Research Article

Seroprevalence and Risk Factors for Brucellosis in Pigs and Pregnant Women in the Western Highlands of Cameroon

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Abstract | Brucellosis is a neglected zoonotic disease that is prevalent in livestock in many developing countries. Though porcine brucellosis has been reported in parts of Cameroon, its threat to human health and public health significance is not known. A cross sectional study was carried to determine the seroprevalence and factors associated with brucellosis in pigs and among vulnerable persons, such as pregnant women, in the Western Highlands of Cameroon. Serum samples from 1,545 pigs and 439 pregnant women were collected and screened for anti-brucella antibodies using Rose Bengal Test (RBT) and ELISA tests. Structured questionnaires were used to collect data on socio-demographics and risk factors. The differences in proportions between reactors were tested using odds-ratio and χ^2 tests. The results showed a brucella seroprevalence of 4.21% at individual pig level (n=1,545; 7.51% RBT, 7.25% i-ELISA), 7.14% at pig herd level (n=98 herds; 10.20% RBT, 8.16% i-ELISA) and 0.68% (n=439; 2.73% RBT and 0.68% i-ELISA) among pregnant women. Brucella seropositivity in pig was significantly ($p<0.05$) influenced by location (OR=2.39) and age (OR=1.93) of the animal, as well as season (OR=1.91), sharing tools between farms (OR=4.99) and history of orchitis in breeding boars (OR=4.58). Brucella seropositivity among pregnant women was strongly associated ($p<0.05$) with their knowledge of symptoms of brucellosis in pigs or humans (OR=172) and history of abortion in their pig farms (OR=27.06). The study reports the prevalence of porcine brucellosis and evidence of human brucellosis in the Western Highlands of Cameroon and therefore, an indication of a real public health problem. Public health awareness campaigns and education based on the One Health Approach especially among vulnerable persons and agro-pastoral communities are essential to disseminate knowledge, risk factors and control of zoonotic brucellosis in the study regions.

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Introduction

Pork is a reliable and affordable source of animal proteins and fats especially for low income countries, due to the short reproductive cycle associated with pig production (Keambou *et al.*, 2010). However, challenges such as poor market structures, high feed costs, inadequate knowledge on pig husbandry, and

high disease pressure constantly hamper the pig sector (Fualefac *et al.*, 2014). In Cameroon, over 26 billion FCFA per annum in direct productivity losses from pig deaths and reproductive failures due to diseases has been estimated (MINEPIA, 2015). In addition, human infections associated to zoonotic pig diseases in endemic areas have been reported (Dean *et al.*, 2012). Porcine brucellosis is a zoonotic bacterial disease of pigs caused by *Brucella* spp. particularly *B. abortus,* and *B. suis*, capable of infecting other livestock, wildlife and humans (Olsen and Tatum, 2017; Guela *et al.*, 2025).

Brucellosis causes reproductive wastage in infected pig herds and manifest as infertility, irregular estrus, abortions, stillbirths and farrowing of weak piglets with a low neonatal survival rate (Franc *et al.*, 2018). The disease is transmitted among pig herds through contaminated ingested materials from uterine discharges, aborted feti or membranes, milk as well as coitus with brucella infected animals and use of infected semen for breeding (El-Sayed and Awad, 2018). The spread of the causative agent is rapid especially among naïve pigs (over 50%) following a primary infection in a farm, and high infections rates ranging from 70–80% have been reported (Pal *et al.*, 2020). The spread and maintenance of the disease within pig herds has been linked to the introduction of un-tested pigs, use of infected boars and/ or semen for breeding, and availability of wildlife (EFSA, 2009). However, human brucellosis has been associated with the consumption of infected and unpasteurized milk and milk products, contamination of skin wounds due to contact with infected animals/ tissues, and inhalation of brucella-contaminated dust/ aerosols (Olsen and Tatum, 2017). The symptoms of the human disease include recurrent fever, malaise, joint and muscle pain, night sweats, and neurologic manifestations (Ducrotoy *et al.*, 2017). Close contact between humans and livestock, the existence of different Brucella species/strains religious, alimentary and cultural habits of the population are major risk factors for human brucellosis (Abdalla, 2016; Cash-Goldwasser *et al.*, 2018).

