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Molecular detection and risk factor analysis of *Brucella* spp. in small ruminants of Chhattisgarh

Vivek K Naik, Sanjay Shakya, Anil Patyal, SL Ali, Subhash K Verma, SD Hirpurkar, Mamta Choudhary and Abhinav Verma

Abstract
Small ruminants, predominantly owned by impoverished farmers in India, serve as significant reservoirs for zoonotic pathogens, demanding a thorough understanding of prevalence. In India, where bovine brucellosis is endemic, sheep and goats are identified as major sources of infection, mirroring clinical manifestations of *B. melitensis* in small ruminants and *B. abortus* in cattle. The risk of human infection arises from contact with infected materials or consumption of contaminated raw milk. PCR, particularly AMOS PCR, is crucial for diagnosing *Brucella* spp., but the lack of epidemiological data hinders effective control. The study, using a risk factor questionnaire and an integrated approach, provides valuable insights for developing intervention programs and policies in Chhattisgarh, India. The cross-sectional study in Chhattisgarh (February 2022 to March 2023) involved obtaining blood samples from 750 small ruminants and additional clinical samples. DNA extraction, PCR techniques, and gel electrophoresis were utilized for molecular detection of *Brucella* spp., targeting *Bcsp31* and AMOS PCR for genus and species confirmation. A risk factor questionnaire assessed farming practices, rearing conditions, and contact, contributing to a comprehensive understanding. Statistical analyses involved Chi- square (χ^2) statistics with Fisher's exact test, univariable logistic regressions, and mixed-effects multivariable logistic regression models to identify risk factors for *Brucella* positivity. The study conducted in Chhattisgarh revealed a 2.26% prevalence of *Brucella* spp., with blood samples showing 1.87% prevalence. Notably, goats had 2.1% prevalence, while sheep exhibited a higher prevalence of 5.13%, and males showed a significantly higher prevalence (4.17%) compared to females (1.87%). Among various risk factors, animals aged 2.5 years had the highest prevalence (6.14%), and the number of small ruminants was identified as a significant predictor of *Brucella* spp. infection at the farm level (OR: 1.103, 95% CI: 1.032-1.178). Additionally, a history of reproductive disorder was significantly associated with a lower odds ratio of *Brucella* spp. infection (OR: 0.040, 95% CI: 0.002-0.836), while the introduction of new animals and proper disposal of placenta did not reach statistical significance in this analysis. These findings contribute to our understanding of *Brucella* spp. epidemiology in Chhattisgarh and provide a foundation for tailored strategies to mitigate transmission risks and safeguard both animal and public health.

Keywords: *Brucella,* molecular detection, risk factor, small ruminants, Chhattisgarh

Introduction
Sheep and goats play a crucial role in supporting the livelihoods of numerous impoverished rural households in India, with an estimated 98% of small ruminants being owned by small, landless, and often illiterate farmers (Kanani *et al*., 2018; Sahu *et al*., 2018)[17,31]. Despite their socioeconomic significance, these animals, serving as reservoirs for various zoonotic pathogens of substantial concern to both animal and public health in India, necessitate a comprehensive understanding of zoonotic prevalence.
In India, where Bovine brucellosis is endemic and poses a significant risk to public health affecting both humans and animals, sheep and goats are identified as major sources of infection (Khurana *et al*., 2020; Renukaradhya *et al*., 2002)[18,28]. The infection caused by *B. melitensis* in small ruminants closely mirrors that of *B. abortus* in cattle, exhibiting similar clinical manifestations such as abortion, stillbirth, and typically occurring once in the animal's lifetime (Blasco and Molina-Flores, 2011; Elzer *et al*., 2002) [5, 9]. The shedding of bacteria in their discharges by infected animals is recognized as a critical source for spreading the infection among susceptible hosts (Hosein *et al*., 2018; Jamil *et al*., 2020) [15, 16].
For humans, the risk of infection arises when they come into contact with infected aborted materials, such as the placenta or dead foetus, through abraded skin. However, the most prevalent route of infection is through the consumption of contaminated raw, unpasteurized milk and milk products (Georgiou's *et al*., 2005; Rahman *et al*., 2011) [11, 27].

