BJMHR
British Journal of Medical and Health Research
Journal home page: www.bjmhr.com

Molecular detection of *Brucella abortus* in hygromas and cow's milk in the Chari Department of Chad

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ABSTRACT

Brucellosis is a major notifiable zoonotic disease that affects domestic, wild, and marine animals, as well as humans. It is caused by a bacterial infection of the genus *Brucella*. The objective of this study was to detect *Brucella abortus* in bovine hygromas and cow's milk using conventional Polymerase Chain Reaction (PCR). Two primer pairs were used to amplify DNA fragments present in the collected samples. Gel electrophoresis results showed electrophoretic profiles for the *Brucella abortus* strain at approximately 223 base pairs and another strain at approximately 100 to 120 base pairs. Of 50 hygromas and 50 raw milk samples collected from cattle, 36 samples were PCR-positive, representing an overall prevalence of 72%. The study revealed a co-infection with *Brucella abortus* and *Brucella melitensis,* with a prevalence of 34.0%. Females were more frequently infected with *Brucella abortus* than males, with a rate of 76.2% in females and 50% in males. Transhumant cattle had an infection rate of 83.3%, while sedentary cattle had a rate of 65.6%, resulting in a crude odds ratio of 2.62 (χ^2=1.021; p=0.312). Prevalence ranged from 52.9% (Medina villages) to 87.5% (Shilo camps). The study showed that two strains of *Brucella abortus* circulate in the Chari department with high prevalence. Control and prevention strategies will be necessary for effective management, as brucellosis is included on the Chadian government's list of priority diseases to be monitored and eradicated.

Keywords: Molecular detection, *Brucella abortus*, hygroma, milk, Chad.

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Received 25 May 2026, Accepted 3o June 2026

Please cite this article as: Zachée *B et al*., Molecular detection of *Brucella abortus* in hygromas and cow's milk in the Chari Department of Chad. British Journal of Medical and Health Research 2026.

INTRODUCTION

Brucellosis is a major zoonosis caused by a Gram-negative bacterium of the genus *Brucella*. The species *Brucella melitensis, B. abortus, B. suis, B. canis, B. inopinata, B. ovis, B. neotomae, B. pinnipedialis, B. ceti,* and *B. microti* are isolated from terrestrial domestic animals, wild animals, marine animal species, and humans (Steven and Nammalwar, 2019; Ajana *et al.,* 2022; Aubry and Gaüzère, 2022). Infections caused by *Brucella abortus, Brucella melitensis,* and *Brucella suis* are the most pathogenic to humans and are considered biological weapons due to their low infectious doses (10 to 100 bacteria), their ability to persist in the environment, their rapid transmission via various routes-including aerosols and, finally, their resistance to antibiotic treatment. They are classified as Category B priority pathogens by the Centers for Disease Control (CDC) which is why brucellosis is a notifiable endemic zoonosis, as it affects more than 1.6 to 2.1 million human cases annually worldwide (Ajana *et al.,* 2022; Aubry and Gaüzère, 2022; Lavigne, 2025). The bacterium is present in the biological fluids and secretions of infected individuals, such as milk, blood, urine, hygroma, fetal fluid, feces, and invades organs such as the spleen, lungs, liver, and ovaries (OMS, 2020; Khurana *et al.,* 2021 ; Ajana *et al.*, 2022 ; Aubry et Gaüzère, 2022). Scientific advances, new laboratory diagnostic technologies, the implementation of strict biosecurity measures, and preventive health protocols have made it possible to better control and even eradicate brucellosis (Jansen *et al.,* 2019; Zeybek *et al.,* 2020). In Africa, brucellosis persists in ruminants and is transmitted to humans through several routes of exposure, including the consumption of raw, unpasteurized milk, undercooked meat, direct contact with infected animals, handling of aborted fetuses, and laboratory-related risks associated with handling samples (Garshasbi *et al.,* 2014; Khan *et al.,* 2017; Ajana *et al.,* 2022; Aubry and Gaüzère, 2022). Indeed, PCR proves to be particularly useful when antibiotic therapy prevents the isolation of *Brucella spp*. It enables a faster diagnosis to guide effective intervention strategies in terms of specific medical treatments once the strains are isolated (Lesley-Anne et *al.,* 2017; Daugaliyeva *et al.,* 2024). Molecular studies conducted in sub-Saharan Africa in ruminants and humans have identified *Brucella abortus, Brucella melitensis,* and *Brucella suis* biovars 1, 2, 3, and 6 as the most commonly isolated species (Sidi *et al.,* 2024).