Though the prevalence of porcine brucellosis in sub Saharan Africa is considered to be low (Diaz, 2013), there is limited information on the epidemiology and zoonotic potentials of the disease (Njeru *et al.*, 2016; Ducrotoy *et al.*, 2017) and ineffective management of pig diseases have been recorded (MINEPIA, 2023). Cameroon has the largest pig industry in the Central African sub-region with an estimated 4 million pig heads, with pork contributing over 16.0% of the meat production capacity in the country (MINEPIA, 2021). However, there are concerns of the pig industry contributing substantially to Cameroon's economic policy (MINEPAT, 2020). Also, human brucellosis among risk professions (herdsmen and abattoir staff) and vulnerable groups (pregnant women) (Awah-Ndukum *et al.*, 2018a; Kamga *et al.*, 2021), and prevalence of porcine brucellosis ranging from 1.49–3.35% (Awah-Ndukum *et al.*, 2018b; Kamga *et al.*, 2020; Dinayen *et al.*, 2021; Guela *et al.*, 2025) have been reported in the country. Among the disorders reported on pig farms in the country, there are increasing veterinary concerns to investigate the incidences of notifiable abortion cases, particularly in the Western Highlands Region. In addition, the epidemiological and economic burdens of the zoonotic disease in pigs and humans, and factors that influence its spread and magnitude in pig farms and at the human pig interfaces in Cameroon are unknown.

Therefore, this study was carried out to contribute knowledge to the epidemiology of porcine brucellosis and estimate the seroprevalence in live and slaughtered pigs, and pregnant women in the Western Highlands of Cameroon. The study also assesses the risk factors for evidence-based control of the disease in Cameroon.

Materials and Methods

Description of study areas

A cross sectional study was carried out from October 2020 to September 2021 within pig farms, pig holding facilities, slaughterhouses, and two hospitals in eight of the ten administrative subdivisions that make up Mezam and Mifi Divisions of the Western Highlands Region of Cameroon (Figure 1). Mezam division (5°20′–6°15′N and 9°7′–10°21′E) covers a surface area of 1,745 Km^2 with a population of 524,127 and is comprised of seven subdivisions. Mifi division (5°25'–5°45'N and 10°11'–10°20'E) covers a surface area of 402 Km^2 with a population of 301,456 and is comprised of three subdivisions (MINEPAT, 2009). Overall, the Western Highlands Region has a typical equatorial type of climate with two major seasons: A long wet season (mid-March to mid-October) and a short dry season (mid-October – mid-March) (Tume, 2021).