Understanding and addressing these aspects of zoonotic transmission in small ruminants is of paramount importance for safeguarding both animal and public health in the context of the intricate relationships between these animals and vulnerable human populations.
Among various diagnostic tools, PCR has emerged as a promising and superior technique for diagnosing infectious diseases caused by fastidious or slow-growing bacteria (Romero *et al*., 1995; Bricker, 2002) [29, 6]. Molecular assays that target the IS711 insertion element and the *Bcsp31* gene, responsible for encoding a 31-kDa immunogenic outer membrane protein conserved across all *Brucella* spp., are widely utilized in clinical applications (Baily *et al*., 1992) [4]. For identifying species and biotypes within the *Brucella* genus, AMOS PCR is frequently employed due to its high reliability in detecting various species in a single reaction (Sonekar *et al*., 2018) [33].
Nevertheless, the absence of sufficient epidemiological data on the seroprevalence of *Brucella* and associated risk factors can hinder the establishment of effective strategic control programs (Gwida *et al*., 2015; Eltholth *et al*., 2017) [13, 8]. To address this gap, a risk factor questionnaire was utilized to gain deeper insights into farming practices, rearing conditions, and contact between small ruminants and other species—identified as crucial risk factors for *Brucella* infection (Megahed *et al*., 2022; Leahy *et al*., 2020) [24, 20]. Through an integrated approach that combined blood and other clinical sampling with farmer interviews, this study achieved a comprehensive understanding of the epidemiology and risk factors for *Brucella* positivity. These findings contribute valuable insights for the development of future intervention programs and policies aimed at mitigating transmission risks in Chhattisgarh, India.

Material and methods

Sampling Methodology

This cross-sectional study was executed in the Indian state of Chhattisgarh spanning from February 2022 to March 2023. Blood samples were obtained from 750 small ruminants through the jugular vein, drawn into sterile syringes, and subsequently transferred to EDTA vial vacutainer tubes. Additionally, 33 aborted fetus samples, 9 placental tissue samples, and 3 vaginal swabs were collected. These samples were carefully transported to the laboratory in a cold chain, where they were triturated and mixed in PBS before undergoing DNA extraction. A total of 795 samples were gathered from 131 households across various districts of Chhattisgarh for comprehensive analysis.

Data Collection

Consent was meticulously obtained from all participants in the study. A pre-tested household questionnaire, paired with a risk factor analysis tool featuring closed-ended questions, was distributed to a diverse group of stakeholders, including animal owners, handlers, veterinarians, and paravets, during the various sampling procedures. The survey aimed to comprehensively assess the knowledge base related to zoonotic diseases, with a specific focus on brucellosis.

