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Molecular evidence of *Brucella abortus* circulating in cattle, goats, and humans in Central Equatoria State, South Sudan

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Brucellosis is a neglected zoonotic disease in most developing countries, including South Sudan. Precise identification of *Brucella* species is crucial for addressing public health and epidemiological concerns associated with brucellosis. The study aimed to identify *Brucella* species using real-time polymerase chain reaction (qPCR) from seropositive samples that were acquired from an earlier investigation. A total of 143 genomic DNA samples were extracted from brucellosis Rose Bengal plate test (RBPT) seropositive samples from humans (n = 7), cattle (n = 103) and goats (n = 33). The samples were collected from Terekeka and Juba counties, Central Equatoria State (CES), South Sudan. The qPCR targeting the *Brucella*-specific IS*711* insertion gene at the genus level was performed. Samples with a cycle threshold (Ct) of ≤ 35 were considered positive and subjected to further *Brucella* speciation assays. Out of 143 DNA samples tested for genus-specific *Brucella*, 15 (10.5%) were positive including 4 (2.8%) from humans, 10 (6.9%) from cattle, and 1 (0.7%) from goats. *Brucella abortus* was identified in 5 (33.3%) of the positive samples at the genus level. The overall individual species infection rates with *B. abortus* were 6.6% (1/15) in humans, 20% (3/15) in cattle, and 6.6% (1/15) in goats. There was no *B. melitensis* detected in this study. This study identified *B. abortus* in cattle, goats and humans in CES, South Sudan. The findings suggest that cattle are probably the primary reservoirs for transmission of *B. abortus*, with infections occurring in goats and humans primarily resulting from cattle spillover.

Keywords *Brucella abortus*, Spill-over, Brucellosis, Cattle, Goats, Humans, South Sudan

Brucella species, the etiological agents of brucellosis, remain the major global zoonotic pathogens responsible for enormous economic losses and human morbidity in several endemic countries[1]. The overall burden of brucellosis remains under-estimated and neglected[2]. The 500,000 globally reported annual cases of human brucellosis are under-estimated[3]. The global incidence of brucellosis has been estimated at 2.1 million cases per year[4].The under-estimation is due to under reporting, misdiagnosis caused by non-specific clinical symptoms, lack of physician awareness, and laboratory capacity for diagnosis. The genus *Brucella* comprises 12 recognized species, each varying according to host preference, biochemical characteristics, and virulence[5].

The main *Brucella* spp. that affect livestock species include *B. abortus (cattle), B. melitensis* (goats and sheep), *B. suis* (domestic pigs) and *B. ovis* (sheep)[6]. Humans usually get infected by *B. melitensis*, which causes the most severe disease, *B. abortus*, *B. suis*, and *B. canis*[7]. Each *Brucella* species has a primary host, but *B. suis* has been

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reported in cattle[8] and spillover of *B. abortus* and *B. melitensis* into camels from small ruminants and cattle have been reported[9].The chances of cross-species transmission often increase in herds with different livestock species, such as cattle, sheep, and goats as is the case in most developing countries[10].

Brucella infection is transmitted between animals and humans by contact with infected materials like aborted fetuses, placentas, fetal fluids, and vaginal discharges; by ingestion of contaminated animal products as well as inhalation of the agent[11]. *Brucella* infection and transmission can be prevented by vaccination of young animals with *B. abortus* S19 or RB51 vaccine strains for cattle and *B. melitensis* Rev 1 vaccine for small ruminants[5]. Human brucellosis is almost exclusively associated with transmission from animal reservoirs. Thus, when brucellosis is controlled in the animal reservoirs, there is always a significant decline in the incidence in humans[12]. Mass vaccination of livestock against brucellosis offers substantial benefits to both agricultural and health sectors, leading to healthier animals, reduced human incidences, reduced treatment costs, and minimized financial burden on health care systems[13,14].

The gold standard test for diagnosing brucellosis is the isolation of *Brucella* bacteria from blood, tissues, or other body fluids. However, *Brucella* cultures have low sensitivity, are time-consuming, require specialized laboratory facilities, are not always successful and also present risk of infection to humans collecting samples and laboratory personnel[5,15]. Therefore, molecular techniques such as qPCR are now widely used due to their reliability, rapidity, specificity, and increased sensitivity for pathogen DNA detection[16–19]. Molecular identification of *Brucella* at the genus level is done by targeting specific gene sequences such as, the IS*711* insertion sequence, 16S-23S ribosomal RNA operon, 31-kDa protein, and bcspP31[15,20]. The IS*711*-based qPCR is a specific, highly sensitive, efficient, and reproducible method for rapid detection of the genus *Brucella*[21].