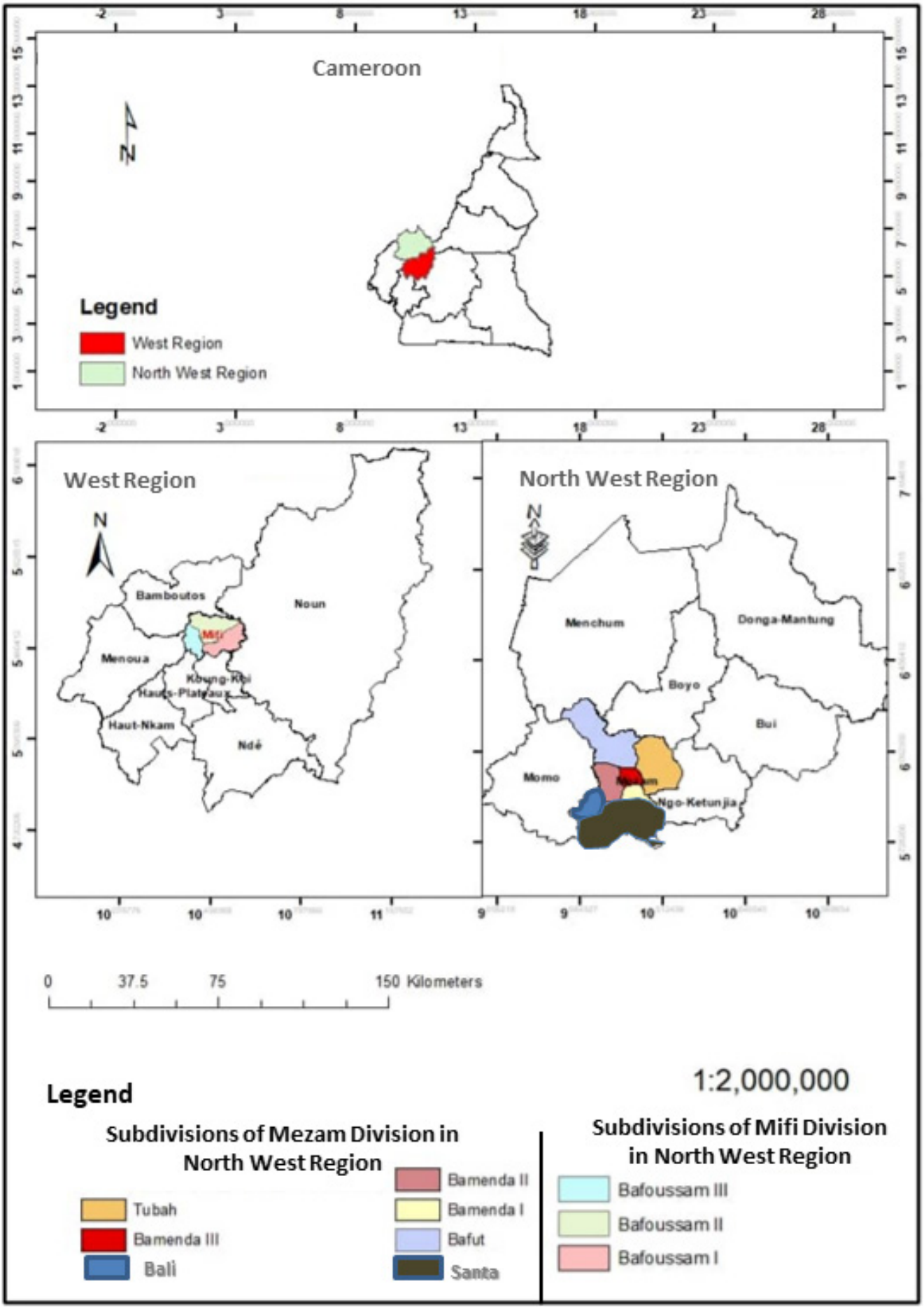

Figure 1: *Maps showing the administrative regions in Cameroon and study areas (Subdivisions) in Mezam Division of the North West Region and Mifi Divisions West Region.*

The choice of study area was due to the following: (1) The study areas are located in the high-density pig producing areas of the Western Highlands of the country which has witnessed a 50% drop in its pork production capacity within the last decade due to many factors including outbreaks of various porcine diseases (MINEPIA 2021, 2023). (2) There is massive settlement of internally displaced persons, an upsurge in pig keeping and increased contact between humans and pigs in urban and peri-urban areas in the Western Highlands of Cameroon (NWLDF-PULCCA, 2024). (3) Though health implications of brucellosis have been reported in livestock and humans (vulnerable persons and risk professionals) in parts of the country and elsewhere (Awah-Ndukum *et al*., 2018a; Kamga *et al*., 2021; Rajala, 2016), the public health significance of porcine brucellosis in Cameroon, particularly in the high-density pig producing areas in the Western Highlands is not known.

Selection of animals for the study

The sample size needed to estimate the prevalence of porcine brucellosis with a desired 95% confidence and precision of ≤ 5% was calculated using a porcine brucella prevalence rate of 3.35% in Cameroon (Awah-Ndukum *et al*., 2018b) and the following equation:

$$n = \frac{1.96^2 P_{exp}(1 - P_{exp})}{d^2}$$

Where; n= required sample size; P_{exp}= expected prevalence; and d= desired absolute precision (Thrusfield, 2007).

On farm

The selection of on-farm pig herds was based on random-number generations of lists of pig farmers in the study localities obtained from the Delegations of Livestock, Fishery and Animal Industries (DDEPIA) of Mezam and Mifi Divisions. In addition, purposive samplings through referencing by the selected farmers within their localities of other farms at reasonable distances (at most 150 meters) were included in the study. A farm was considered as an epidemiological unit, and was categorized based on herd size as follows: small scale (≤ 10 adult pigs), medium scale (11 to ≤ 25 adult pigs) and large scale (> 25 adult pigs).

Pig holding facilities and slaughter areas

Pigs originating from the subdivisions of Mezam and Mifi Divisions and other Divisions in the North West and West regions of Cameroon and imported into the pig markets in the Bamenda and Bafoussam metropolis were targeted in the study. However, traders and/ or butchers present in the markets were listed during visits, and animals destined for transportation to other parts of the country such as Yaoundé and Douala and/ or slaughter whose traders-butchers presented verbal informed consent were included in the study.

Selection of humans for the study

The targeted human populations were plausible vulnerable humans who interact with livestock including pigs in the study regions and their environs. However, pregnant women on antenatal consultations and women (at least 15 years old) at the Obstetrico-gynaecological unit of Regional Hospital (RH) and the Saint Mary Soledad Catholic Hospital (SMSH) in Bamenda were plausible candidates for the study. A default expected prevalence of 50% was used to determine the sample size required to estimate the prevalence of brucellosis among pregnant women in the study region as previously described (Thrusfield, 2007).

Blood sampling

Apart from procedural restraining manipulations for safety purposes and jugular venipuncture for blood sampling (≥5 ml) using sterile vacutainer, the animals were humanely handled. Blood was collected by jugular veno-puncture from the chosen animals on the selected farms and animals at the pig markets in the Bamenda and Bafoussam metropolis that were destined for transportation to other parts of the country such as Yaoundé and Douala.