Molecular detection of *Brucella* spp. from small ruminants

DNA extraction utilized the Qiagen Blood and Tissue DNA Extraction Kit, and the isolates were subsequently validated through PCR technique. For molecular identification, genomic DNA from the isolates was extracted using the QIAamp DNA Mini Kit (QIAGEN) following the manufacturer's instructions. The DNA samples were evaluated for concentration and optical density (OD) using a Nanodrop.
The extracted DNA samples underwent analysis for *Brucella* genus specificity by targeting the *Bcsp31* gene (Baily *et al*., 1992) [4]. The primers utilized were *Bcsp*-F: TGGCTCGGTTGCCAATATCAA and *Bcsp*-R: CGCGCTTGCCTTTCAGGTCTG, with a resulting product size of 223 bp. PCR was employed to validate the presence of the genus-specific *Bcsp31* gene. The PCR reaction mixture, with a volume of 25 µl, comprised 2.5 µl of 1X Dream Taq Green buffer, 1 µl of dNTP (200 µm), 0.5 µl of 10 pmol/µl of both forward and reverse primers, 1.5 µl of DNA template, 0.5 µl of Taq polymerase, and 18.5 µl of nuclease-free water. The PCR cycling conditions involved an initial denaturation step at 95 °C for 3 minutes, followed by 35 cycles of denaturation at 95 °C for 45 seconds, annealing at 60 °C for 45 seconds, extension at 72 °C for 2 minutes, and a final extension at 72 °C for 10 minutes. Subsequently, the PCR products underwent gel electrophoresis on a 1.5% agarose gel, visualized under a UV transilluminator, and captured using the Bio-rad GelDoc system. Isolates displaying amplification at 223 base pairs were deemed positive for the *Bcsp31* gene, confirming their affiliation with the genus *Brucella* spp.
After confirming the genus as *Brucella* spp., the isolates underwent species verification through AMOS PCR, a multiplex PCR employing five primers (with a common reverse primer, IS711). This targeted specific species within *Brucella*, including *B. abortus*, *B. melitensis*, *B. ovis,* and *B. suis*. The primer sequences and corresponding product sizes are as follows: AMOS IS711 (R): TGCCGATCACTTAAGGGCCTTCAT, *B. abortus* (F): GACGAACGGAATTTTTCCAATCCC, *B. melitensis* (F): AAATCGCGTCCTTGCTGGTCTGA, *B. ovis* (F): CGGGTTCTGGCACAATCGTCG, *B. suis* (F): GCGCGGTTTTCTGAAGGTTCAGG. Each species yielded different-sized amplicons at 498, 731, 976, and 285 base pairs, respectively.
The PCR reaction mixture for AMOS PCR, with a volume of 25 µl, included 2.5 µl of 1X Dream Taq Green buffer, 1 µl of dNTP (200 µm), 0.5 µl of each of the five primers at 10 pmol/µl, 1.5 µl of DNA template, 0.5 µl of Taq polymerase, and 17 µl of nuclease-free water. The cycling conditions comprised an initial denaturation at 95 °C for 2 minutes, followed by 30 cycles of denaturation at 95 °C for 1.15 minutes, annealing at 55.5 °C for 2 minutes, extension at 72 °C for 2 minutes, and a final extension at 72 °C for 10 minutes. Following amplification, the products were subjected to gel electrophoresis using a 1.6% agarose gel, visualized under a UV transilluminator, and documented using the Bio-rad Gel Doc system.

Statistical analysis

The data were inputted into Microsoft Excel and analyzed utilizing SPSS version 25. Individual animal-level and flock-level risk factors were methodically organized in an Excel sheet, and coding was applied for conducting various tests in SPSS. Initial univariable analyses were performed using chi-square statistic (χ^2) with Fisher's exact test and univariable logistic regressions. This was done to initially identify each potential risk variable for *Brucella* positivity. Subsequently, mixed-effects multivariable logistic regression models were constructed, commencing with all independent variables that demonstrated a p-value < 0.1 in the univariable analysis (Leahy *et al*., 2020) [20].

Results

The study involved DNA sample extraction and PCR analysis targeting the *Bcsp31* gene for the genus *Brucella*. Confirmation of the genus was achieved through the amplification of a 223 base pair fragment (Fig 1). During the execution of AMOS-PCR for the species identification of *Brucella* genus-positive samples, all tested specimens exhibited positive results for *B. melitensis.* This outcome indicates the prevalence of *B. melitensis* within the small ruminant flock (Fig 2).