Other underdeveloped African countries face difficulties in using PCR technology due to the high financial costs of acquiring the equipment, kits, and reagents required for this technique, which pose a significant constraint for laboratory researchers (Boukary *et al.,* 2014).

In Chad, the revolutionary molecular biology technique for diagnosing brucellosis remains a challenge for researchers, as the genus and species of *Brucella* circulating within Chad's national territory are not well understood. It is with this in mind that we initiated this first

research project, the objective of which was the molecular detection of *Brucella abortus* in bovine hygromas and cow's milk in the Chari Department of Chad.

MATERIALS AND METHOD

Study Area and duration of the study

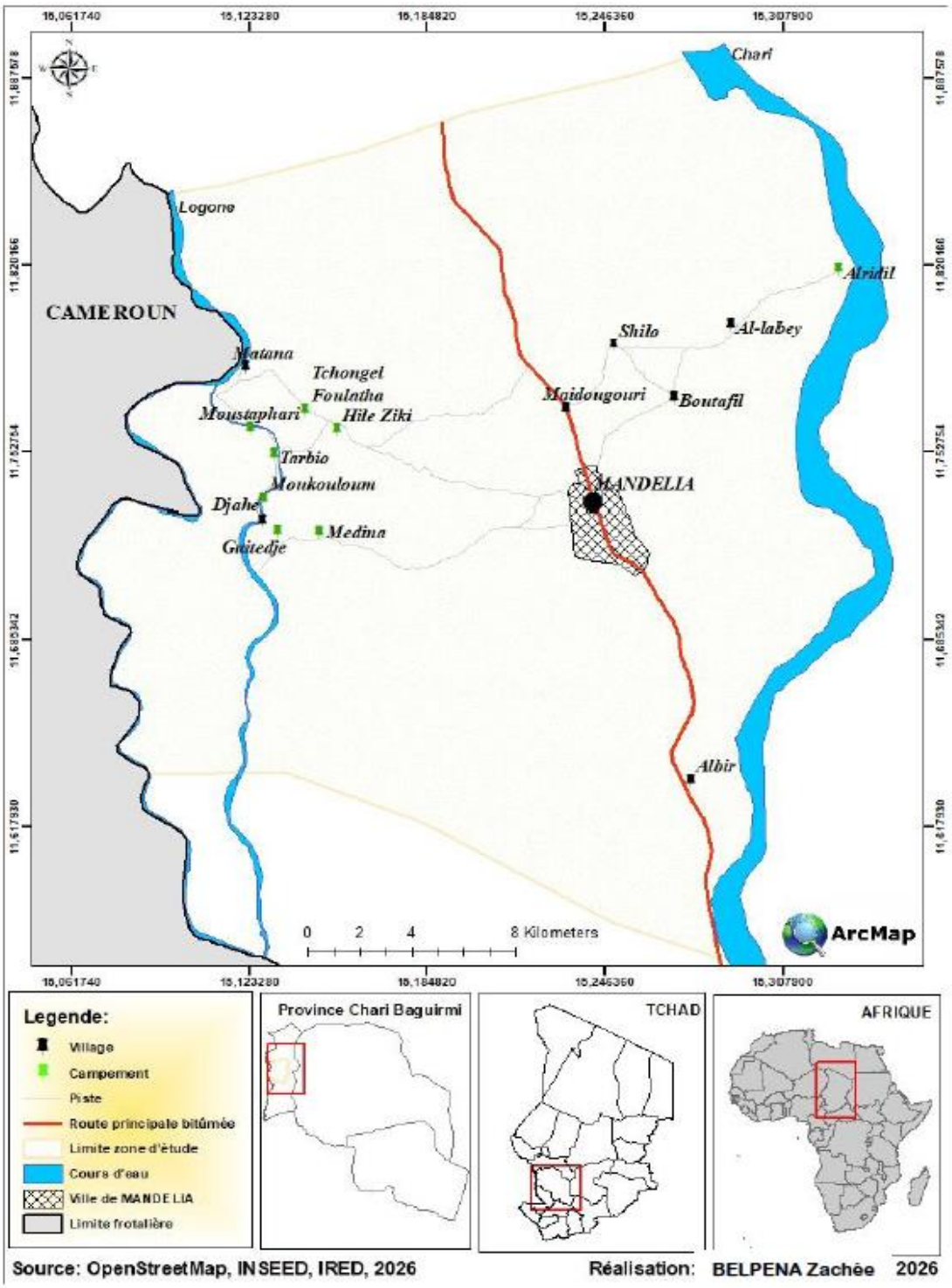

Figure 1: Map of the study area showing the villages, Town and camps visited

The study was conducted from February to March 2023 in 52 pastoralist households in the Mandiagho Canton, Chari-Baguirmi Province, Chad. The Mandelia Prefecture is located between the Chari and Logone rivers, approximately 55.7 km from the capital, N'Djamena.