Based on a calculated sampling fraction of five, up to two and five pigs were randomly selected without replacement for small and medium scale farms and at least six pigs for large-scale farms, respectively. A total of 210 pigs in 54 farms (23 small scale, 27 medium scale and 04 large scale farms) were sampled in Mezam division and 162 pigs in 44 farms (22 small scale, 18 medium scale and 04 large scale farms) in Mifi division.

For animals that were destined for slaughter, the researcher accompanied the traders-butchers and

their animals (irrespective of the number) to the slaughter points. Blood samples were collected by jugular veno-puncture from the chosen animals on the chain before slaughter or from severed jugular veins of pigs at slaughter. Briefly, about 20% of 20–40 pigs slaughtered daily at the slaughter points in Bamenda and Bafoussam metropolis was randomly selected each day (i.e. 3–4 days per week), except on Friday, Saturday and Sunday for Bafoussam and Saturday, Sunday and Monday for Bamenda, were included in the study. Based on a calculated sampling fraction of five (every fifth animal was sampled) for daily use, the first animal was selected by picking one animal by random generation method of the first five animals on the slaughter chain. Thereafter, every fifth animal (adding 5 to previous picked number) was chosen. A total of 1,173 slaughtered pigs (690 in Mezam division and 483 in Mifi division) were sampled in the study.

Overall, 1,545 serum samples was extracted from the collected blood samples (900 in Mezam division and 645 in Mifi division) and stored at –20 °C until laboratory analysis at the National Veterinary Laboratory (LANAVET) Annex Yaoundé Cameroon. However, pigs under six months of age due to poor reactions to various serological tests for brucellosis (OIE, 2016), pregnant sows in their third trimester of gestation and animals such as nursing sows which could not be restrained safely for bleeding were excluded from the study.

Human blood samples were obtained by a team of qualified medical laboratory technicians recruited for the task at each institution. Blood (≥5 ml) was obtained from the median and cephalic veins using a sterile vacutainer, causing little or no discomfort to the human subjects. The participant's name, age, and date of sampling were recorded to prevent collecting blood from the same person during subsequent antenatal appointments. Serum extraction and storage at –20 °C was done at the respective hospitals until laboratory analysis. A total of 439 pregnant women (predominantly from Mezam division and its environs) on antenatal and Obstetrico-gynaecological consultations at Regional Hospital (RH) and Saint Maria Soledad Catholic Hospital (SMSH) in Bamenda were sampled during the study period.

Serological tests

Following Rose Bengal test (RBT) screening of all pigs (1,545) and human (439) samples, Indirect-Enzyme-Linked Immunosorbent Assay (i-ELISA) was performed on the pig and human samples to detect anti-brucella antibodies (OIE, 2016; Nielsen and Yu, 2010). Each round of tests contained both a positive and a negative control. An individual was seropositive when the serum tested positive for RBT and / or i-ELISA.

Rose Bengal test (RBT)

RBT was performed as previously described (Alton *et al.*, 1988; OIE, 2016). Briefly, the sera and antigen were brought to room temperature (22 ± 4°C) before use. Equal volumes (30 µl) of standardized *B. abortus* antigen Weybridge strain 99 and test serum were mixed thoroughly and rotated on the RBT card using a wooden splint, and the card rocked gently for 4 min. The appearance of agglutination, recorded as positive within 1 min was scored 4+ (++++) and between 1 and 4 min was scored 1+ to 3+ (+, + +, and + + +) according to the different degrees of agglutination. The absence of agglutination within 4 min was regarded as negative (–).

Detection of pig brucella antibodies by indirect enzyme linked immunosorbent assay (i-ELISA)

Antibrucella (*B. abortus, B. melitensis, B. suis,* and *B. canis*) antibodies (IgG) in pig serum were detected using a commercial indirect multispecies ELISA (i-ELISA) (ID.Vet, Innovative Diagnostics, Grabels, France) according to the manufacturer's instructions and as previously described (Limet *et al.*, 1988; Saegerman *et al.*, 2004). Briefly, all reagents and test sera were brought to room temperature (22 ± 4°C) before use. Thence, 100µl of diluted buffer dispensed into each of 96-well polysterene plates pre-coated with purified standard smooth lipopolysaccharide antigen prepared from *B. abortus* isolates. This was followed by adding 10µl of positive control serum and 10µl of negative control serum (provided by the manufacturer) into two different wells of the plate and 10µl of each of the test serum samples to the remaining wells. Each plate was sealed and incubated at room temperature for 45 minutes on a rotary shaker to ensuring homogenization. After, each plate was washed four times with PBS-Tween washing solution, and 100µl of anti-multi-species-IgG- horseradish peroxidase (HRP) conjugate was added to each well; incubated at room temperature for 30 minutes and washed four times to eliminate excess of conjugate. Thereafter, 100µl of chromogen