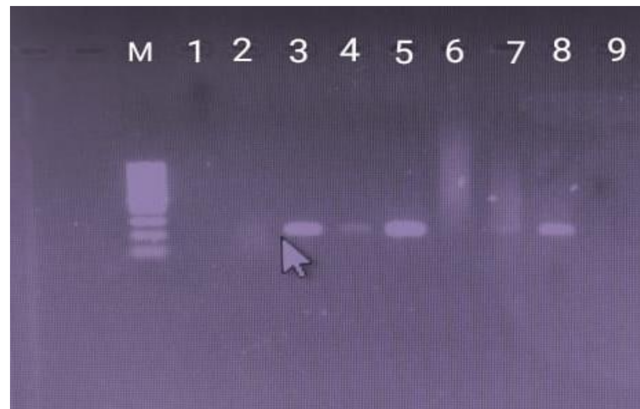

Fig 1: Genus-specific PCR for *Brucella* detection. Lane M- 100-bp DNA ladder; Lane 2- Negative control, Lane 3- Positive control for *Brucella* genus; Lanes 4,5 and 8- *Brucella* genus DNA samples for *Bcsp31* (223 bp).

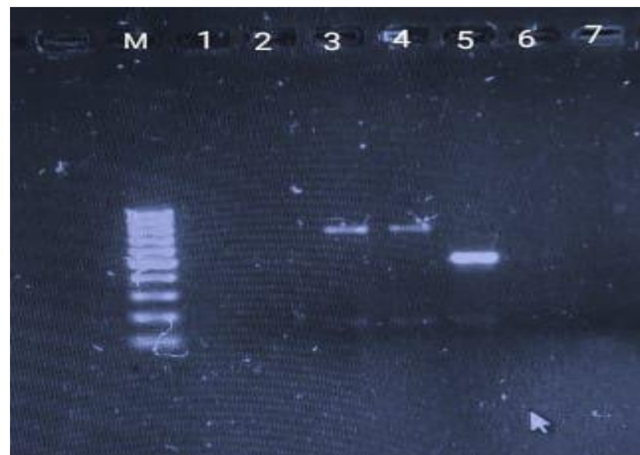

Fig 2: *B. melitensis* detection by AMOS PCR. Lane M- 100-bp DNA ladder; Lane 3- *B. melitensis* DNA samples (731 bp); Lane 4- positive control for *B. melitensis,* Lane 5- Positive control for *B. abortus* (498 bp), Lane 6- Negative control.

Among the total samples analysed for *Brucella* spp., 2.26% of the collected samples were identified as positive cases. In the blood samples, which constituted the majority (94.34%) of the collected specimens, the prevalence was 1.87%. Notably, three positive cases were found among the aborted foetuses, while one positive case was detected in the vaginal swabs. Species-wise prevalence revealed that goats had a prevalence of 2.1%, while sheep exhibited a slightly higher prevalence of 5.13%. Sex-wise prevalence indicated a higher percentage in males (4.17%) compared to females (1.87%). Indigenous breeds showed a prevalence of 2.11%, whereas crossbreeds exhibited a prevalence of 3.33%. Age-wise prevalence varied across different age groups. While no positive cases were detected in 1 and 4-year-olds, prevalence percentages for the other age groups were as follows: 2 years (3.84%), 2.5 years (3.51%), and 3 years (4.68%).

Among the 131 small ruminant flocks, 14 flocks were found positive showing prevalence rate of 6.2%.

Analysis of factors associated with *Brucella* infection at animal level

In the univariable analysis detailed in Table 1, the prevalence of *Brucella* spp. at the individual animal level was scrutinized, and various risk factors were evaluated. Under the variable "Species," goats (756 samples) exhibited a prevalence of 2.12%, with an odds ratio (OR) of 2.50 (95% CI: 0.554-11.278) and a p-value of 0.219. In contrast, sheep (39 samples) displayed a higher prevalence of 5.13%. In relation to the "Breed" variable, indigenous breeds (705 samples) demonstrated a prevalence of 2.27%, with an OR of 0.979 (95% CI: 0.221-4.328) and a p-value of 1.0. Crossbred animals (90 samples) had a slightly lower prevalence of 2.22%. Regarding "Sex," both females (747 samples) and males (48 samples) exhibited a prevalence of 2.27%. The OR for females was 0.914 (95% CI: 0.119-7.014) with a p-value of 1.0. For the "Age" variable, animals of different age groups displayed varying prevalence rates. Animals aged 2.5 years had the highest prevalence at 6.14%, with an OR of 2.025 (95% CI: 1.276-3.213) and a significant p-value of 0.003. Lastly, the "Type of sample" variable revealed diverse prevalence rates, with blood samples (750 samples) indicating a prevalence of 1.87%, an OR of 2.289 (95% CI: 1.131-4.635), and a p-value of 0.21. Aborted foetus samples (33 samples) and vaginal swab samples (9 samples) displayed higher prevalence rates of 9.09% and 11.11%, respectively, while placental tissue samples (3 samples) showed no prevalence.

