

Original Research Article

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## Serodetection and Bacteriological Study of Brucellosis in Goats

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### ABSTRACT

The present study was conducted with the aim to record seroprevalence and microbiological studies in serum and milk samples and vaginal swabs respectively, of brucellosis in goats. A total of 110 serum samples, 13 milk samples and 50 vaginal swabs were collected randomly from various organized and unorganized goat farms, regardless of breed and age (1 year-9 years) of goats. Out of total 110 serum samples examined, 06 (05.45%) and 04 (03.63%) samples were found to be positive by Rose Bengal Plate Test and Standard Tube Agglutination Test, respectively. Out of 13 milk samples 04 (30.76%) were found positive by Milk Ring Test. Vaginal swabs were inoculated for isolation and identification of *Brucella* organisms in selective media. Three isolates (06.66%) were obtained and confirmed as *Brucella* spp by morphological and biochemical characters. The results show the occurrence of brucellosis in the studied area and are of public health significance.

#### Keywords

ABST, Brucella, MRT, isolation, RBPT, STAT

#### Article Info

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### Introduction

Brucellosis is a chronic infectious disease of livestock, rodents, marine animals and human beings and is caused by facultative intracellular coccobacilli of genus *Brucella*. Caprine brucellosis caused by *Brucella melitensis* is most pathogenic for humans and the second most important zoonotic disease after rabies (Saxena *et al.*, 2018). In goats clinically the disease is characterised by

chronic infections leading to abortion, retention of placenta, metritis, subclinical mastitis, hygroma, orchitis, epididymitis and infertility or sterility forcing the owners to cull the animals and thus poses a serious threat to the livestock economy (Gogoi *et al.*, 2017).

The conventional assays such as Rose Bengal Plate Test (RBPT), Milk Ring Test (MRT), Serum Agglutination Test (SAT), indirect Enzyme Linked Immunosorbent Assay

(iELISA) etc. is widely used in various combinations for the diagnosis of brucellosis. However, as unequivocal diagnosis is by bacteriological identifications of the causative agent, for confirmation of brucellosis, isolation and culture of *Brucella* organisms is the gold standard test (Saxena *et al.*, 2018).

The present study was carried out to determine the prevalence of *Brucella* antibodies in goats and isolation, identification and biochemical characterization of organisms from goats having a history of abortion and vaginal discharge.

## **Materials and Methods**

### **Samples**

The samples were collected randomly from female goats regardless of breed and age (1 year- 9 years) belonging to various organized and unorganized goat farms, in and around Jabalpur, Madhya Pradesh, India. Approximately 5 ml of blood was collected aseptically from jugular vein of 50 goats with history of abortion (20) and vaginal discharge (30) whereas, 60 samples were collected randomly without any history of reproductive disorder and serum was separated from these blood samples. All the collected serum samples were tested for *Brucella* antibodies by RBPT and STAT. For MRT, milk samples were collected aseptically from 13 goats with a history of abortion. Vaginal swabs from 50 live goats with the history of abortion (20) and vaginal discharge (30) were collected aseptically using transport swabs in Amies medium with charcoal (HiMedia) and processed for bacteriological isolation.

### **Rose Bengal Plate Test**

The RBPT antigen was procured from Biological Products Division, Indian Veterinary Research Institute, Izatnagar.

Twenty five microlitre of serum was taken on a glass slide. Twenty five microlitre of RBPT coloured antigen was added. The antigen and serum were mixed thoroughly using sterile toothpick and the slide was rotated for four minutes and the results were read immediately. Definite visible clumping was considered as positive, whereas no clumping/ agglutination was considered as negative (Singh *et al.*, 2016).

### **Standard Tube Agglutination Test**

The plain *Brucella abortus* Serum Agglutination Test (SAT) antigen was procured from Biological Products Division, Indian Veterinary Research Institute (IVRI), Izatnagar. Serial dilution upto ten tubes were made with 0.5% phenol saline and eleventh tube was kept as control without adding serum and the tubes were placed in a rack. The tube rack was also shaken manually and was incubated at 37°C for 24 hours. Positive reaction was determined by observing a clear supernatant with matt formation at the bottom. Negative reaction was determined by turbid supernatant with button formation at the bottom. The highest dilution showing agglutination was considered as end titre. Tubes showing agglutination at 1:20 dilution and above or antibody titre of 40 I.U. and above were considered as positive.