substrate solution (tetramethylbenzidine in substrate buffer containing Hydrogen peroxide) (TMB + DMSO + H_2O_2) was added to each well and the plate incubated at room temperature for 10 minutes under dark conditions. The reaction was stopped after adding 100µl of 1 N-hydrochloric acid. The coloration of antigen-antibody conjugate-peroxidase complex formed depended on the quantity of anti-Brucella antibodies that was present in the specimen tested. Thus, in the presence of antibodies, a blue solution appeared which became yellow after addition of the stop solution, while in the absence of antibodies, no coloration appeared. An automatic micro plate reader (BioTek ELX800 absorbance reader) read the optical density (OD) of each well at 450 nm and for each sample tested S/P% was calculated as follows:

$$\frac{S}{P}\% = \frac{(OD_{sample} - OD_{nc})}{(OD_{pc} - OD_{nc})} \times 100$$

Where; OD_{sample}, OD_{nc}, and OD_{pc} are the readings of Optical Densities (OD) for the sample, negative control, and positive control, respectively. The samples were classified as positive if S/P% ≥120%, negative if S/P% ≤ 110%, and doubtful if 110% < S/P% < 120%. Also, the fact that OD pc > 0.350 and OD_{pc}/OD_{nc} > 3 indicated that the tests were working properly.

Detection of human brucella antibodies by indirect enzyme linked immunosorbent assay (i-ELISA)

For the qualitative and quantitative measurement of IgG antibodies against Brucella in human serum, the commercial Brucella IgG ELISA kit (IBL International GMbH, Hamburg, Germany) was used in accordance with the manufacturer's instructions and as previously described (Esmaeili *et al.*, 2013). Briefly, the ELISA was based on the sandwich principles. The wells were coated with antigen and specific antibodies of the sample binding to the antigen-coated wells were detected by secondary enzyme conjugated antibody specific for human IgG (horseradish peroxidase-conjugated anti-human IgG). After tetra methyl benzidine (TMB) substrate reaction, a Brucella antibody-antigen reaction was indicated by a blue coloration. The intensity of the blue coloration that developed proportional to the quantity of IgG-specific antibodies detected. An automatic micro plate reader (BioTek ELX800 absorbance reader) read the optical density (OD) of the well at 450 nm. Positive, negative and cut-off controls were included in the test. Antibody activities were calculated using a standard curve according to the manufacturer's guidelines. However, the Cut-off value was obtained from the optical density (OD) of the Cut-off control and the Cut-off index (COI) was calculated from the optical densities of the sample and Cut-off value as follows:

$$COI\% = \frac{OD_{sample}}{OD_{Cut-off\ control}} \times 100$$

Where; OD_{sample} and $OD_{Cut\text{-}off\ control}$ are the readings of optical densities for the sample and cut-off control, respectively. The samples were classified as positive if COI % ≥120%, negative if COI % ≤ 80%, and doubtful if 80% < COI % < 120%. The samples were classified quantitatively as positive if IgG concentration (IgG) ≥1.2 U/mL, negative if (IgG) ≤ 0.8 U/mL, and doubtful if 0.8 U/mL < (IgG) < 1.2 U/mL.

Risk assessment

Risk factor assessment for brucellosis in pigs and humans was done through examination of individual pigs and questionnaire interview of pig farm owners in the study areas and personnel at the Bamenda and Bafoussam pig markets and slaughter points (pig traders and/ or pig butchers); and also women on antenatal and obstetrico-gynaecological consultations at the RH and SMSH in Bamenda. The questionnaires were structured to collect information on a range of variables including lifestyle, socio-demographic data and disease awareness status of the humans included in the study; breed, sex, age, and health status of the study pigs; as well as husbandry and biosecurity practices and breeding history of the pig farms.

Ethical considerations

To avoid threats to all individuals and animals participating in the study, the researchers undertook the project's risk assessment. Following validation by the College of Technology of The University of Bamenda (Ref N°: 408/2020/UBa/COLTECH/D/tsf of 16/10/2020; N°: 468/2020/UBa/COLTECH/D/tsf of 11/11/2020; N°: 853/21/UBa/COLTECH/D/HoD/APT of 12/03/2021), ethical clearance (Ref N°: 2021/099H/UBa/IRB of 28/05/2021) and permission from the required authorities in Cameroon including the Delegations of Livestock, Fisheries and Animal Industries (Ref No: 038/MINEPIA/DDEPIA/MEZAM of 21/10/2020 and Ref N°: 73/20/L/DREPIA-O/SRAG of 20/10/2020), Regional Delegation of Public Health for the North West region (Ref N°: 14/L/NWR/RDPH/CSGA of 11/06/2021), and Regional Hospital Bamenda (Ref

N°: H004/MPH/RDPH/RHB/186 of 23/06/2021) were obtained to carry out the study in Mezam and Mifi divisions. The purpose of the study was explained to the targeted participants (pig farmers, pig traders, pig butchers, women on antenatal and obstetrico-gynaecological consultations) in the present study usually with the assistance of resident veterinary and medical practitioners, community leaders and/ or trusted intermediaries. The targeted participants were used in the study after giving their written informed consent. An animal was included in the study after the pig owner or trader-butcher gave an informed verbal consent. Apart from procedural restraining manipulations for safety purposes, the animals used in the present study were not subjected to any stress and suffering.