Table 1: Univariate analysis demonstrating risk factors associated with the prevalence of *Brucella* spp. at the animal level.

Variable	Category	Total number(N)	Positive	Prevalence (%)	OR (95% CI)	P-value
Species	Goat	756	16	2.12	2.50(0.554-11.278)	0.219
	Sheep	39	2	5.13		
Breed	Indi.	705	16	2.27	0.979(0.221-4.328)	1.0
	Crossbr.	90	2	2.22		
Sex	Female	747	17	2.27	0.914(0.119-7.014)	1.0
	Male	48	1	2.08		
Age	1 Yr	99	0	0	2.025(1.276-3.213)	0.003
	1.5 Yr	44	0	0		
	2 Yr	365	4	1.09		
	2.5 Yr	114	7	6.14		
	3 Yr	171	7	4.09		
	4 Yr	2	0	0		
Type of sample	Blood	750	14	1.87	2.289(1.131-4.635)	0.21
	Aborted Foetus	33	3	9.09		
	Vaginal Swab	9	1	11.11		
	Placental Tissue	3	0	0		

p-value below 0.05 was considered to be statistically significant

Analysis of factors associated with *Brucella* infection at the farm level

The univariable analysis investigating risk factors linked to the prevalence of *Brucella* spp. at the small ruminant farm or flock level are given in Table 2. The variable "Number of small ruminants kept" reveals that farms with more than 10 small ruminants (106 samples) had a higher prevalence of 13.21%, with an odds ratio (OR) of 1.072 (95% CI: 1.025-1.121) and a significant p-value of 0.002. In contrast, flocks with 5-10 small ruminants (22 samples) showed no positive cases. Concerning "Flock location," rural areas (111 samples) exhibited a prevalence of 9.91%, with an OR of 1.604 (95% CI: 0.405-6.353) and a p-value of 0.449. Urban areas (20 samples) had a higher prevalence of 15%. The presence of "New animals introduced in the last 12 months" was linked to a higher prevalence, with farms introducing new animals (73 samples) showing a prevalence of 17.81%, compared to 1.72% for those without new introductions (58 samples). The OR was 12.350 (95% CI: 1.565-97.481), and the p-value was 0.003. The variable "Other species on the farm" did not demonstrate a significant association with *Brucella* spp. prevalence. "Vaccination" also did not exhibit a significant association. "Husbandry system" indicated that semi-intensive systems (19 samples) had a higher prevalence of 15.79%, while extensive (58 samples) and intensive (54 samples) systems showed prevalence of 8.62% and 11.11%, respectively.

The absence of "Quarantine of animals" was linked to a higher prevalence (14.44%) compared to farms with quarantine measures (2.44%). "Floor spacing," "Presence of ticks," "Biosecurity measures adopted," and "Whether to bury a dead animal" did not show significant associations with *Brucella* spp. prevalence.

However, several factors, including "History of reproductive disorder," "Proper disposal of placenta," and "Mastitis," significantly associated with *Brucella* spp. prevalence, with p-values of 0.000. For instance, farms with a "History of reproductive disorder" (39 samples) had a prevalence of 2.56%, with an OR of 45.50 (95% CI: 5.68-364.249). Similarly, "Proper disposal of placenta" (34 samples) was linked to a lower prevalence of 2.06%, with an OR of 0.039 (95% CI: 0.008-0.185) and a significant p-value of 0.000. "Mastitis" (27 samples) was associated with a higher prevalence of 18.52%, with an OR of 2.39 (95% CI: 0.732-7.866) and a p-value of 0.164.