### **Milk Ring Test**

Abortus Bang Ring / MRT coloured antigen was obtained from Biological Products Division, Indian Veterinary Research Institute (IVRI), Izatnagar. One ml of milk was taken in two test tubes each and 30-40 µl (1-2 drops) of antigen was added to one tube and mixed well. The tubes were incubated at 37°C for 1 hour (Khan *et al.*, 2018). Milk was completely white and cream layer was coloured - high positive (+++). Milk was slightly coloured and cream layer was distinctly coloured- positive

(++). Milk was distinctly coloured and cream layer was white- negative.

### **Bacterial isolation, identification and biochemical characterization**

Each swab was separately inoculated into *Brucella* broth aseptically for enrichment of the organisms and incubated at 37°C for 24 hours. The broth cultures were streaked on *Brucella* Agar Medium (BAM) plates (with Hemin and Vitamin K) with 5 per cent v/v sterile inactivated horse serum (HiMedia) and rehydrated contents of *Brucella* selective supplement in duplicate. One plate was incubated aerobically in incubator at 37°C and the other plate was incubated at 37°C under 5 per cent CO<sub>2</sub> in an anaerobic candle jar and incubated at 37°C for minimum of 72-96 hours (03-04 days). The plates were observed at every 24 hours for the growth. The suspected colonies of *Brucella* were picked from *Brucella* agar medium and were further inoculated on MacConkey agar and blood agar and incubated at 37°C for 72 hours for their cultural characteristics. The organisms were identified by colony morphology like colour, shape, size, surface, edges.

The isolates were confirmed by staining characters with Gram's and modified Ziehl-Neelsen (MZN) staining and agglutination with *Brucella abortus* antiserum biochemical tests like catalase, oxidase, urease, nitrate reduction and growth in the presence of dyes {(thionin 20µg/ml (1:50,000) and basic fuchsin 20µg/ml (1:50,000)} (Alton *et al.*, 1988).

The isolates were tested for their sensitivity pattern against 07 antibiotics. Antibiotics discs (HiMedia): Rifampicin (RIF, 30 mcg), Ceftriaxone (CTR, 30 mcg), Enrofloxacin (EX, 10 mcg), Gentamicin (GEN, 10 mcg), Streptomycin (S, 10 mcg), Oxytetracycline (O, 30 mcg) and Penicillin (P, 10 units) were used.

## **Results and Discussion**

### **Seroprevalence of *Brucella* antibodies in serum and milk samples**

The prevalence of *Brucella* antibodies in female goats was found to be 5.45% (06/110) comprising of 20% (4/20) cases of abortion and 06.6% (2/30) cases of vaginal discharge by RBPT, 3.63% (04/110) comprising of 15.00% (3/20) cases of abortion and 03.33% (01/30) cases of vaginal discharge by STAT and 30.76% (04/13) cases of abortion by MRT. Four samples were positive by both RBPT and STAT. Three of the STAT positive samples showed titres of 40 I.U/ml at 1:20 dilution and one sample showed a titre of 80 I.U/ml at 1:40 dilution.

The prevalence was found to be higher in goats belonging to the age group of 3-5 years (Table 1) and goats of unorganized farms as compared to the organized farms (Table 2).

Highest percentage of *Brucella abortus* positive reactors was observed among the goats having a history of second day to two months post abortion (Table 3).

### **Bacterial isolation from vaginal swabs**

A total of 03 isolates (02- abortion cases, 01- vaginal discharge) were obtained from 50 vaginal swabs collected and were confirmed as *Brucella* spp.

### **Identification of *Brucella* spp**

All the isolates were identified as *Brucella* spp based on colony morphology with typical characteristics as small, pinpoint, glistening, smooth and circular colonies on *Brucella* agar with hemin and vitamin K1, no growth on MacConkey agar and non-hemolytic colonies on blood agar. The organisms were able to grow aerobically without CO<sub>2</sub>.

Smears from all the isolates revealed pale red coloured coccobacilli by Gram’s staining and pink coloured coccobacilli by MZN staining. The isolates showed moderate agglutination with *Brucella abortus* antiserum. The isolates were positive for catalase, oxidase, urease, nitrate reduction and growth on Tryptic Soya agar with thionin and basic fuchsin dyes 20 µg/ml concentrations as listed in table 4.