Data analysis

Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS) for windows® version 20.0 (SPSS Inc.). The proportions of positive reactors for the disease and their 95% confidence interval were calculated using Wilson's equation:

$$p \pm z\sqrt{\frac{p(1-p)}{n}}$$

Where p is the sample proportion, n is the sample size and z is the critical z-value of 1.96 for a 95% confidence interval. The chi-square test was used to test significant levels (fisher test where observations were less than 5) within factors on brucellosis seroprevalence rates, odds-ratios and regression analysis were used to assess the strength of association of different factors with porcine and human brucellosis along 95% confidence intervals and statistical significance set at P< 0.05 (Thrusfield, 2007).

Results

Seroprevalence rates of porcine and human brucellosis in the western highlands of Cameroon

Combination of tests results of 1,545 individual pigs showed an overall apparent brucellosis seroprevalence of 65 (4.21% (2.82–6.32)) with 116 (7.51 % (6.36–11.22)) for RBT and 112 (7.25 % (5.73–10.31)) for i-ELISA. A total of 63 (5.37 %) combined (106 (9.04 %) RBT and 104 (8.87 %) i-ELISA) positive results for slaughtered pigs and 2 (0.54 %) combined (10 (2.69 %) RBT and 8 (2.51 %) i-ELISA) positive results for on-farm pigs were recorded (Table 1). The brucellosis seropositive results for 98 pigs herds were 7 (7.14 %) for combined tests (10 (10.20 %) for RBT and 8 (8.16 %) for i-ELISA). For the human study, the tests results of 439 pregnant women gave an overall combined apparent brucellosis seroprevalence of 3 (0.68 % (0.12–1.88)), with 12 (2.73 % (0.93–3.54) for RBT and 3 (0.68 % (0.12–1.88)) for Brucella IgG ELISA (Table 1).

Table 1: *Brucellosis seropositivity among pigs and vulnerable persons in the Western Highlands of Cameroon according to combined results of Rose Bengal test and indirect enzyme linked immunosorbent assay.*

Serological results	Number of Cases	Prevalence (% (95% CI))
Individual pig level: Total (n = 1,545)		
RBT (+)	116	7.51 (6.36 – 11.22)
RBT (–)	1,429	92.49 (90.15-94.79)
iELISA (+)	112	7.25 (5.73 – 10.31)
iELISA (–)	1,433	92.75 (89.15-95.27)
RBT (+) iELISA (+)	65	4.21 (2.82 – 6.32)
RBT (+) iELISA (–)	51	3.30 (2.50 – 5.86)
RBT (–) iELISA (–)	1,382	89.45 (87.80 – 96.85)
Individual pig level: Slaughtered (n = 1,173)		
RBT (+)	106	9.04 (6.83 – 12.36)
RBT (–)	1,067	90.96 (87.83 – 94.16)
iELISA (+)	104	8.87 (6.74 – 11.33)
iELISA (–)	1,069	91.13 (89.15 – 94.79)
RBT (+) iELISA (+)	63	5.37 (4.22 – 7.99)
RBT (+) iELISA (–)	43	3.67 (1.12 – 5.81)
RBT (–) iELISA (–)	1,026	87.47 (84.14 – 92.45)
Individual pig level: Live on-farm (n = 372)		
RBT (+)	10	2.69 (0.83 – 3.25)
RBT (–)	362	97.31 (94.66 – 100)
iELISA (+)	8	2.15 (0.15 – 3.89)
iELISA (–)	364	97.85 (95.94 – 100)
RBT (+) iELISA (+)	2	0.54 (0.03 – 2.88)
RBT (+) iELISA (–)	8	2.15 (0.15 – 3.89)
RBT (–) iELISA (–)	356	95.70 (94.4 – 99.37)
Herd pig level: Total (n = 98)		
RBT (+)	10	10.20 (7.55 – 22.06)
RBT (–)	88	89.80 (87.80 – 96.85)
iELISA (+)	8	8.16 (4.82 – 19.92)
iELISA (–)	90	91.84 (88 – 96.84)
RBT (+) iELISA (+)	7	7.14 (5.11 – 12.33)
RBT (+) iELISA (–)	3	3.06 (0.6 – 6.06)
RBT (–) iELISA (–)	85	86.73 (83.69 – 91.25)
Pregnant women: Total (n = 439)		
RBT (+)	12	2.73 (0.93 – 3.54)
RBT (–)	427	97.27 (93.77 – 100)
iELISA (+)	3	0.68 (0.12 – 1.88)
iELISA (–)	436	99.32 (96.82 – 100)
RBT (+) iELISA (+)	3	0.68 (0.12 – 1.88)
RBT (+) iELISA (–)	9	2.05 (0.05 – 7.05)
RBT (–) iELISA (–)	426	97.04 (95.31 – 100)