The multivariable analysis scrutinized several risk factors associated with *Brucella* spp. infection at the small ruminant farm/flock level (Table 3). In Step 1a of the analysis, the following variables were considered: "Number of small ruminants kept" emerged as a significant predictor (p-value = 0.004). For each unit increase in the number of small ruminants kept, the odds of *Brucella* spp. infection increased by a factor of 1.103 (95% CI: 1.032-1.178). "New animal introduced in the last 12 months" did not attain statistical significance (p-value = 0.085), but it exhibited a negative association with *Brucella* spp. infection. The odds of infection decreased by a factor of 0.102 (95% CI: 0.008-1.368) for farms introducing new animals. "History of reproductive disorder" was identified as a significant predictor (p-value = 0.038). Farms with a history of reproductive disorder had a lower odds ratio of 0.040 (95% CI: 0.002-0.836) for *Brucella* spp. infection. "Proper disposal of placenta" did not demonstrate statistical significance (p-value = 0.358). The odds ratio for this variable was 3.093 (95% CI: 0.278-34.379). The constant term in the model was -4.250 (p-value = 0.008), representing the intercept when all other predictor variables are zero. The odds of *Brucella* spp. infection were 0.014 times the reference value when the constant term was considered.

In summary, the results indicate that the number of small ruminants kept and a history of reproductive disorder are significant predictors of *Brucella* spp. infection at the small ruminant farm/flock level. However, the introduction of new animals and the proper disposal of placenta did not reach statistical significance in this analysis.

Table 2: Univariate analysis revealing risk factors associated with the prevalence of *Brucella* spp. at the small ruminant farm/flock level.

Variable	Category	Total number of flock (N)	Positive	Prevalence (%)	OR(95% CI)	P-value
Number of small ruminant kept	< 5	3	0	0	1.072(1.025-1.121)	0.002
	5-10	22	0	0		
	>10	106	14	13.21		
Flock location	Rural	111	11	9.91	1.604(0.405-6.353)	0.449
	Urban	20	3	15		
New animal introduced in last 12 month	No	58	1	1.72	12.350(1.565-97.481)	0.003
	Yes	73	13	17.81		
Other species on farm	No	48	3	6.25	2.292(0.606-8.663)	0.254
	Yes	83	11	13.25		
Vaccination	No	109	13	11.93	0.352(0.044-3.837)	0.463
	Yes	22	1	4.54		
Husbandry system	Extensive	58	5	8.62	-	0.680
	Semi Intensive	19	3	15.79		
	Intensive	54	6	11.11		
Quarantine of animal	No	90	13	14.44	0.148(0.019-1.173)	0.063
	Yes	41	1	2.44		
Floor spacing	Inadequate	41	4	9.76	1.156(0.340-3.929)	1.0
	Adequate	90	10	11.11		
Presence of ticks	No	38	1	2.63	6.013(0.758-47.693)	0.06
	Yes	93	13	13.98		
Biosecurity measures adopted	No	103	13	12.62	0.256(0.032-2.050)	0.30
	Yes	28	1	3.57		
History of reproductive disorder	No	92	1	1.09	45.50(5.68-364.249)	0.000
	Yes	39	13	2.56		
Proper disposal of placenta	No	34	12	35.29	0.039(0.008-0.185)	0.000
	Yes	97	2	2.06		
Mastitis	No	104	9	8.65	2.39(0.732-7.866)	0.164
	Yes	27	5	18.52		
Whether bury dead animal	No	42	5	11.90	0.83(0.261-2.657)	0.767
	Yes	89	9	10.11		

p-value below 0.05 was considered to be statistically significant

Table 3: Multivariable analyses of risk factors for *Brucella* spp. infection at the small ruminant farm/flock level.

