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Seroprevalence of Brucellosis in Goats and Sheeps in Different Regions of India, using *B. melitensis* Soluble Antigen in Plate-ELISA

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ABSTRACT

In present study, we investigated 50 serum samples of goats from CIRG, Makhdoom and 470 serum samples of sheep from an organized animal shed of Northern India. These samples were tested for detection of anti-Brucella antibodies in goats and sheep using plate-ELISA. At total of 24% serum samples (24.4% females and 20% males) from CIRG, Makhdoom and 39.4% serum samples (41.2% females and 20% males) from an organized shed of Northern India were found seropositive respectively. This data indicate a high prevalence of disease in Northen India followed my CIRG, Makhdoom. This data also indicate that females are highly susceptible to infection than males.

Keywords: *Seroprevalence brucellosis, goats, sheeps*

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1. INTRODUCTION

Brucellosis has been recognized in Latin America since the early 1900, and it continues to be one of the most prevalent zoonosis in the region [1]. It causes severe economic losses by way of abortion and still births besides potentially hazardous to handlers. Unequivocal definitive diagnosis is made by bacteriological identification of the causative agent [2].

However, this procedure is time consuming, expensive and weakly sensitive. Control eradication programmers are then generally based partly or totally on serology, and then success largely depends on the accuracy and reliability of the tests employed, Numerous serological tests have been described [2–4].

Singh *et al*. (1994) reported 13.4% kids due to abortion and stillbirth in organized goat herds. Screening of these herds by SAT detected only 0.6% goats as positive reactors. Use of species-specific reagents in diagnostic tests increases the sensitivity of the detection. Among the newer assays for serological diagnosis of brucellosis, the plate-ELISA has become increasingly popular as it is less complex, more time saving and sensitive procedure and also overcomes many of the shortcomings encountered in other assays like SAT, RBPT, and CFT employed for routine diagnosis of brucellosis in animals [5, 6].

We use *Brucella melitensis* 16M antigens, because these antigens are twenty times more dominant than 'A' antigen which is dominant in *B. abortus.* Serology was performed using ELISA. For ELISA, *B. melitensis* 16M extract prepared by ultrasonication was used as antigen [7–9].

2. MATERIAL AND METHODS

Serum samples of goats and sheep's were collected from the animals shed of Animals Health Division, CIRG, Makhdoom and an organized shed of Northern India. A total of 50 serum samples of goats were collected from CIRG Makdoom, out of which 45 are females and 5 are males. A total 470 serum samples of sheep's were collected from an organized shed of Northern India, out of which 430 are females and 40 are males.

Soluble antigens were prepared as per the method of *Gupta et al*. [10]. Logarithmic phase of *B. melitensis* 16M were sedimented by centrifugation at 10,000 rpm for 10 min at 4ºC, washed twice with normal saline solution and resuspended in 20 mL NSS. The suspended culture was treated with ultrasonic waves in an ultrasonicator for 10 cycles, centrifuged at 10,000 rpm for 10 min at 4 ºC. Cell debris was removed and supernatant were collected, concentrated in vacuum concentrator, stored at 4 ºC. Protein concentration in the antigen was estimated by Lowry's method using Folin-Ciocalteu's phenol reagent [11].

ELISA was performed as per the method of Engvall and Pearlman (1971) [7]. The wells of polystyrene plates coated overnight at 4 ºC with *B. melitensis* antigens (50 µl per well) at a final conc. of 20 µg /mL in 0.2 M coating buffer (carbonate–bicarbonate buffer). After washing three times with PBST, the uncoated sites of the wells were blocked by adding 100 µl of blocking buffer, plates were incubated at 37 ºC for 1 h. Nonadsorbed material was removed by three washing with 0.05% Tween-20 PBS. Serum dilution (1:50) were made in dilution buffer (1% BSA-PBST), and 50 µl of individual serum sample was taken into the plates in duplicate wells and incubated at 37 ºC for 2 h. The serum from nonimmunized goats were used as negative control. After washing three times, 50 µl per well of 10,000 fold diluted rabbit HRPO anti-goat immunoglobulin G conjugate was added to the polystyrene plates. After 1 h incubation at 37 ºC, plates were gently washed three times with PBST. Finally 100 µl per well of substrate buffer (phosphate-citrate buffer) containing 1.5 ml of 30% hydrogen peroxide per litre was added to monitor the peroxidase activity. The reaction was stopped after 20 min by addition of 2 M H_2SO_4 and the optical density was measured at 450 nm. The color development is directly proportional to the bounded antigen-antibody complex.

3. RESULTS AND DISCUSSION

Out of 45 females and 5 male goat samples collected from CIRG, Makhdoom 11 females and 01 male were found seropositive respectively, against *B. melitensis*. It indicates that 24.4% females and 20% males were found seropositive in ELISA, when *B. melitensis* antigen was used.

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Out of 430 females and 40 male sheep samples collected from an organized shed of Northern India, 177 females and 08 males were found seropositive respectively, against *B. melitensis*. It indicates that 41.2% females and 20% males were found seropositive in ELISA, when *B. melitensis* antigen was used. In ELISA mean O.D was taken, and it was compared by known positive control. The hyperimmune sera were collected from goats which were by injected with a killed *B. melitensis* with complete adjuvant (Figures 1 and 2, Table I).

The serum samples were collected from those herds in which most of the animals having history of abortions. *B. melitensis* uses the sheep and goats as its preferred natural hosts but other animals may also be infected. It is an important disease because it undermines animal health and productivity. It is transmitted from animal reservoirs, wastes, or their products. In domestic animals, brucellosis is mainly an abortive disease that results from a long lasting after unapparent infection. *B. melitensis* (biovar 1,2,3) is the main causative agent of Caprine and Ovine brucellosis, causes abortion during the third trimester of pregnancy.