In the present study all the isolates were variably sensitivity to antimicrobial drugs tested. All the isolates (100%) were sensitive to rifampicin, ceftriaxone and enrofloxacin whereas all the isolates (100%) were resistant to oxytetracyclin and penicillin. While 02 isolates (66.66%) were sensitive and 01 isolate (33.33%) were resistant to gentamicin and 01 (33.33%) of the isolates were sensitive whereas 02 isolates (66.66%) were resistant to streptomycin.

The findings in the present study by RBPT has close association with the findings of Priya *et al.*, (2010), Akbarmehr and Ghiyamirad (2011) and Reddy *et al.*, (2014) as they reported 05.70%, 05.00% and 05.15% prevalence among goats. A higher prevalence was reported by Aworh *et al.*, (2017) as 19.60% from Nigeria, Albert *et al.*, (2018) as 14.80%, by Saxena *et al.*, (2017) as 07.30% from Punjab and by Kanani *et al.*, (2018) as 15.99% from Gujarat.

The prevalence of *Brucella* antibodies by STAT (03.63%) observed in the present study is corroborated with the findings of Sharma *et al.*, (2017) who reported a prevalence of 03.42% by STAT in goats in and around border areas of Jammu, India. Higher prevalence of brucellosis in goats by STAT as 06.53% from Punjab by Saxena *et al.*, (2017), as 10% from Gujarat by Padher *et al.*, (2017) 05.86% from Sudan by Mohamed *et al.*, (2018) and 04.45% from China by Rahman *et al.*, (2019). Lower prevalence rates, 02.00% and 01.45% was reported by Uddin *et al.*, (2007) and Gogoi *et al.*, (2017), respectively from Bangladesh and Assam.

A lower prevalence was observed in goat milk as 26.00%, 09.16%, 06.00%, 11.50% and 11.67% by Kaltungo *et al.*, (2013), Najum (2014), Khan *et al.*, (2018), Al – Mashhadany (2018) and Rahman *et al.*, (2019) respectively by MRT.

Of the 110 goat serum samples, 06 (05.45%) were found positive by RBPT, of these 04 (03.63%) were also positive by STAT. More number of samples was positive by RBPT than STAT. However as compared to RBPT, STAT is more reliable and accurate as it involves serial dilution which gives both qualitative and quantitative results about the titre of the antibodies against *Brucella abortus* SAT antigen (Din *et al.*, 2013).

**Table.1** Seroprevalence of *Brucella* antibodies in goats of different age groups

Age group	Serum					Milk		
	No. collected	RBPT		STAT		No. Collected	MRT	
		No. positive	Per cent prevalence	No. positive	Per cent prevalence		No. positive	Per cent prevalence
1-2 yrs	61	02	03.27	01	01.63	09	02	22.22
3-5 yrs	41	04	09.75	03	07.30	03	02	66.66
6-9 yrs	08	00	00.00	00	00.00	01	00	00.00

**Table.2** Sero-prevalence of *Brucella* antibodies in goats of organized and Unorganized goat farms

Farm types	Serum					Milk		
	No. collected	RBPT		STAT		MRT		
		No. Positive	Per cent prevalence	No. positive	Per cent prevalence	No. collected	No. positive	Per cent prevalence
<b>Organized</b>	40	01	02.50	01	02.50	04	01	25.00
<b>Unorganized</b>	70	05	07.14	03	04.28	09	03	44.44

**Table.3** Reproductive disorders in positive reactors

Reproductive disorder	Serum					Milk		
	No. collected	RBPT		STAT		MRT		
		No. Positive	Per cent	No. positive	Per cent	No. collected	No. positive	Per cent
<b>Abortion</b>	20	04	20.00	03	15.00	13	04	30.76
<b>Vaginal discharge</b>	30	02	06.66	01	03.33	00	00	00.00

**Table.4** Cultural, biochemical and serological characteristics of isolates

S. No.	Name of the test	Isolates							
		L1	L2	L3	U1	U2	U3	F	
<b>01.</b>	CO <sub>2</sub> requirement	-	-	-	-	-	-	-	-
<b>02.</b>	Growth on MacConkey	-	-	-	-	-	-	-	-
<b>03.</b>	Hemolysis on blood agar	NH	NH	NH	NH	NH	NH	NH	NH
<b>04.</b>	Agglutination with positive serum	M	M	M	M	M	M	M	M
<b>05.</b>	Catalase test	+	+	+	+	+	+	+	+
<b>06.</b>	Oxidase test	+	+	+	+	+	+	+	+
<b>07.</b>	Urease test	+	+	+	+	+	+	+	+
<b>08.</b>	Nitrate reduction test	+	+	+	+	+	+	+	+
<b>09.</b>	Methyl Red	-	-	-	-	-	-	-	-
<b>10.</b>	Voges-Proskauer	-	-	-	-	-	-	-	-
<b>11.</b>	Indole production	-	-	-	-	-	-	-	-
<b>12.</b>	H <sub>2</sub> S production	-	-	-	-	-	-	-	-