		B	df	Sig.	Exp(B)	95% C.I. for EXP(B)	
						Lower	Upper
Step 1[a]	Number of small ruminants kept	.098	1	.004	1.103	1.032	1.178
	New animal introduced in last 12 month(Yes)	-2.282	1	.085	.102	.008	1.368
	History of reproductive disorder(Yes)	-3.225	1	.038	.040	.002	.836
	Proper disposal of placenta(Yes)	1.129	1	.358	3.093	.278	34.379
	Constant	-4.250	1	.008	.014		

p-value below 0.05 was considered to be statistically significant

Discussion

Investigating the prevalence of *Brucella* spp. in the small ruminant population, crucial insights were obtained using molecular techniques, notably PCR. This method facilitated a direct evaluation of the genetic material of *Brucella* spp. within the study cohort. The results uncovered a positivity rate of 2.26%, elucidating the molecular prevalence of *Brucella* spp. among the small ruminants under examination. Leahy *et al*. (2020) [20] highlighted significantly higher seroprevalence in Odisha (14%) and Assam (6%) contrasting sharply with our study's overall lower prevalence of 2.26%. Megahed *et al*. (2022) [24] found no significant gender-based differences in seroprevalence, aligning with our results that indicated a higher prevalence in males (4.17%) than in females (1.87%). Megahed *et al*. (2022)[24] and various earlier studies noted increased seroprevalence in older animals, consistent with our findings where animals aged 2.5 years exhibited the highest prevalence (6.14%). Kumar *et al*. (2016) [19] suggested age as a debatable risk factor, a viewpoint contrasted by our study's significant influence of age, particularly at 2.5 years. Megahed *et al*. (2022) [24] identified a history of abortion as a significant risk factor, aligning with our study, which indicated that farms with a "History of reproductive disorder" had a higher prevalence (2.56%). Leahy *et al*. (2020)[20] and our study noted species-wise variations, with sheep exhibiting a higher prevalence. Our findings align with Megahed *et al*. (2022) [24] where sheep had a higher prevalence (5.13%) than goats (2.1%). Megahed *et al*. (2022) [24] highlighted flock size as a major risk factor, consistent with our findings where farms with more than 10 small ruminants showed a higher prevalence (13.21%). Our study, along with Godfroid *et al*. (2010) [12] and Deka*et al*. (2018) [7] all suggested that higher seroprevalence rates may be attributed to infected animals remaining carriers throughout their lives. Megahed *et al*. (2022) [24] contradicted earlier studies suggesting a higher prevalence in female animals, aligning with our findings where males showed a higher prevalence. Our multivariable analysis identified several significant predictors of *Brucella* spp. infection at the small ruminant farm/flock level, with the number of small ruminants kept and a history of reproductive disorder emerging as significant factors. The introduction of new

animals and the proper disposal of placenta did not reach statistical significance. The confidence intervals in our study, as well as in Leahy *et al*. (2020) [20] and Megahed *et al*. (2022) [24] provide a measure of the precision and reliability of the reported prevalence rates. In summary, while specific prevalence rates may vary, the overall trends in our study align with existing literature, emphasizing the significance of factors such as age, reproductive history, and flock size in understanding and mitigating *Brucella* spp. prevalence.

Mugizi *et al*. (2015) [25] observed that older animals were more likely to be seropositive, aligning with Megahed *et al*. (2022) [24]. Asmare *et al*. (2013) [3] linked *Brucella* infection to sexual maturity due to sex hormones and placenta erythritol. In contrast, Kumar *et al*. (2016) [19] reported more common *Brucella* infection in younger calves, suggesting age as a debatable risk factor.