The department is located in the western part of the country and borders Cameroon. (Figure 1) It is bounded to the far north by Guera, to the south by the Moyen Chari Province, and to the east by the Hadjer Lamis and Bahr El-Ghazel Provinces. It covers an area of (45,432 km²) with an estimated population of 84,615 inhabitants spread across more than 500 villages, resulting in a population density of 32 inhabitants per km². The average temperature is 28.7°C, and average annual precipitation is 726.2 mm.

Sample Collection

Samples of hygromas and milk were collected from Arabian cattle. Hygroma samples (10 mL) were obtained by aspiration from the knee joints and, in some cases, from the hock joints, and collected in 15mL conical tubes. In a herd, depending on the herd size (100 head or more), three (03) cows were selected for milk collection into a sterile 60-ml bottle. When the herd size was less than 50 head, two (02) cows were selected for milk collection. All these samples were stored in an insulated cooler at 4°C and transported to the Bacteriology Laboratory at IRED. A total of fifty hygroma puncture fluids and fifty milk samples were collected in sterile tubes (Figure 2).

Traite de lait dans un flacon stérile

Prélèvement de liquide d'hygroma

Les échantillons des hygromas collectés

Les échantillons de lait de vaches

Figure 2: Showing hygroma sampling and milk collection from cattle.

DNA Extraction

Protocol for Extracting *Brucella* DNA from Bovine Hygroma Fluids

Brucella DNA was extracted from bovine hygromas and milk using the Qiagen extraction kit (*GmbH, Germany*). A volume of 20µl of proteinase K, 10µl of hygroma sample, and 190µl of Phosphate Buffered Saline (PBS) were pipetted and added to each cryotube corresponding to the total number of samples. Next, 200µl of AL lysis solution was added to the same cryotubes. The mixture was incubated for 10 minutes at 56°C in a *BioSan TDB-120 Dry Block Thermostat*. A volume of 200µl of Ethanol (96%) was added to the mixture and then vortexed. The lysate was transferred to a 2ml Mini Column Spin and centrifuged for 1minute at 8,000rpm. The column was placed in a new empty collection tube, and then 500µl of AW1 wash solution was added to the column and centrifuged for 1minute at 8,000rpm. The collected supernatant was discarded, and then 500µl of AW2 wash solution was added to the column and centrifuged for 3minutes at 14,000rpm. The QIAamp Mini centrifugation column was transferred to a 1.5ml Eppendorf tube, and then 200µl of Elution Buffer (AE) was added to the center of the column and incubated for 1minute at room temperature. The DNA was eluted after centrifugation at 8,000 rpm for 1minute. The DNA extracts obtained were stored at -20°C for future use (IRED SOP, 2025a).

Protocol for Extracting *Brucella* DNA from Cow Milk

Milk samples (2mL) were pipetted and centrifuged at 10,000 rpm for 15 minutes at 4°C to obtain a bacterial concentration in the pellet. The upper lipid layer containing the cream was carefully removed, and the pellet was resuspended in 1mL of PBS, vortexed, centrifuged again, and the supernatant removed.

A volume of 200µl of Buffer AL and 20µl of Proteinase K were added to the pellet, mixed by vortexing for 15 seconds, and incubated at 56°C for 30 minutes in a *BioSan TDB-120 Dry Block Thermostat*. After incubation, 200 µl of Ethanol (96%) was added to the solution and vortexed again for 15seconds. After the DNA was precipitated by the Ethanol, the AL/Proteinase K/Ethanol mixture was transferred to the QIAamp column in a collection tube and then centrifuged at 8,000rpm for 1minute to remove the supernatant. The AW1 wash solution was added to the collection tubes at 500µl and centrifuged at 8,000 rpm for 1minute to remove the supernatant, while the AW2 wash solution was pipetted at 500µl and centrifuged at 14,000rpm for 3minutes. An additional 1minute centrifugation was performed to remove traces of Ethanol, and the membrane was dried.

Finally, *Brucella* DNA was collected by eluting 100µl of Buffer (AE) onto the membrane, incubating it for 3minutes at room temperature, and then centrifuging it at 8,000rpm for 1minute (IRED SOP, 2025b).

Amplification of DNA Extracts

The sequence primers listed in Table 1 below were used to amplify DNA extracted from hygromas and milk.