+= Positive; - = Negative; NH= Non hemolytic colonies; M= Moderate agglutination

Isolates L1, L2, L3- vaginal swabs from live animals; U1, U2, U3- uterine swabs; F- stomach content swab from foetus.

Higher prevalence of the disease as detected by MRT, could be due to less number of samples screened or because of false positive reactions that can occur in samples containing abnormal milk (such as colostrum) as the samples were collected from goats after abortion or infection with other cross reacting bacteria like *Yersinia enterocolitica*, *Escherichia coli*, *Francisella tularensis*, *Salmonella* etc. (Albert *et al.*, 2018).

A higher prevalence of *Brucella* antibodies in goats was observed in unorganized farms as compared to organized farms. This could be due to better managerial practices like routine screening of goats for brucellosis in organized farms.

The present study showed that goats of higher age group are more affected than young goats. With advancing age the reproductive organs are more functional, hence, adult goats are more susceptible to brucellosis. Tegegn *et al.*, (2016) stated that brucellosis is mainly a disease of sexually mature and pregnant animals which, may be due to the presence of erythritol that stimulates the growth and multiplication of *Brucella* organisms.

The present findings are in agreement with Radostits *et al.*, (2007) and Jones *et al.*, (1997) who stated that with time duration of pregnancy, production of erythritol increases, which promotes the growth of *Brucella* organisms as a result there is increased level of antibodies in the serum. Moreover, proliferation of organisms in trophoblasts leads to placentitis, infection of the foetus and abortion in the late stage of pregnancy.

The 03 isolates obtained from different samples in the present study were confirmed as *Brucella* species by cultural, morphological and biochemical tests similar to earlier reports by Alton *et al.*, (1988) and Koneman *et al.*, (1997). Babuhai (2012) also obtained 07

isolates (02 from vaginal swabs and 05 from tissue samples) from goat samples. Sumathi *et al.*, (2018) isolated the organisms from 05 samples and identified them as *Brucella*, based on colony morphology, Gram's reaction, acriflavine test and agglutination tests which can be compared to the present study. In another study by Tekle *et al.*, (2019) of the 64 samples cultured, eight samples (five vaginal swabs and three milk) were positive for *Brucella* species based on colony morphology, growth characteristics, modified acid fast staining and biochemical tests results.

In the present study only 03 isolates could be obtained from the samples with reproductive disorder which could be attributed to the fastidious nature of *Brucella* species, it could also be influenced by stage of the disease and quantity of bacteria shed in the uterine discharge which determines the amount of viable organisms in the sample for culture (Tekle *et al.*, 2019). Also the low culture positivity from the present study may be because the causes of reproductive problems could be by any etiology other than *Brucella*. Morales-Estrada *et al.*, (2016) reported that 09 of the 10 isolates were sensitive to rifampicin which is comparable to the present study. In another study by Nagal *et al.*, (1994) it was reported that *Brucella melitensis* biotype III was sensitive to tetracycline and gentamicin but similar to the present study resistance to penicillin G and streptomycin was observed. In contrast to the present study Ghodsara *et al.*, (2011) reported that 100 per cent of the isolates were sensitive to penicillin-G, streptomycin, gentamicin, oxytetracycline, whereas 60 per cent to rifampicin and 40 per cent isolates were found sensitive to ceftriaxone.

Seroprevalence against *Brucella abortus* in goats was determined as 03.63 per cent by Standard Tube Agglutination Test. The

prevalence was found to be higher in goats belonging to the in unorganized farms as compared to the organized farms. A higher prevalence was observed age group of 3-5 years. 03/50 isolates of *Brucella* spp were recovered from vaginal swabs. All the isolates had typical colony characteristics of *Brucella* and showed MZN positive staining, catalase, urease, oxidase and nitrate reduction positive.

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