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Short Communication

SERODIAGNOSTIC APPROACHES TO DETECT BRUCELLOSIS IN GOATS IN AN OUTBREAK IN KADAPA DISTRICT, ANDHRA PRADESH

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The present study addresses an outbreak of brucellosis in a goat flock which showed a high abortion rate. Serum samples of the affected animals were screened by various serological tests viz. Rose Bengal plate test (RBPT), Serum agglutination test (SAT), Indirect enzyme linked immunosorbent assay (i-ELISA) and Lateral flow assay (LFA). Except LFA, all other tests proved effective. The present study shows the need for effective screening and control programmes to eradicate the disease in small ruminants.

Key words: Brucellosis, Goats, i-ELISA, LFA, RBPT, SAT

Goats in India are considered as poor man's cow. Marginal and below marginal farmers rear goats as a means of their livelihood and contribute to national economy through various products and by products. Caprine brucellosis is the major cause of abortion in goats and also accounts for large number of human brucellosis cases (Mantur and Amarnath, 2008 and Awad, 1998). The infection in small ruminants is wide spread in India (Isloor *et al.*, 1998).

Brucellosis is an infectious bacterial disease caused by the members of the genus *Brucella*. *Brucella melitensis* is a dominant causative agent of brucellosis in sheep and goats in many countries including India. Polding (1942) first reported the isolation of *B. melitensis* in goats. Three biotypes exist in *B. melitensis*. Among them *B. melitensis* biotype 1 is the most common type. In India, *B. melitensis* biotype 1 was isolated in the states of Karnataka, Andhra Pradesh, Maharasthra and Gujarat.

Brucellosis is a disease of the sexually matured animals with predilection for placentas, fetal fluids and testes of male animals. The disease is transmitted by direct or indirect contact with infected excreta (Verger and Plommet, 1985 and Radostits *et al.*, 2000). Abortion usually occurs in the fourth or fifth month of gestation. Clinical mastitis is common with other symptoms of un-thriftiness, bronchitis with hacking cough, lameness, pyrexia and hygroma and orchitis in males. The disease also has great significance because of its transmission to

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animal owners and consumers through direct contact and consumption of contaminated milk (Smits and Kadri, 2005). Diagnosis of Brucellosis is based on observations of clinical signs (abortions, infertility and orchitis) associated with positive serological tests (Praud *et al*., 2012).

An outbreak of brucellosis was recorded in goats of Osmanabadi breed in Kadapa district. To investigate the case, all the goat population in and around the affected region are screened for brucellosis by various diagnostic tests. There was a sudden surge of abortions among does of Osmanabadi breed under age group of 1.5 years during the last trimester of gestation in a goat flock of about 32 animals. This outbreak lasted for two weeks and a total of 25 pregnant does in the farm at the time of outbreak were affected. The buck used for breeding also exhibited typical orchitis.

Paired serum samples were collected from all the aborted and normal does and the buck aseptically. No single gold standard test is currently available to be used for routine diagnosis of brucellosis. Hence, each serum sample was subjected to four serological tests: Rose Bengal plate test (RBPT), Serum agglutination test (SAT), Indirect enzyme linked immunosorbent assay (i- ELISA) and Lateral flow assay (LFA).

Rose Bengal plate test (RBPT) was carried out according to standard protocol (Stemshorn *et al*., 1985). Rose Bengal antigen was obtained from National Institute of Veterinary Epidemiology and Disease Informatics, Bangalore. Antigen and serum samples at room temperature are mixed in equal volumes (25-30 µL), agitated gently and any visible agglutination reaction should be considered positive after 4 minutes period.

Serum samples considered positive by RBPT were tested by SAT (OIE, 2011) and Brucella plain antigen was obtained from Indian Veterinary Research Institute, Izatnagar, Uttar Pradesh. Serum samples were serially diluted from 1:10 to 1:5120, incubated at 37°C for 18-24 hrs. A titre of 1:40 IU and above was considered as a positive reactor.