Table 1: Primers used to amplify DNA extracts from hygroma samples

N°	Primer code	Nucleotide sequences	Target band
1	BAbort-F1	5'-TGC CGA TCA CTT AAG GGC CTT CAT-3'	498bp
	BAbort-R1	5'- GAC GAA CGG AAT TTT TCC AAT CCC-3'	
2	BAbort-F2	5'- TGG CTC GGT TGC CAA TAT CAA-3'	223bp
	BAbort-R2	5'- CGC GCT TGC CTT TCA GGT CTG-3'	

The BAbort-F1/BAbort-R1 primers were used to prepare the amplification reaction by pipetting 21µl of Master Mix (dATP, dCTP, dTTP, and dGTP; KCl; $MgCl_2$; *Taq polymerase*) 1µl of each primer, and 2µl of the DNA extract, resulting in a total reaction volume of 25µl. The PCR tubes containing the reactions to be amplified were placed in a *BIORAD T100TM Thermal Cycler* and programmed according to the different amplification phases of the reaction. Polymerase activation was performed at 95°C for 3minutes, standard DNA denaturation at 95°C for 1minute, annealing at 63°C for 1minute, extension at 72°C for 1minute, and final extension at 72°C for 10 minutes. The reaction volume was set to 25µl, and the reaction cycle was 35, according to Koichi *et al.* (2007).

For BAbort-F2/BAbort-R2, the Master Mix volume was 20.5µl. Then, 1µl of each primer and 2.5µl of DNA were added. The amplification conditions were as follows: polymerase activation was performed at 95°C for 5minutes, standard DNA denaturation at 95°C for 1minute, annealing at 57°C for 1minute, extension at 72°C for 1minute, and final extension at 72°C for 5 minutes. The reaction volume was 25µl, and the number of reaction cycles was 35, according to Mol *et al.* (2020).

Gel Electrophoresis

A 1.5% gel was prepared by measuring out 1.88g of Agarose powder and 125mL of 1X Tris-Borate-EDTA (TBE) Buffer. The mixture was heated in a microwave for 1 minute and 30 seconds to obtain a homogeneous solution. After cooling, 3µl of *GrenGel DNA/RNA* stain (containing Ethidium Bromide) was pipetted into the solution and then poured onto the gel plate containing the combs. After 45minutes, the gel had solidified, and the combs were removed, leaving the wells for loading the PCR products or amplified DNA.

In a tank, 1.5liters of 1X concentrated TBE solution was poured, and the solidified gel was placed in the solution, ensuring the migration direction was from the negative terminal to the positive terminal. The Loading Dye reagent (2µl) was pipetted and mixed with 12µl of amplified DNA, then loaded into the gel well. *The FastRuler Middle Range (MR) 500-bp size marker* was used as a reference. The *Cleaver Scientific nanoPAC-300P voltmeter* was set to 110 amperes and the gel was run from the negative (-) pole to the positive (+) pole for up to

45minutes. The gel was imaged using an *Analytik Jena UVP Transilluminator (UVP PhotoDoc-It™ Imaging System)*.

RESULTS AND DISCUSSION

Molecular Detection of *Brucella abortus* in Bovine Hygromas and Milk

Molecular identification of *Brucella abortus* was carried out on 50 DNA extracts from hygromas and 50 DNA extracts from milk of Arabian cows. The primer pair BAbort-F2/BAbort-R2 successfully amplified a *Brucella abortus* DNA fragment of approximately 223 base pairs in samples no. 10, 12, and 14, with the bands visible but of low intensity. A positive control and a negative control were used to confirm the reaction (Figure 3).