In Megahed *et al*.'s (2022) [24] study, a history of abortion emerged as a significant risk factor for cattle brucellosis, consistent with earlier studies (Samaha *et al*., 2009; Lindahl *et al*., 2014; Alhaji *et al*., 2016) [32, 22, 2]. No link was found between *Brucella* infection and abortion or placenta retention in some studies (Asmare *et al*., 2013; Mugizi *et al*., 2015) [3, 25]. Worldwide seroprevalence in small ruminants varied, with 0.5% of sheep in Ethiopia testing positive for brucellosis (Lemu *et al*., 2014) [21]. In Bangladesh, seroprevalence in sheep was 2.31%, and in goats, it was 3.15% (Rahman *et al*., 2011) [27]. In Egypt, 18.09% positivity for *B. melitensis* in sheep was recorded (Mahboub *et al*., 2013) [23]. Seroprevalence results varied in different studies in India, with Sonekar *et al*. (2018) [33] reporting higher seroprevalence than other studies. Flock size appeared to be a major risk factor for brucellosis transmission. Investigation in apparently healthy sheep flock showed a seroprevalence of 50%. Seroprevalence in a healthy sheep flock in Tajikistan was 28%. Vaginal swabs were considered the best sample for isolating *B. melitensis* from sheep. *B. melitensis* was isolated from the outbreak and confirmed by PCR.

Numerous earlier studies, including Samaha *et al*., (2009) [32], Lindahl *et al*., (2014) [22], Alhaji *et al*., (2016) [2], affirmed a history of abortion as a significant risk factor for cattle brucellosis. O'Callaghan (2013) [26], highlighted *Brucella* organisms as a major cause of abortion due to the presence of erythritol in the uterus. Asmare *et al*., (2013) [3] and Mugizi *et al*., (2015) [25], found no link between *Brucella* infection and abortion or placenta retention. Holt *et al*., (2011) [14] emphasized that infected animals may remain carriers throughout their lives. Lemu *et al*., (2014) [21] reported that in Ethiopia, 0.5% of sheep tested positive for brucellosis. Rahman *et al*., (2011) [27] documented seroprevalence in sheep at 2.31% and in goats at 3.15% in Bangladesh. Mahboub *et al*., (2013) [23] recorded 18.09% positivity for *B. melitensis* in sheep in Egypt. Sonekar *et al*., (2018) [33], reported higher seroprevalence in India than other studies. Suryawanshi *et al*., (2014) [34], recorded seroprevalence up to 17.68% in sheep from Maharashtra. Sutariya *et al*., (2014) [35], documented seropositivity for *B. melitensis* up to 4.41% in Gujarat. Sadhu *et al*., (2015) [30] reported an overall seroprevalence in small ruminants of 11.30% by RBPT and 8.80% by i-Elisa in Northern Gujarat. Abbas and Agab, (2002) [1] found that flock size is a major contributing factor to the disease. Gameel *et al*., (1993) [10] highlighted that contamination of the environment with *Brucella* organisms occurs during abortion, aiding rapid transmission.

In conclusion, our study contributes valuable insights to the understanding of *Brucella* spp. prevalence and associated risk factors in small ruminants. The observed variations in prevalence across regions, age groups, and genders emphasize the complexity of *Brucella* infection dynamics. Contrary to some earlier suggestions, our findings underscore the significance of age, reproductive history, and flock size as critical determinants of *Brucella* prevalence.

Conclusions

Our findings align with specific studies, strengthening the robustness of our conclusions. Notably, the identification of a history of reproductive disorders and the influence of flock size as significant predictors of *Brucella* spp. infection at the farm/flock level offers practical insights for effective control measures. While recognizing variations in specific prevalence rates, our research underscores overarching trends that resonate with existing literature. This underscores the importance of tailored strategies considering factors such as age, reproductive history, and farm management practices. The incorporation of confidence intervals in our analysis enhances the reliability of the prevalence rates we report. In summary, our study adds to the collective understanding of *Brucella* spp. prevalence, providing valuable insights for informed interventions and control measures in small ruminant populations.

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