Comparatively, the qPCR is widely used, it is rapid and more sensitive than conventional PCR[17]. Moreover, it doesn't require post-amplification handling of PCR products, reducing the risk of laboratory contamination and false-positive results. However, it also has potential drawbacks, such as cost, specificity of primers, optimization, and sample quality requirements[22]. Additionally, most of the PCR-based methods used to detect brucellosis were developed using *Brucella* spp. DNA prepared directly from cultured bacteria or extracted from the culture. Hence, their sensitivity and specificity are not well established and their real value of use with clinical samples and diagnosis has not been validated[23].

Several studies have been conducted to investigate the molecular epidemiology of brucellosis in East Africa. In Tanzania, molecular studies have highlighted the presence of both *B. abortus* and *B. melitensis* in livestock with significant human cases reported particularly in rural areas where close contact with livestock is common[24–26]. In Kenya, studies have shown that *B. abortus* is more common in cattle, while *B. melitensis* is predominant in goats and sheep[27,28]. Both *B. abortus* and *B. melitensis* DNA were detected in humans and multiple livestock host species, suggesting cross-transmission in Kenya[27]. Knowing the circulating species of *Brucella* is a cornerstone for successfully controlling the disease.

In South Sudan, brucellosis has been reported in cattle and humans in the Bahr el Ghazal region in the north-western part[29,30] and cattle, goats, and humans in the CES[31–33]. The seroprevalence of brucellosis reported in South Sudan in humans ranges from 4.8%–46.0%[32,34], in cattle from 21.7%–23.2%[30,31,33] and in goats at 11.8%[33]. Controlling brucellosis infections in livestock is the most effective means of mitigating human brucellosis[35]. So far, there is no information regarding the type of *Brucella* species responsible for the infections in livestock and humans in CES, South Sudan. Therefore, this study used molecular methods to identify the *Brucella* species accountable for infections in livestock and humans in Terekeka and Juba counties, CES, South Sudan.

Methods and materials

Study area

The study was conducted in Terekeka and Juba counties of CES, South Sudan. Both counties were purposively chosen because of a huge livestock population and pastoral activities. In Terekeka County, three payams (villages) namely Reggo, Nyori, and Terekeka were selected due to presence of huge cattle camps as indicated by Lita et al.[33]. In South Sudan, cattle camps are traditional, rural, and temporary settlements where pastoralist communities raise and manage their animals including cattle, sheep, and goats. The camps are often located in remote areas, close to grazing lands, water sources, and seasonal migration routes[36]. In cattle camps, herding is usually done collectively, with some camps having 3,000—10,000 head of cattle, involving 30 – 80 families[36,37]. A payam is the second-lowest administrative division, below counties, in South Sudan. Terekeka County is located on both the east and west banks of the White Nile River north of Juba, as indicated by Lita et al.[33]. A total of 17 cattle camps were randomly selected, of which 13 were from Terekeka County and four from Juba County as shown in Fig. 1.

Sample collection

A total of 986 sera, from humans (n = 143), cattle (n = 478), sheep (n = 86), and goats (n = 279) were randomly collected from 17 cattle camps in CES as indicated by Lita et al.[33]. This study included only Rose Bengal plate test (RBPT) seropositive samples from cattle (n = 103), goats (n = 33) and humans (n = 7). All the sheep serum samples (0/86) tested negative on RBPT as reported by Lita et al.[33].

Extraction of genomic DNA

Genomic DNA was extracted from serum samples that were positive for RBPT as reported by Lita et al.[33]. The 143 sera, were subjected to genomic DNA extraction using Quick-gDNA™ Blood MiniPrep (Zymo Research, USA) kit. The concentration and purity of the extracted DNA were assessed by using a Nanodrop spectrophotometer (Biochrom LTD, Cambridge, England) at 260 nm and 280 nm.The extracted DNA was stored at—80 °C until further analysis.

