 1: Samples size and Percentage of positivity per type of animal

S. NO	Type of animal	No. of samples Collected	% of positivity on RBPT& SAT(NO)
1	Pregnant ewes	10	80 % (8)
2	Non-Pregnant ewes	27	22.2% (6)
3	Rams with orchitis	3	66.66% (2)
4	Rams without orchitis	2	0% (0)
5	Aborted ewes	8	75.0% (6)

Table 2: Degree of agglutination and their percentage on RBPT.

S.N0	Degree of agglutination	Percentage (NO)
1	Highly positive	59.09% (13)
2	Positive	22.7% (5)
3	Weakly positive	18.0 % (4)

Table 3: SAT titres and their percentage

S.NO	SAT titres	Percentage (NO)
1	1:20	18.18% (4)
2	1:40	22.7% (5)
3	1:80	36.6% (8)
4	1:160	13.63% (3)
5	1:320& above	9.09% (2)

Conclusion

The present study reports an outbreak of brucellosis in sheep flock. Pregnant ewes reported with abortion and breeding rams with orchitis were positive for brucellosis on RBPT and SAT. It draws a conclusion, that breeding rams are the main source of infection to the ewes. Additional diagnostic techniques are required for further confirmation. Regular screening of suspected samples is necessary to eradicate the brucellosis and at the same time awareness should be created among the farmers for regular vaccination.

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