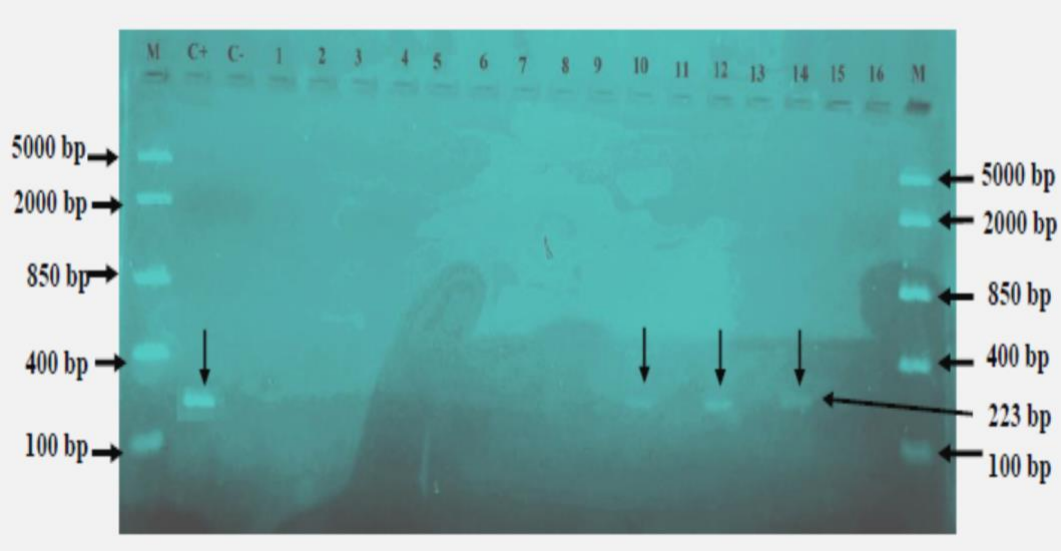

Figure 3: Electrophoretic profile showing amplified hygroma DNA bands in cattle.

M : Marqueur FastRuler Midlle Range DNA ladder mix (Thermo Scientific) ; From 1 to 16 amplicon of hygroma. C+ : Positif Control, C- : Negatif Control.

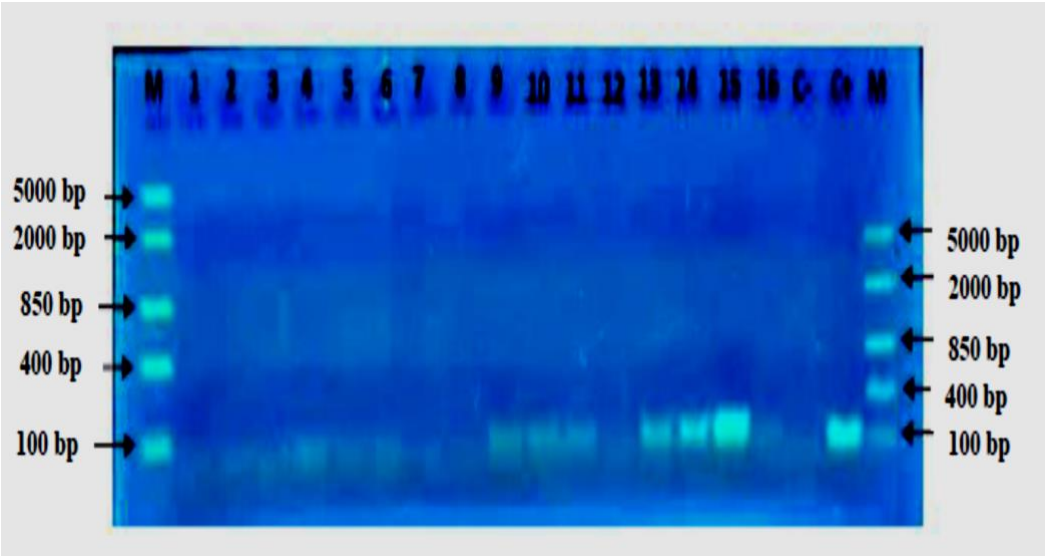

Figure 4: Electrophoretic profile showing amplified hygroma DNA bands in cattle

The primer pair BAbort-F1/BAbort-R1 was also used to confirm the presence of *Brucella abortus* in DNA extracts from hygromas and milk. The expected band size of the strain was 498 base pairs. Gel migration after amplification showed that samples no. 13, 14, and 15 were positive, with a target band fragment of approximately 100 to 120 base pairs. Samples in wells no. 4, 9, 10, and 11 showed weaker bands but were still positive for *Brucella abortus* (Figure 4).

*M : Marqueur FastRuler Midlle Range DNA ladder mix (Thermo Scientific) ; From 1 to 8 amplicon of hygroma; From 9 to 16 amplicon of milk ; C+ : Positif Control, C- : Negatif Control.***Prevalence of *Brucella abortus* in Bovine Hygromas and milk**

The results in Table 2 show the proportions of *Brucella abortus* and *Brucella melitensis* strains isolated in the Chari Department. Out of 50 hygroma samples collected from cattle, 36 were positive by conventional PCR, corresponding to an overall prevalence of 72%. Among these, 30.0% (95% CI: 19.1-43.8%) of animals were positive for *Brucella abortus* alone, 8.0% (95% CI: 3.2–18.8%) were infected with *Brucella melitensis* only, while co-infection with *B. abortus* + *B. melitensis* was the most frequently detected strain (34.0%, 95% CI: 22.4-47.8%). The PCR-negative rate was 28.0% (95% CI: 17.5-41.7%).

