Gynaecological disorders associated with *Brucella melitensis* in goat flocks with potential risk of occupational zoonoses in Central India

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Brucellosis is an occupational infectious disease affecting both animals and humans. It causes abortions, retention of placenta, stillbirth and infertility in small and large ruminants. It is considered as a disease of economic importance to the society engaged in animal husbandry. The present study was aimed to estimate the prevalence of brucellosis in goats exhibiting abortions, retention of placenta and still births and in occupationally exposed individuals, to type the circulating *Brucella* species in Central India and risk factors for its spillage to human host. Seroprevalence of 34.72% (RBPT), 33.33% (SAT and S-LPS ELISA) was recorded in goats and 2.38% (RBPT, SAT and S-LPS ELISA) in occupationally exposed individuals. The clinical samples revealed three *Brucella melitensis* isolates as confirmed by bcsp31, IS711 and AMOS PCR. Early diagnosis, general awareness, restricted animal movement, hygiene, secure handling and disposal of infectious waste is warranted.

Keywords: *Brucella melitensis*, goats, gynaecological disorders, occupational zoonoses, seroprevalence.

BRUCELLOSIS is an occupational infectious disease caused by the Gram-negative, capnophilic and facultative intracellular bacteria belonging to the genus *Brucella* and is distributed worldwide in both animals and humans[1]. The genus *Brucella* comprises of ten different species, viz. *B. abortus*, *B melitensis*, *B. ovis*, *B. suis*, *B. canis*, *B. neotomae*, *B. microti*, *B. inopinata*, *B. pinnipedialis* and *B. ceti*[2,3]. *B. melitensis* is considered as the most potential zoonotic agent.

India ranks second and third in goat and sheep populations respectively[4]. These animals support the livelihood of marginal farmers and landless labourers in rural areas. Diseases like brucellosis are one of the major constraints to increase profit margin. Economic losses are incurred due to abortions and stillbirths. About 13%–14% kids are lost because of caprine brucellosis in India[5]. Successful parturition from infected parents causes further spreads of this disease among the flocks[6,7]. The occupationally exposed humans and humans consuming animal products are at risk of acquiring this disease[8]. The magnitude of infection and type of prevailing *Brucella* species among the livestock are important for successful control. The present study was conducted to elucidate the magnitude and type of circulating *Brucella* species among unorganized goat flocks of central India and risk factors for its possible spillage to humans.

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Materials and methods

Sample collection

A cross-sectional study was conducted in ten unorganized goat flocks from central India during 2018. A total of 216 samples (72 sera, 72 deep vaginal swabs and 72 blood samples) from goats and 84 samples (42 sera and 42 blood) from occupationally exposed persons were collected and stored at –20°C until further use.

Serological tests

The serum samples were screened for brucellosis by rose bengal plate agglutination test (RBPT), standard tube agglutination test (SAT) and indirect IgG ELISA following standard procedures described earlier[9–11]. The RBPT reactions were observed for agglutination within 4 min and recorded as a positive test. SAT titres of 40 IU (goat) and 80 IU (human) and above were recorded as positive. The purified *Brucella abortus* S99 smooth lipopolysaccharide (S-LPS) antigen (WHO *Brucella* referral laboratory, Indian Veterinary Research Institute, Izatnagar), rabbit anti-goat and rabbit anti-human HRP conjugated antibodies (Sigma, USA), *o*-phenylenediamine dihydrochloride and hydrogen peroxide (Qualigen, India) were used in ELISA. The working concentrations of the control serum (1 : 100), S-LPS antigen and rabbit anti-goat HRPO conjugated antibodies were established by checkerboard titration. Positive to negative ratio ≥3 was recorded as

Table 1. Sequences of oligonucleotide primers for PCR

Gene/PCR	Primer	Sequence (5′ to 3′)	Product size (bp)
BCSP31	B4 (F)	tgg ctc ggt tgc caa tat caa	223
	B5 (R)	cgc gct tgc ctt tca ggt ctg	
IS711	IS313 (F)	ctg gct gat acg ccg gac ttt gaa	350
	IS639 (R)	gga acg tgt tgg att gac ctt gat	
AMOS	IS711 (F)	tgc cga tca ctt aag ggc ctt cat	
	Brucella abortus (R)	gac gaa cgg aat ttt tcc aat ccc	498
	Brucella melitensis (R)	aaa tcg cgt cct tgc tgg tct ga	731
	Brucella suis (R)	gcg cgg ttt tct gaa ggt tca gg	285

positive result. The known positive and negative controls (National Institute for Veterinary Epidemiology and Disease Informatics (NIVEDI), Bengaluru) were used.