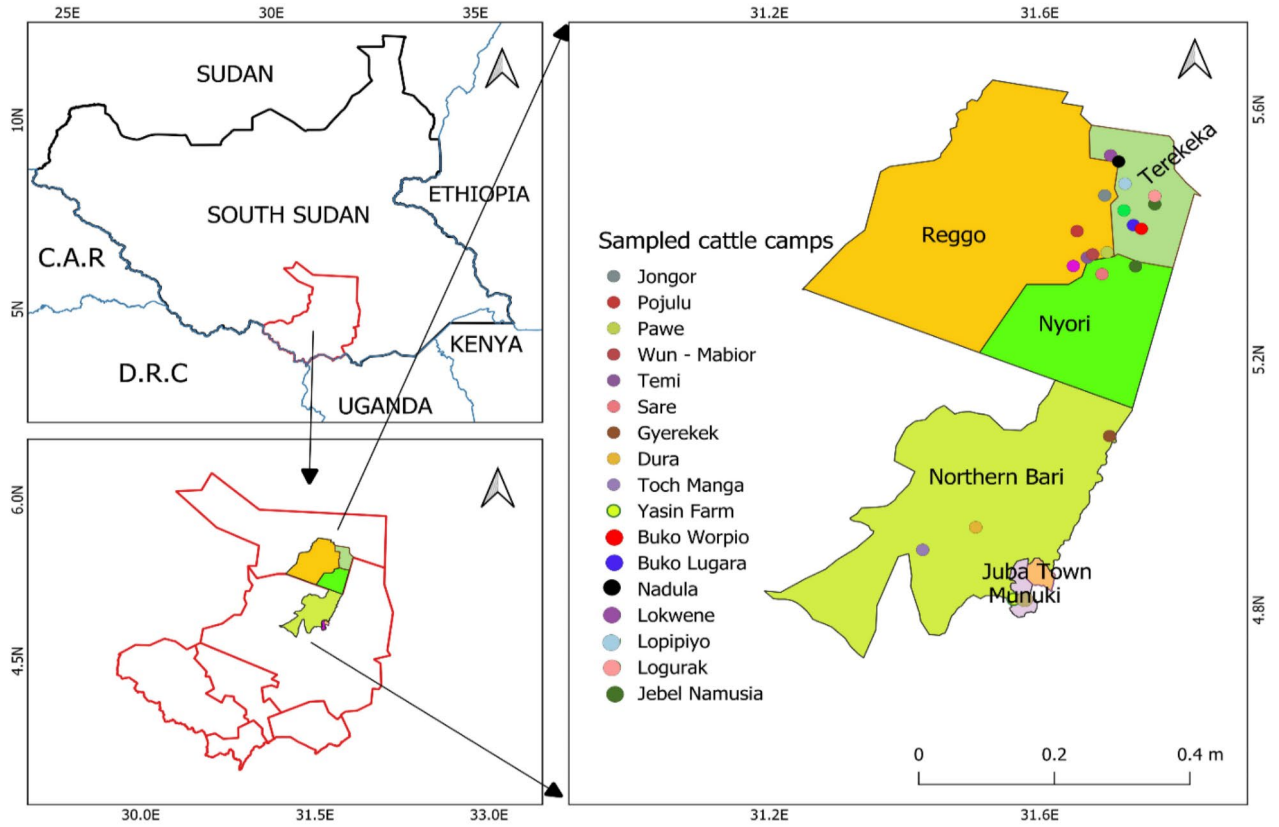

Fig. 1. Map of South Sudan showing the study areas in Juba and Terekeka counties, Central Equatoria State, South Sudan. Source: Lita et al.[33] Map generated using QGIS software version 3.32.1. Shapefiles for administrative boundaries from Humanitarian Data Exchange https://data.humadata.org/dataset/cod-ab-ssd.

Brucella genus-specific DNA identification

The genus *Brucella* was identified by targeting the IS*711* gene specific to *Brucella* using qPCR primers (Forward: GCTTGAAGCTTGCGGACAGT, Reverse: GGCCTACCGCTGCGAAT, IS*711*-FAM probe: AAGCCAACACC CGGCCATTATGGT). The IS*711*-based qPCR assay is a specific, sensitive, efficient, and reproducible method for the rapidly detecting of the genus *Brucella*[21]. The qPCR reaction was carried out in a total volume of 20 µl, containing 10 µl of 2X Prime-Time Gene Expression Master Mix (Integrated DNA Technologies (IDT)), Primer/ Probe assay 1 µl, DNA template 2 µl and nuclease-free water 7 µl. The reaction was performed in the Applied Biosystems® 7500/7500 fast real-time PCR apparatus using the following parameters, initial activation at 95 °C for 3 min followed by 35 cycles. Each cycle comprised of denaturation at 95 °C for 30 s followed by annealing and extension at 60 °C for 30 s. *Brucella abortus* S19 and *B. melitensis* were used as positive controls. In the negative control, nuclease-free water was used instead of DNA. Samples with a cycle threshold (Ct) of ≤ 35 were considered positive and subjected to further *Brucella* speciation assays.