Table 2: Prevalence of *Brucella abortus* in bovine hygromas and milk

Isolated strain	NT	P(%)	IC 95% (Wilson)	% Negatif
Brucella abortus	15	30.0%	19.1%- 43.8%	—
B. abortus + B. melitensis	17	34.0%	22.4% -47.8%	—
Brucella melitensis	4	8.0%	3.2%-18.8%	—
Negative for both strains	14	28.0%	17.5%-41.7%	100%
Total positifs	36	72.0%	58.3%-82.5%	—

NT : Total Number ; P(%) : Percentage ; IC : Confidence Interval

Prevalence of *Brucella abortus* according to Risk Factors in the Mandelia Prefecture.

Risk factors such as sex, age, farming system, and location were considered in this study. The results show that the prevalence was 76.2% in females compared to 50.0% in males (χ^2 = 1.172; p=0.279). Although the difference is biologically plausible, as females are more exposed to *Brucella abortus* infection during gestation and reproduction, it was not statistically significant, likely due to the small number of males (n = 8) included in this study. Regarding the farming system, transhumant animals had a prevalence of 83.3%, higher than sedentary animals (65.6%), with a crude odds ratio of 2.62 (χ^2 = 1.021; p = 0.312). This indicates that transhumant animals are twice as likely to be exposed to *Brucella abortus* infection as sedentary ones. The high infection rate among cattle in transhumant systems can be explained by several factors. During transhumance, animals are continuously exposed and at risk of contamination. Infected animals deposit feces, urine, and other biological secretions

on pastures and in water sources, which promotes the multiplication and spread of *Brucella* bacteria, facilitating widespread contamination of other herds during their movement.

Considering the study sites, prevalence varied, with the highest proportion in Shilo village (87.5%), followed by Alridil camp (83.3%) and Boutafil village (71.4%). Médina camp showed a rate of 52.9%. The chi-square test with 3 degrees of freedom was not significant ($\chi^2 = 5.164$; $p = 0.160$), but Médina stood out as the least affected site.

In terms of age, cattle aged 6-8 years had an *Brucella abortus* infection rate of 80.0%, while those older than 12 years had a prevalence of 75.0%. In contrast, cattle aged 9-11 years showed a prevalence of 66.7%. Brucellosis is a reproductive disease, so the peak infection of 80.0% among cattle aged 6-8 years in this study can be explained by the fact that animals at this age are in full reproductive activity. Indeed, *Brucella abortus* preferentially localizes in the placenta, testes, and mammary glands.

In cattle aged 9 to 11 years, the *Brucella abortus* infection rate of 66.7% can be explained by several factors related to physiology, the immune system, the persistence of *Brucella*, and the dynamics of its transmission within the herd. Indeed, having spent many years in the herd could increase the likelihood of repeated exposure to the bacterium. Moreover, the longer the contact time with infected animals, the higher the cumulative risk of infection. Between 9 and 11 years of age, cows may have had multiple calvings or experienced infectious abortions, thereby releasing a large number of bacteria into the environment and promoting contamination of other cattle. The mere fact that older cattle can become chronic carriers constitutes a major source of infection for younger animals, as well as serving simultaneously as a reservoir within the herd.

Table 3: Prevalence of *Brucella abortus* According to Risk Factors

Variable	Modality	Pos/N	P(%)	OR brut	IC 95% (Woolf)	p (Fisher)	Interpretation
Sex-ref. = Male							
Sex	Male	4/8	50,0%	1,00(réf)	—	—	Reference
	Female	32/42	76,2%	**3,20**	0,67-15,19	0,197	NS
Farming system-ref. = Sedentary							
Livestock	Sedentary	21/32	65,6%	1,00(réf)	—	—	Ref.
	Transhumant	15/18	83,3%	**2,62**	0,62-11,04	0,211	NS
Study site — ref. = Médina (lowest prevalence)							
Site	Médina	9/17	52,9%	1,00(réf)	—	—	Ref.
	Boutafil	5/7	71,4%	**2,22**	0,33-14,80	0,653	NS
	Alridil	15/18	83,3%	**4,44**	0,93-21,22	0,075	Tendance(p≈0,08)
	Shilo	7/8	87,5%	**6,22**	0,62-62,16	0,182	NS
Age group — réf. = 9–11 years							
Âge	9–11 years	16/24	66,7%	1,00(réf)	—	—	Ref.
	6–8 years	8/10	80,0%	**2,00**	0,34-11,70	0,683	NS
	≥ 12 years	12/16	75,0%	**1,50**	0,36-6,17	0,729	NS