The occurrence of brucellosis seropositivity among pregnant women and pigs in the study regions revealed that the presence of brucellosis in animals including pigs presents a non-negligible threat for the disease in humans.

Temporal patterns of porcine brucellosis in the Western Highlands of Cameroon

This study showed that porcine brucellosis occurred throughout the study period with peak incidences of seropositive cases recorded in January, April and June for both RBT and i-ELISA tests. The least seropositive incidences were in October, November and December for both tests (Figure 2).

Risk factors for porcine brucellosis in the Western Highlands of Cameroon

This study showed location and age of animals as well as season, sharing of farm tools between farms and animals with infected testes to significantly ($p \leq 0.05$) influence brucella seropositivity in pigs (Table 2). Seropositive reactors were significantly higher in Mifi division (OR= 2.39; 95%CI: 1.61 – 3.54, $p < 0.0001$), among young pigs < 12 months old (OR = 1.93, 95%CI: 1.20-3.09, p= 0.006), during rainy season (OR= 1.91, 95% CI: 1.20 – 3.03, p= 0.006), as well as in farms that share tools with other farms (OR = 4.99, 95%CI: 1.09-22.76, p= 0.043), and farms that have experienced inflamed testes (OR= 4.58, 95%CI: 0.91-23.02, p = 0.050) compared to Mezam Division, other age groups, dry season, farms that do not share tool with other farms and farms that have not experienced inflamed testes, respectively. Factors such as herd size, housing structures, exchange of breeding boars and afterbirth materials and carcasses disposal methods, abortion and repeat breeding on farms did not significantly influence ($p>0.05$) the seroprevalence rate of porcine brucellosis in the present study (Table 2). However, seropositive reactors were recorded in farms without footbath, no free roaming pigs, and farms where the management was not aware of brucellosis and its signs in pigs and not in farms with footbaths, free roaming pigs and farms where the management was aware of the brucellosis and its signs in pigs (Table 2).

Risk factors for human brucellosis in the Western Highlands of Cameroon

This study found brucella seropositivity in pregnant women to be significantly ($p<0.05$) associated with women having knowledge of symptoms of brucellosis in infected pigs and humans (OR= 172, 95%CI: 13.36-224.06, p= 0.0007), experienced an abortion on their farm (OR= 27.06, 95% CI: 2.39 – 307.13, p = 0.015) (Table 3). Brucella seropositivity was observed only among women who were married, in active age group (30 – 44 years old), had experience miscarriages and consume fresh milk and fresh milk products.