Species-specific identification of *Brucella*

All positive samples at the genus *Brucella* IS*711*gene amplification were further subjected for speciation using qPCR assay as described by[20]. The assay used species-specific primers for *B. abortus* (Forward: GCACACTCAC CTTCCACAACAA, Reverse: CCCCGTTCTGCACCAGACT, *Brucella abortus* FAM probe: FAM-TGGAACGA CCTTTGCAGGCGAGATC) and *B. melitensis* (Forward: TCGCATCGGCAGTTTCAA, Reverse: CCAGCTTT TGGCCTTTTC, *Brucella melitensis* FAM probe: CCTCGGCATGGCCCGCAA).

The tests were performed with the following PCR condition, initial activation at 95 °C for 3 min followed by 35 cycles. Each cycle comprised of denaturation at 95 °C for 30 s followed by annealing and extension at 60 °C for 30 s. *Brucella abortus* and *B. melitensis* positive controls, negative control, and templates were loaded in duplicates. *Brucella. abortus* S19 and *B. melitensis* were used as positive controls. In the negative control, nuclease free water was used instead of DNA. The thresholds were set using the auto-baseline and threshold feature in 7500 software for 7500 and 7500 fast real-time PCR apparatus version 2.0.4 (Applied Biosystems®). Any sample with a cycle threshold (Ct) of ≤ 35 on the speciation assay was considered positive.

Data analysis

Data were analyzed using Statistical Package for Social Sciences (SPSS) version 25. Descriptive statistic was run to obtain the frequency distribution and percentages.

Variable	Category	Number of DNA tested	IS711 qPCR positive (*n*/*N*) %	Species-specific qPCR
Species	Cattle	103	10 (10/143)	3
	Goats	33	1 (1/143)	1
	Humans	7	4 (4/143)	1
Total		143	15 (15/143)	5

Table 1. Detection of *Brucella* species from DNA extracted from seropositive blood samples using real-time PCR.

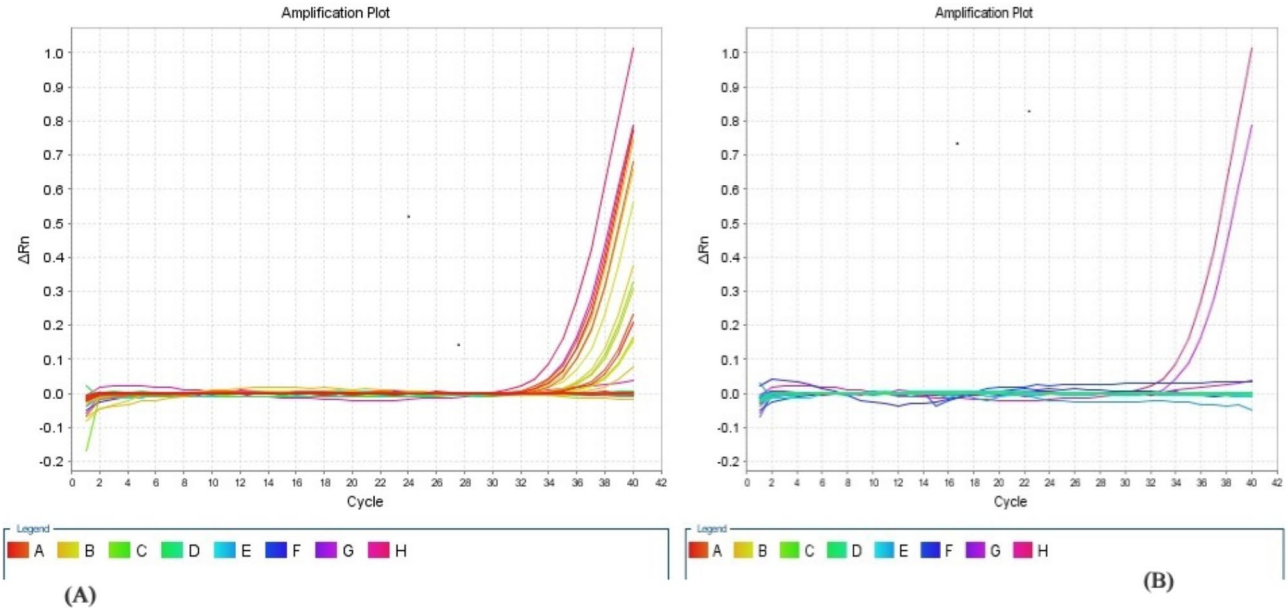

Fig. 2. Amplification plots of the real-time PCR for speciation *Brucella* spp. (**A**): *B. abortus* and *B. melitensis* (**B**).