For detection of anti-brucella antibodies, i-ELISA kit was obtained from Genomix Molecular Diagnostics Pvt. Ltd., Hyderabad and the procedure was followed as per the manufacturer's protocol. A primary dilution of 1/100 of all the test and control serum samples was prepared by adding 2µL of serum to 198 µL of sample diluting buffer. Each serum sample (100 µL) was dispensed into each well of the antigen coated plate. Every sample was tested in duplicate. The plates were incubated at 37° C for one hour. After the incubation period, the plates were washed 4 times with 300 µL wash buffer provided with the kit. The conjugate solution (100 µL) was added into each well and incubated at 37° C for one hour. The plates were washed once again (4 times) with 300 µL wash buffer. o-Phenylene diamine dihydrochloride (OPD) solution was freshly prepared (1mg/1 mL of PBS) and substrate

(30% H_2O_2) was added just before dispensing OPD to the wells. The plate was incubated in dark at room temperature for 10-15 minutes until the colour developed. The reaction was stopped by adding 100 µL of 1M H_2SO_4 provided with the kit and the OD values were read at 492 nm using Multiskan Go (Thermo Scientific).

Lateral flow immune chromatographic assay is a rapid antibody detection test kit obtained from Genomix Molecular Diagnostics Pvt. Ltd., Hyderabad and the procedure was followed as per the manufacturers' protocol. The testing device was placed on a level surface. The sample (5 µL) and 2 drops of sample diluent buffer provided with the kit were placed in the sample well. Appearance of purple coloured band both at the test and control slots indicated positive test.

The abortion rate in the affected flock recorded was 78% (25/31). All the aborted does (25), normal does (2) and the buck were positive by RBPT. All the samples were further tested by SAT, i-ELISA and LFA. Six of the samples positive by RBPT were diagnosed negative by SAT and i-ELISA. SAT positive samples revealed very high titres (1:160 to 1:640). i-ELISA positive samples also showed high OD readings (1.340 to 1.650) in comparison to negative controls (0.2). LFA kit used in the study could not detect all the positive samples and cannot be recommended as a screening test. LFA, a simple qualitative test has been used since long time in detecting a wide variety of infections but with low sensitivity warranting the need to confirm with another serological test. Among the four serological tests, i-ELISA was the most sensitive and specific diagnostic test. The incidence of brucellosis in the village was recorded as 11% (30/270).

Recently, commercial goat farming in India has picked up at a fast pace. These goat flocks are serving as nuclei for several infectious diseases including brucellosis. Brucellosis is a disease of economic importance as it adversely affects reproduction and productive potential of affected animals in terms of loss of kids, infertility as well as reduction or complete loss of milk yield. Unlike in cattle, there is no specific control programme for brucellosis in sheep and goat. Mixed farming and contact between several herds will increase the chance for disease transmission to susceptible animals (Al-Majali, 2005).

The present study reports an outbreak of brucellosis in goats in a private farm. The abortion percentage was very high indicating rapid transmission of infecting organisms from one animal to the other. The buck used for natural service of the herd was also a positive reactor and might have led to the infection from served contaminated does. The buck suffered severe orchitis reflecting the seriousness of the disease. One of the most prominent clinical sign of goats affected with brucella is abortion of dead or weak fetus usually in the last trimester (Acha and Szyfres, 2003) which was in accordance to the present

finding. Other signs include stillbirths, decreased fertility, and low milk production. There was an estimated reduction up to 25% in milk and the organisms localized in the supra mammary lymph nodes and mammary glands.

Food products of sheep and goats are one of the sources of human infections. Transmission may occur when humans come in contact with infected animals or by consumption of milk and milk products like cheese and rennet from unpasteurized milk of sheep and goat (Seleem *et al.*, 2010). Hence, routine surveillance of goat flocks is essential to eradicate the disease. At the same time, the farmers should be convinced and educated enough to cull the infected animals from the flock, a task which is a bit difficult. *B. melitensis* strain Rev1 vaccine has proven to be very useful in controlling brucellosis in small ruminants. Hence the authors advocate the need to conduct exhaustive epidemiological surveys in the state to identify the infected areas and plan a mass vaccination campaign to effectively control brucellosis in small ruminants.

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