Isolation and identification of bacteria

The vaginal swabs and blood samples were inoculated on *Brucella* selective agar (HiMedia, India). The agar plates were incubated at 37°C with 5% CO_2. The plates showing no growth after 21 days of inoculation were discarded after decontamination. The suspected colonies showing no growth on MacConkey's agar and no haemolysis on 5% sheep blood agar were subjected to Gram and modified Ziehl Neelsen (MZN) staining followed by biochemical tests (nitrate reduction, oxidase, urease, catalase and H_2S production).

DNA was extracted from fresh bacterial cultures using DNA isolation kit (Promega, USA), quantified by Nanodrop 1000 spectrophotometer (Thermo Scientific, USA) and subjected to amplification of *BCSP31* and *IS711* genes and AMOS-PCR using specific primers (Table 1) in a thermal cycler (Eppendorf, Germany)[12–14]. The reaction comprised of 100 ng of DNA, 1× PCR buffer, 1.5 mM $MgCl_2$, 200 µM dNTPs, 10 µM of each (forward and reverse) primer and 1 U of *Taq* DNA polymerase. The cycling conditions for amplification of *BCSP31* gene included an initial denaturation step at 95°C for 3 min; 35 cycles at 95°C for 45 sec, 60°C for 45 sec and 72°C for 2 min, and a final elongation step at 72°C for 10 min. The annealing temperature of 55°C was used for amplification of the *IS711* gene. The cycling conditions for AMOS-PCR included an initial denaturation step at 95°C for 3 min; 35 cycles at 95°C for 1 min, 55.5°C for 2 min and 72°C for 2 min, and a final elongation step at 72°C for 10 min. Three positive (*B. abortus* S19, *B. melitensis* and *B. suis* DNA) and a negative (*Escherichia coli* DNA) control were used. The amplicons were visualized in gel documentation system (BioRad, USA) after submarine gel electrophoresis in 1.5% agarose with ethidium bromide.

Results

The results revealed that out of the 72 goat serum samples, 25 (34.72%) were positive by RBPT, and 24 (33.33%) by SAT and S-LPS ELISA. A human serum sample (2.38%) was found to be positive by all three tests. The history of seropositive humans indicated occupational exposure to infected flocks, intermittent fever and joint pain (Table 2).

A total of three *Brucella* isolates (29V, 32V and 43V) were recovered from goat vaginal swabs. The cultural characteristics included small, circular, light honey to white-coloured, opaque colonies on *Brucella* agar. Microscopic observations included Gram-negative and weak acid fast cocco-bacilli arranged singly or in pairs, occasionally in small chains. The biochemical tests revealed positive catalase, oxidase, urease and H_2S production.

PCR yielded specific 223 and 350 bp amplicons for universally accepted marker genes *BCSP31* and *IS711*. AMOS-PCR yielded specific 285, 498 and 731 bp amplicons for *B. suis*, *B. abortus* and *B. melitensis* respectively (Figure 1).

Discussion

The results revealed high seropositivity (34.72%) in goats having a history of abortions, retention of placenta and repeat breeding. Our previous reports suggested high seropositivity (66.24%, 28.1% and 17%) in migratory sheep flocks of Akola[15] and Latur[16] districts in Maharashtra, followed by a human case of zoonoses from western India[17]. Variable seroprevalence was reported in goat flocks from different states, viz. 7.6% in Karnataka, 11.30% in Gujarat, 22% in Odisha, 24% in Uttar Pradesh, 30% in western Rajasthan and 55% in Andhra Pradesh[18–20]. Studies indicated high incidence of brucellosis in flocks that are reared in the unorganized sector and showing a history of gynaecological disorders[19,21,22], in agreement with the findings of the present study. More seropositivity in the goats of central India may be because of the conventional goat-rearing system, as well as poor hygienic and disposal practices in the study area. Moreover, unrestricted animal movements in search of pasture and water, trade within and between districts and States, the mixing of animals at marketplaces and watering points, especially during the dry season may have enhanced the spread of infection as reported in the present study. Further studies may be impregnable on individual and flock prevalence