Ethical clearance

The study methods were carried out following ARRIVE guidelines https://arriveguidelines.org. The study protocol for the animal subjects was approved by the Institutional Review Board of Sokoine University of Agriculture under reference number (DPRTC/R/186/16) as indicated by Lita et al.[33]. All methods were performed following the relevant guidelines and regulations. The study protocol for the human subjects was approved by the National Ministry of Health Research Ethics Review Board (RERB-P No: 13/14/02/2023), South Sudan. All methods were performed by the relevant guidelines and regulations. Moreover, Export and Import permits for shipment of the biological samples were obtained from the National Ministry of Livestock and Fisheries, South Sudan, and the Ministry of Livestock and Fisheries, United Republic of Tanzania. Informed consent was obtained from each participant before data collection.

Results

Brucella genus-specific IS*711* gene amplification

A total of 143 genomic DNA was extracted from cattle (n = 103), goats (n = 33), and humans (n = 7). Out of 143 DNA, 10.5% (15/143) were positive at the genus level using genus-specific IS*711* qPCR. The number of *Brucella*-positive reactors from the previous study[33] were 103, 33 and 7 for cattle, goats, and humans, respectively as shown in Table 1. The individual animal species prevalence was 6.9% (10/103) for cattle and 0.7% (1/143) for goats. In contrast, the infection rate in humans was 2.8% (4/143), as shown in Table 1.

Species-specific identification of *Brucella*

Out of the 15 positive samples, five (33.3%) samples were found to be positive for *B. abortus* which included 6.66% (1/15) in humans, 20% (3/15) in cattle, and 6.66% (1/15) in goats. There was no *B. melitensis* detected in this study, as shown in Fig. 2.

Discussion

The current study detected *Brucella* in cattle, goats, and humans by qPCR in South Sudan. The study identified *B. abortus* from these host species as the leading cause of brucellosis in CES, South Sudan. This finding provides

a significant epidemiological understanding of the brucellosis dynamic by demonstrating the prevalent *Brucella* species and this is crucial for the mitigation of the disease in livestock and humans.

Brucellosis has been reported in humans in South Sudan[30,32,33] and cattle[31,33,38]. These studies were serological and were unable to identify the circulating *Brucella* spp. The current study detected *Brucella* spp. in sera of 15 (10.5%) of the tested samples by qPCR targeting the IS*711* gene. The IS*711* gene has a greater analytical sensitivity for the detection of *Brucella* at the genus level[21]. The identification of circulating species of *Brucella* is a cornerstone for successful control of the disease.

The prevalence of *Brucella* infection was higher in cattle (6.9%/10/143) than in goats (1/0.7%). The high prevalence could be explained by the fact that cattle are the dominant species of livestock and their population is larger compared to goats in South Sudan. Moreover, cattle are kept in the herds for a longer period and hence are more prone to manifest cumulative exposures over time with *Brucella* infection[39].

Additionally, the study identified 5 (33.3%) *B. abortus* DNA at the species-specific amplification. Comparatively, the percentage of *Brucella* identified in the species-specific assay is lower than that detected at the genus level. The low percentage of species-specific *Brucella* detected in this study is in agreement with the results of several other studies. A similar finding of minimal numbers of *Brucella* was detected from a PCR assay of the blood and serum of animals suspected of being infected with brucellosis[40]. Additionally, the design of PCR techniques directed towards the identification of species or biovars of *Brucella* is difficult due to deletions and rearrangements in the genome[41].

The identification of *B. abortus* in cattle was as expected, cattle are the classic and preferred host for this *Brucella* species[5]. A previous study conducted in Kenya has reported that *B. abortus* is more often detected in cattle[27] than in camels, sheep, and goats. The finding shows that cattle are the primary reservoirs of *Brucella* and a source for transmission of the disease to humans and other livestock species.