Pos/N: Positif /Negatif ; P(%) : Percentage ; IC : Confidence Interval; OR: Odds Ratio; P: Probability

DISCUSSION

The 223-base-pair *Brucella abortus* strain was detected in hygroma and milk samples from cows in this study. These results could be explained by the fact that PCR is a method that amplifies and quantifies DNA in each cycle, yielding results through the detection of emitted fluorescence that is proportional to the amount of DNA from the detected strain. However, it does not allow for the precise quantification of the number of sequences of interest (Marion Holzapfel, 2018). Nevertheless, the conventional or classical PCR method enables the amplification of the target sequence and the detection of amplicons via electrophoresis, a well-established technique (Marion Holzapfel, 2018). The gene sequence specifically encoding the BCSP31 protein, which targets *Brucella spp*, from the BAbort-F2/BAbort-R2 primer pair, and the *IS711* gene sequence from the BAbort-F1/BAbort-R1 primer pair were used to confirm the presence of *Brucella abortus* in the hygromas and milk of cows in this study. The PCR method assay enabled us to detect a 223-base-pair amplicon of *Brucella abortus*. These results are supported by several authors who detected *Brucella abortus* in cattle using the same primers as ours in their studies (Ingle *et al.,* 2023; Himeidou *et al.,* 2025).

Our results are consistent with those of molecular studies conducted in cattle by Garshasbi *et al.* (2014) and Thorat *et al.* (2017) and may be explained by the fact that *Brucella abortus* infects cattle. Furthermore, according to Holzapfel (2018) and López-Goñi and Moriyón (2005), the primer sequence used in this study encodes a gene specific to a 31-kDa protein present in the outer membrane of *Brucella* (BCSP31) and found in all *Brucella* species and biovars, it is the most common molecular target in clinical applications. These findings may explain the success of the 223-base-pair amplicon obtained in our study.

Our PCR amplification reactions yielded amplicons ranging from 100 to approximately 120 base pairs. Our results are roughly identical to the 99-base-pair amplicons reported by Alamian *et al.* (2017) in Iran and similar to the 113-base-pair amplicons published by Alwan *et al.* (2010) and Dehkordi *et al.* (2012). However, our results are lower than those of 178 base pairs and 180 base pairs obtained by Moussa *et al.* (2011) and Ardiyanto *et al.* (2015), respectively.

An overall prevalence of 72% for the *Brucella abortus* strain was detected in hygroma and milk samples from cows in this study using conventional PCR. These results are lower than the 82.6% reported by Saadat *et al.* (2017) in Iran but higher than the 62.3% reported by Lesley-Anne *et al.* (2017) in South Africa. The results of research published by Dehkordi *et*

al. (2012) in Iran, Lita *et al.* (2025) in South Sudan, Mahmood *et al.* (2016) in Pakistan, Sarker *et al.* (2018) in Bangladesh, Ntivuguruzwa *et al.* (2022) in Rwanda, and Himeidou *et al.* (2025) in the region. The prevalence rates reported in the peri-urban areas of Bamako are lower than our results, which showed prevalence rates of 31.5%, 20%, 21%, 21.43%, 16.7%, and 22.3% in blood, milk, and hygroma samples from cattle, respectively. The high prevalence of *Brucella abortus* in this study is likely correlated with the high serological contamination of cattle reported by Zachée *et al.* (2025) in Chad who show that a circulation of *Brucella* in this area is very important. The tendency of livestock farmers to frequently gather their animals in a communal pen in the village or often herd them together to spend the night poses a very serious risk of *Brucella* infection. Furthermore, the clinical signs observed-notably hygromas, mastitis, orchitis, and abortions as well as the cases of infertility reported by farmers in their herds during the field survey are believed to be linked to this prevalence.

Brucella abortus alone accounted for 30.0% of cases in cattle in this study. These results are higher than the 21.56% and 23.5% rates reported by Dehkordi *et al.* (2014) in Iran and Thenamutha *et al.* (2017) in Malaysia, but lower than the 33.3% and 56.9% reported by Lita *et al.* (2025) in South Sudan and Lesley-Anne *et al.* (2017) in South Africa. The results of 60.5% and 80% published by Akoko *et al.* (2021) in Kenya and the findings of Awais *et al.* (2024) in the peri-urban and rural areas of Multan District, in southern Punjab, Pakistan, are significantly higher than our results.

This study reported an infection rate of 8.0% for *Brucella melitensis* alone. While a *Brucella abortus* + *Brucella melitensis* co-infection rate of 34.0% represented the most frequently isolated strain. Compared to our results, studies by Akoko *et al.* (2021) in Kenya reported a high prevalence of 16.3% for *Brucella melitensis* in cattle, whereas a low rate of 5.45% for co-infection was found (Awais *et al.,* 2024) in Pakistan. Thenamutha *et al.* (2017) in Malaysia found a 23.5% rate of mixed infection.