Table 2. Results of serology and culture identification

Sample ID	Species	History	Serology			Culture			Staining		Biochemical identification					Molecular identification (PCR)		
			RBPT	STAT (IU)	ELISA	BSA	BA	MA	G	MZN	N	O	H_2S	U	C	*BCSP*31	*IS*711	AMOS
G2V	Goat	ROP, RB	+	80	++	–	–	ND	ND	ND	ND	ND		ND	ND	ND	ND	ND
G6V	Goat	AB	+	40	+	–	–	ND	ND	ND	ND	ND		ND	ND	ND	ND	ND
G7V	Goat	AB	++	80	++	–	–	ND	ND	ND	ND	ND		ND	ND	ND	ND	ND
G14V	Goat	AB	++	160	+++	–	–	ND	ND	ND	ND	ND		ND	ND	ND	ND	ND
G15V	Goat	AB	+	40	+	–	–	ND	ND	ND	ND	ND		ND	ND	ND	ND	ND
G16V	Goat	AB	++	80	++	–	–	ND	ND	ND	ND	ND		ND	ND	ND	ND	ND
G21V	Goat	AB	++	80	++	–	–	ND	ND	ND	ND	ND		ND	ND	ND	ND	ND
G24V	Goat	ROP, RB	+	80	++	–	–	ND	ND	ND	ND	ND		ND	ND	ND	ND	ND
G26V	Goat	AB	+	40	++	–	–	ND	ND	ND	ND	ND		ND	ND	ND	ND	ND
G29V	Goat	AB	+	-	-	+	NH	–	–	±	+	+		+	+	+	+	*B. melitensis*
G31V	Goat	AB	++	80	++	–	–	ND	ND	ND	ND	ND		ND	ND	ND	ND	ND
G32V	Goat	AB	++	160	+++	+	NH	–	–	±	+	+		+	+	+	+	*B. melitensis*
G40V	Goat	AB	+	80	++	–	–	ND	ND	ND	ND	ND		ND	ND	ND	ND	ND
G43V	Goat	AB	++	320	+++	+	NH	–	–	±	+	+		+	+	+	+	*B. melitensis*
G45V	Goat	ROP, RB	+	80	++	–	–	ND	ND	ND	ND	ND		ND	ND	ND	ND	ND
G49V	Goat	ROP, RB	++	80	++	–	–	ND	ND	ND	ND	ND		ND	ND	ND	ND	ND
G53V	Goat	ROP, RB	+	80	+	–	–	ND	ND	ND	ND	ND		ND	ND	ND	ND	ND
G55V	Goat	ROP, RB	+	40	+	–	–	ND	ND	ND	ND	ND		ND	ND	ND	ND	ND
G56V	Goat	ROP, RB	++	80	++	–	–	ND	ND	ND	ND	ND		ND	ND	ND	ND	ND
G65V	Goat	AB	++	80	++	–	–	ND	ND	ND	ND	ND		ND	ND	ND	ND	ND
G67V	Goat	AB	++	160	+++	–	–	ND	ND	ND	ND	ND		ND	ND	ND	ND	ND
G68V	Goat	AB	+	80	++	–	–	ND	ND	ND	ND	ND		ND	ND	ND	ND	ND
G69V	Goat	AB	+	80	++	–	–	ND	ND	ND	ND	ND		ND	ND	ND	ND	ND
G70V	Goat	AB	+	80	++	–	–	ND	ND	ND	ND	ND		ND	ND	ND	ND	ND
G72V	Goat	ROP, RB	++	80	++	–	–	ND	ND	ND	ND	ND		ND	ND	ND	ND	ND
H39V	Human	Fever, joint pain	++	320	+++	–	–	ND	ND	ND	ND	ND		ND	ND	ND	ND	ND

ROP, Retention of placenta; RB, Repeat breeding; AB, Abortion; BSA, *Brucella* selective agar; BA, 5% Sheep blppd agar; MAC, MacConkey agar; G, Gram; MZN, Modified Ziehl Neelsen; N, Nitrate; O, Oxidase; U, Urease; C, Catalase; +++, Strong positive; ++, Positive; +, Moderate positive; ±, Weak positive; –, Negative; NH, Non-hemolytic; ND, Not done.