Following an abortion the infected female excretes copious amount of bacteria in uterine exudates and milk. The antibodies against *B. melitensis* were detected in plate-ELISA and it was detected in the milk samples by PCR, elsewhere including India [9, 12].

Table I: *Showing Positive and Negative Serum Samples of Goats and Sheep's Collected from CIRG and Northern India, respectively.*

S.No.	Animals	Sex	Region/ State	No. of samples	Positive cases	Negative cases
1.	Goats	Females	CIRG, Makhdoom (U.P)	45	11	34
2.	Goats	Males	CIRG, Makhdoom (U.P)	05	01	04
3.	Sheep	Females	Organized farm of Northern India	430	177	253
4.	Sheep	Males	Organized farm of Northern India	40	08	32

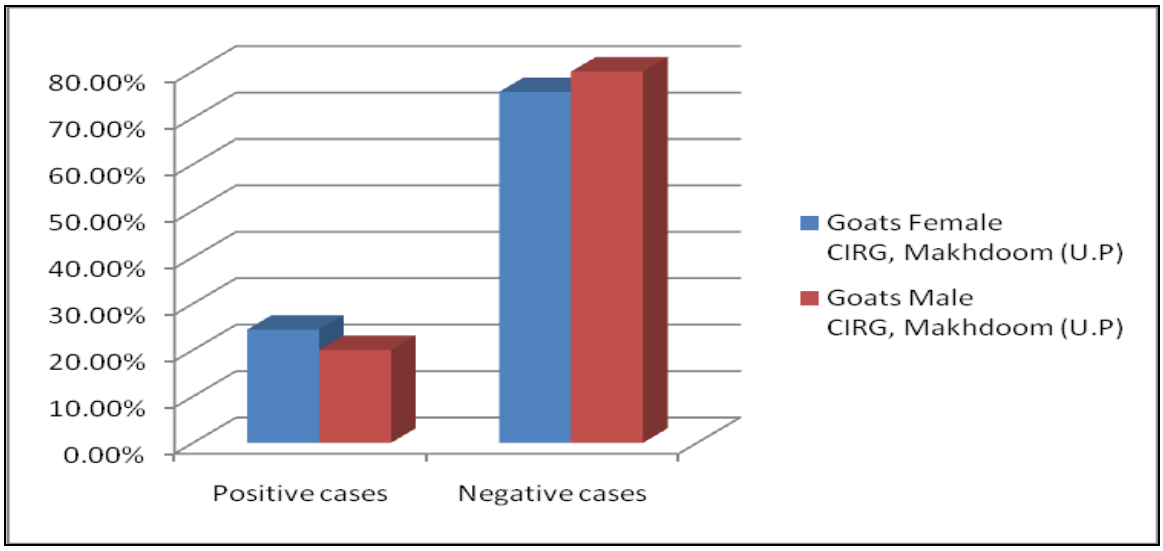

Fig 1: *Data Showing Positive and Negative Serum Samples of Female and Male Goats.*

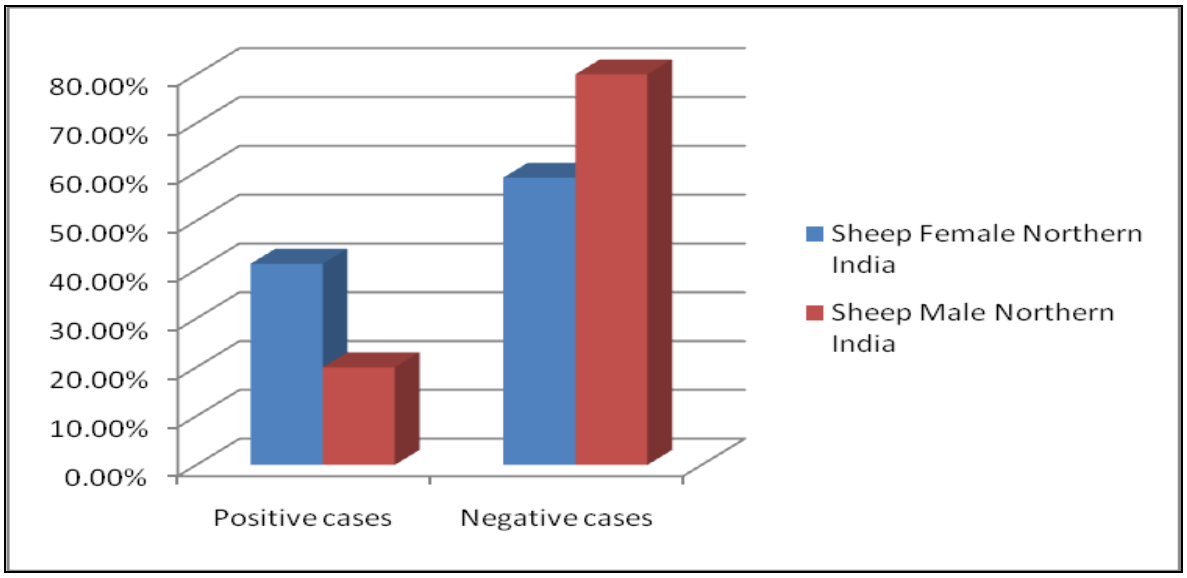

Fig 2: *Data Showing Positive and Negative Serum Samples of Female and Male Sheeps.*

4. CONCLUSION

The present study indicates that comparatively females are more susceptible to *B. melitensis* infection than males. In this study data indicates a high prevalence of disease in Northen India followed my CIRG, Makhdoom. We recommend that before organizing the animals in a shed they should be tested for *anti-Brucella* antibodies in their serum by using plate-ELISA to avoid the transmission of the disease from infected animals to the healthy one.

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