The low rate of *Brucella melitensis* infection observed in cattle in our study is normal and can be explained by the fact that *Brucella melitensis* has sheep and goats as its preferred and specific hosts. However, given that livestock farming in Africa, and in Chad in particular, is mixed and extensive, it is possible that direct and constant contact between cattle and small ruminants due to the sharing of the same watering and grazing areas may facilitate the transmission of *Brucella melitensis* to hosts. These same findings were reported by Parthiban *et al.* (2019) in India and by OMSA (2022).

The prevalence of *Brucella abortus* was 76.2% among females, compared with 50.0% among males. These results are lower than the 32.6% prevalence among females and 42.2% among males reported by Akoko *et al.* (2021) in Kenya. The high prevalence of *Brucella abortus*

among females in this study is likely due to the fact that brucellosis is a reproductive disease; consequently, females are much more susceptible and develop the disease rapidly, leading to abortions. In addition, the sample size of females collected in the field was larger than male cattle. Furthermore, in most cases, female animals in livestock operations are kept in the herd for breeding, which could also be the cause of the observed prevalence.

In this study, transhumant cattle (83.3%) were twice as likely to be infected with *Brucella abortus* (odds ratio = 2.62) as sedentary cattle (65.6%). The results of 87.5% among cattle in sedentary herds published by Himeidou *et al.* (2025) in Mali are higher than our results. Arasoğlu *et al.* (2013) reported a prevalence of 95% in an extensive cattle herd in Turkey. Additionally, Akoko *et al.* (2021) in Kenya reported that 18.9% of cattle in an extensive herd were infected with *Brucella abortus*. There are several reasons for the high infection rate among cattle in transhumant herds. During transhumance, the animals are exposed and at risk of infection at all times. Infected animals excrete feces, urine, and other biological secretions on pastures and in river water or ponds. In these areas, conditions are conducive to the proliferation of germs, facilitating widespread contamination of the herd.

According to the study areas, the village of Shilo has a high *Brucella abortus* infection rate of 87.5%, followed by the Alridil settlement at 83.3% and the village of Boutafil at 71.4%. These results are higher than the 100% rate of *Brucella abortus* strains isolated in peri-urban areas of Bamako, Mali, by Himeidou *et al.* (2025), but significantly lower than those of 12.68% were found in Zhongwei, 14.95% in Shizuishan, 20.43% in Yinchuan, 22.03% in Wuzhong, and 29.91% in Guyuan across the region of China. Indeed, given that brucellosis is a contagious disease and that a heterogeneous distribution of high bacterial loads was observed in each locality, this could likely be the cause of the high prevalence observed. Furthermore, wind, rain, animal movements, aborted fetuses left in the open, and urine and feces deposited on the ground whether on pastures or in water can facilitate the transmission and spread of *Brucella*. This is why, when a herd in a village or camp is found to be positive for *Brucella*, the entire area is at risk.

CONCLUSION

This initial molecular detection study using conventional PCR identified *Brucella abortus* and *Brucella melitensis* circulating among cattle in the Chari Department of Chad. These two strains were detected with high prevalence in hygromas and milk. However, cases of co-infections with *Brucella abortus* and *Brucella melitensis* were also identified in these animals, highlighting the significance of brucellosis as a major zoonosis. The study revealed that Arabian and female cattle were most exposed to *Brucella abortus*. This high rate is due to the lack of vaccination of the animals, inadequate epidemiological surveillance, and the

failure of livestock farmers to implement biosecurity measures, all of which have contributed to this prevalence in the Chari department.

ACKNOWLEDGEMENTS

This study was made possible thanks to funding from the Institute for Livestock Research and Development (IRED). We would like to extend our sincere thanks to Dr. NGANDOLO BONGO NARÉ and to Dr. MAHAMAT NGUINDÉ, Director General of IRED for providing us with the complete molecular biology kits.

Conflicts of Interest:

The authors declare that they have no conflicts of interest related to this study.

Author Contribution Statement:

The project was designed by BZ. Data collection was performed by BZ, and data analysis was conducted by NKA and supervised by NBNR and BBA. Laboratory analyses were performed by BZ, NKA, and DS. Statistical analyses were performed by LGR and DF. Interpreted by LRG, BZ, and NBNR. The first draft was prepared by BZ. All authors contributed to the refinement of the manuscript, proofreading, and the final version of the manuscript.

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