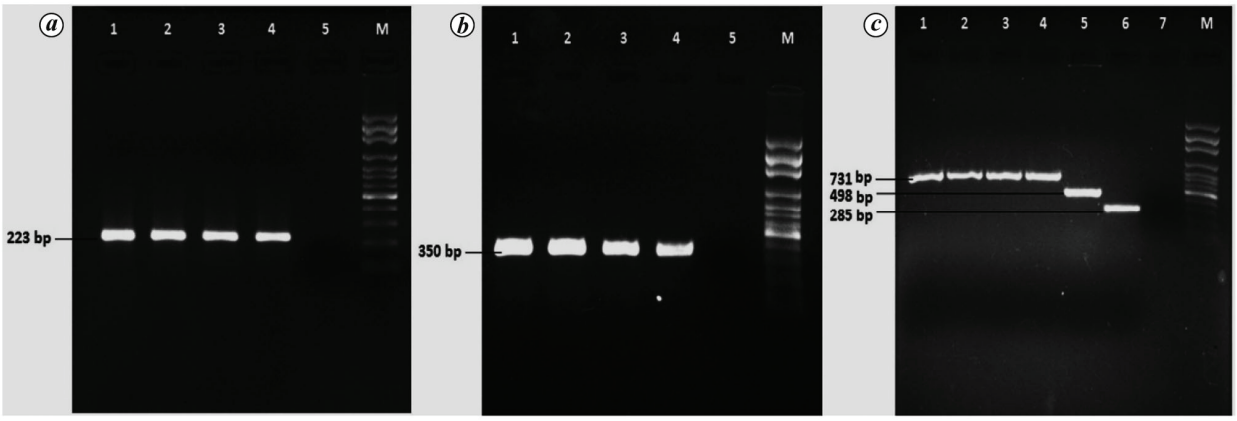

Figure 1. Molecular and differential identification of *Brucella melitensis*. ***a***, *BCSP31* PCR (lanes 1–3, *Brucella* isolates (43V, 32V and 29V); lane 4, Positive control (*Brucella abortus* S19); lane 5, Negative control (*Escherichia coli*); lane M, 100 bp DNA ladder). ***b***, *IS711* PCR (lanes 1–3, *Brucella* isolates (43V, 32V and 29V); lane 4, Positive control (*B. abortus* S19); lane 5, Negative control (*E. coli*); lane M, 100 bp DNA ladder). ***c***, AMOS PCR (lanes 1–3, *Brucella* isolates (43V, 32V and 29V); lane 4, Positive control (*B. melitensis*); lane 5, Positive control (*B. abortus* S19); lane 6, Positive control (*Brucella suis*), lane 7, Negative control (*E. coli*); lane M, 100 bp DNA ladder).

of brucellosis in small ruminants. Recently, high herd prevalence (65.54%) was reported compared to individual animal prevalence (26.50%) in cattle and buffaloes of Punjab and Haryana[23]. National average prevalence of brucellosis in cattle (8.3%), buffalo (3.6%), sheep (7.9%) and goat (2.2%) has been reported in some studies[19,24]. However, considering highly unpredictable rates of infection in geographically diverse regions across a vast country like India, such depiction will mask true health priorities.

All these goats were reared in small flock size by small, marginal or landless farmers, indicating significant role in their livelihood. Tolerance to climatic and nutritional stress, short gestation, less investment, easy maintenance, huge domestic demand and immediate source of additional income make it ideal to support poor families. We report unorganized goat husbandry in study areas with flock size from 5 to 10, as it was found most profitable[25]. For economical goat-rearing, goat health management plays an imperative role. Since it causes abortions, stillbirths and infertility, *B. melitensis* has extensive negative impact in the productivity of a flock[26] and limits the income of livestock farmers. An average annual economic loss of Rs 2860 to Rs 5200 per goat was recorded due to abortion, stillbirth, repeat breeding and veterinary care in India[26].

We could recover three isolates from deep vaginal samples from seropositive goats and subsequently confirmed them as *B. melitensis*. Bacteria could not be isolated from the only seropositive and symptomatic human case. It is a fastidious pathogen, requiring specialized conditions for growth on artificial media, limiting its isolation from every suspected sample. Earlier reports also indicated less isolation rates[27,28].

An occupationally exposed animal health worker (2.38%) who had symptoms like intermittent fever, headache and joint pain was found to be positive for *Brucella* antibodies. Agastya *et al.*[29] reported similar positivity (3.6%) in high-risk groups of Karnataka. Brucellosis is prevalent among all domesticated animals[30] and is a significant public health problem of unknown magnitude in India[31]. Global annual estimate indicates 0.5 million new reported and an equal number of unreported human cases, majority of which are caused by *B. melitensis*[26], the pathogen reported in the present study. It is often mistaken for typhoid, malaria[32] or pyrexia of unknown origin. A report indicated 10.5% overall positivity in rural India[33] with annual median loss of Rs 627.5 million due to human brucellosis[34].

Historically, small ruminants have been the reservoir host for *B. melitensis*[20] and remain a significant burden on human health in India[26]. The susceptible human population engaged in unorganized goat husbandry in the study area lives at risk. The history indicated that all the goat flocks under study were reared in close human, or in the lobby at the entrance of houses. Moreover, handling of infectious waste by family members and animal health workers poses risk for spillage of such a potential pathogen in humans. The results of the present study could facilitate surveillance and eradication programmes, enhancing public health and livestock productivity in central India.

Conclusion

This study confirmed that *B. melitensis*is is circulating among seropositive goat flocks resulting in abortion, retention of placenta, stillbirth and infertility. Seropositivity was also reported in occupationally exposed individuals having a history of handling infected goat flocks. Early diagnosis can help reduce the infection and incidence of zoonoses. Thus general awareness, restricted animal movement, hygiene, and secure handling and disposal of infectious waste are warranted.

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