The study detected the DNA of *B. abortus* in sera of goats and humans. The identification of *B. abortus* in goats and humans is probably an indication of the spill-over of the bacteria from cattle to these species. *Brucella* species have the ability to adapt to different hosts, which is why cattle strains like *B. abortus* can infect other mammals, including humans and goats[42].

As is the case in the study area, the traditional habit of mixing different animal species, such as cattle and small ruminants in camps, communal grazing, and the practice of some pastoralists could likely be the driving factors for the spilling of *B. abortus* from cattle to goats and humans in South Sudan. The presence of *B. abortus* in goats 6.6% (1/5) could be due to close contact of this species with the cattle. The finding is in agreement with[43], who reported a significant proportion of *B. abortus* in goats in the western part of Iran.

The epidemiology and transmission dynamics of brucellosis are complex in Sub-Saharan Africa and differ from region to region. For instance, in West Africa, there is no documented report on the isolation of *B. melitensis* the etiological agent of brucellosis in sheep and goats[44]. In this region, *B. abortus* the common etiological agent of brucellosis in cattle has spilled over from cattle to small ruminants. Our finding is comparable to the situation in West Africa with *B. abortus* infection in cattle having spilled over to goats, and humans and no isolation of *B. melitensis* from small ruminants[44].

In contrast, the epidemiology of brucellosis in some of the East African countries like Kenya[27,28,45], Tanzania[24], Uganda[46,47], and Rwanda[48] is more complex, with *B. abortus* and *B. melitensis* being isolated from both cattle and small ruminants with prevalence reflecting their preferential hosts and spillover events to other livestock species.

In South Sudan, the circulation of *B. abortus* in cattle and its spillover to goats and humans could be of great public health concern. The spillover of *B. abortus* to goats and humans poses significant implications for the control of brucellosis as the S19 vaccine is only effective against bovine brucellosis but not in goats. Additionally, there is no licensed vaccine for brucellosis in humans. The suboptimal hygiene among the pastoralists, and congregations of multiple herds in cattle camps aggravated with lack of *Brucella* vaccination in cattle could result in a public health burden.

The gold standard for diagnosis of *Brucella* spp. in humans and animals is culture. However, it is time-consuming and hazardous[21]. Thus, the main limitation of the current study was the fact that no *Brucella* organism isolated nor characterized as identification of *Brucella* spp. was solely on qPCR. The timing of *Brucella* detection in the blood depends on various factors such as the stage of infection, immune response, and diagnostic methods. In cows experimentally exposed to *B. abortus*, the degree of *Brucella* infection in blood and the genital tract was highest near the termination of the first gestation and the persistence of bacteria were found to be associated with the susceptibility of the animal[49]. A similar experimental study has shown that *B. abortus* could only be detected consistently in the blood of early pregnant heifers during the first week following experimental infection and at the time of abortion[50]. The best clinical sample to isolate *Brucella* from human patients depends on the stage of the infection and the clinical presentation of the patient. In humans, *Brucella* spp. is mainly isolated from blood in acute febrile patients. Therefore, our findings may only represent the lower proportion of infected patients and livestock. We could not identify the *Brucella* species in the entire IS*711* positive DNA very likely because the quantity and the quality of the extracted DNA were not optimal. Nevertheless, a significant finding of our study is that only *B. abortus* has been identified as the etiology of brucellosis in livestock and humans in CES, South Sudan.

Conclusions

This study has for the first time, revealed the circulating *B. abortus*, in cattle, humans and goats in South Sudan. Our findings indicate that cattle are probably the primary reservoirs for transmission of *Brucella* to humans and other livestock species. Therefore, it is advised that control measures be put in place that initially target cattle to minimize production losses and any spillover to goats and humans. Strategies for conducting nationwide sensitization campaigns and adopting the One Health approach are needed to mitigate brucellosis in South

Sudan. Moreover, efforts should be made to isolate and characterize *Brucella* spp. from cattle and goats in CES, South Sudan.

Data availability

All the data generated or analyzed during this study are included in this article and its supplementary information files.

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Author contributions

E.P.L, was involved in data collection, arrangement, data analysis and drafting of the initial manuscript. E.B.O., G.M., R.K., E.M., H.V.H., J.G. & C.M. contributed in supervision, study design and critical reviewing of the final manuscript. All the authors have read and approved the manuscript.

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Declarations

Competing interests

The authors declare no competing interests.

Additional information

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