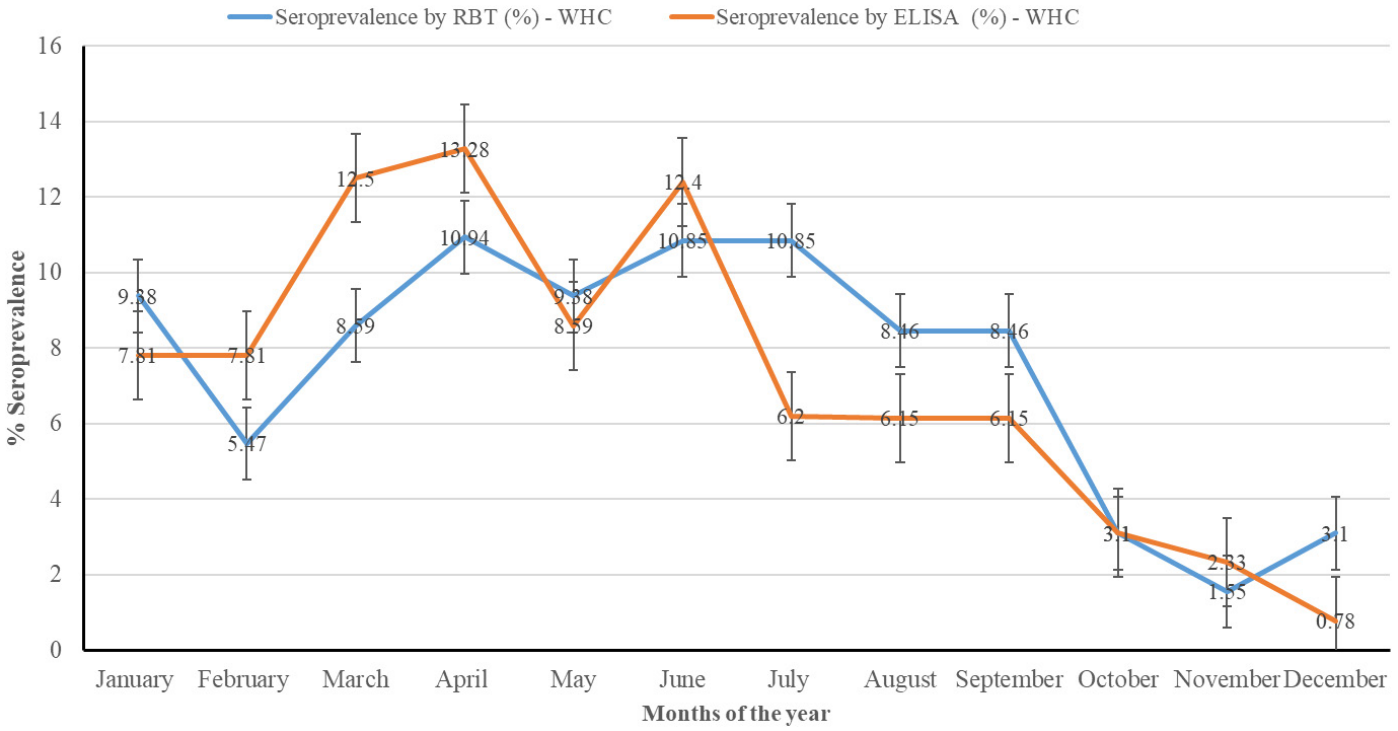

Figure 2: *Temporal distribution patterns for porcine brucellosis seroprevalence according to month in the Western Highlands of Cameroon.*

Table 2: *Brucellosis seropositivity among pigs in the Western Highlands of Cameroon according to potential risk factors.*

Category	Variable	Number examined	Seropositivity by iELISA n (%)	Odds ratio (95%CI)	P – value (χ^2 value)
Location	Mezam	900	43 (4.80)	1	
	Mifi	645	69 (10.70)	2.39 (1.61 – 3.54)	<0.0001 (19.58)
Sex	Male	715	61 (8.53)	1.42 (0.97 – 2.10)	0.071 (3.25)
	Female	830	51 (6.14)	1	
Age (X = months)	≤ 12	1,015	87 (8.57)	1.93 (1.20 – 3.09)	0.006 (7.64)
	12 < X ≤ 24	496	23 (4.64)	1	
	> 24	34	2 (5.88)	1.21 (0.27 – 5.35)	0.517 (Fisher)
Herd size	Small	45	3 (6.67)	1	
	Medium	45	4 (8.89)	0.65 (0.10 – 4.10)	0.50*
	Large	8	1 (12.50)	2.00 (0.18 – 22.06)	0.491 (Fisher)
Season	Dry	514	24 (4.67)	1	
	Rain	1,031	88 (8.54)	1.91 (1.20 – 3.03)	0.006 (7.63)
Pig housing structure	Bare soil floor	14	1 (7.14)	11.54 (0.68 – 195.37)	0.163*
	Suspended wooden floor	207	6 (2.90)	4.48 (0.53 – 37.59)	0.129*
	Cemented floor	151	1 (0.66)	1	
Water source	Stream	296	6 (2.03)	1	
	Others	76	2 (2.63)	1.31 (0.26 – 6.60)	0.511*
Exchange breeding boars	Yes	212	7 (3.30)	5.43 (0.66 – 44.58)	0.076*
	No	160	1 (0.47)	1	
Afterbirth / carcass disposal	Bury	344	7 (2.03)	1	
	Throw away	3	1 (33.33)	24.07 (1.95 – 297.53)	
	Feed to pets	15	0 (0.00)	/	/
Presence of footbath	Yes	131	0 (0.00)		
	No	241	8 (3.32)		
Change farm wear	Yes	236	4 (1.69)	1	
	No	136	4 (2.94)	1.76 (0.43 – 7.14)	0.326*
Share farm tools	Yes	81	4 (6.17)	4.99 (1.09 – 22.76)	0.043*
	No	291	3 (1.03)	1	
Free roaming pigs in neighbourhood	Yes	78	0 (0.00)		
	No	294	8 (2.72)		
Farmer is aware of porcine brucellosis	Yes	150	0 (0.00)		
	No	222	8 (3.60)		
Farmer is aware of signs of the disease	Yes	134	0 (0.00)		
	No	238	8 (3.36)		
Experience abortion on farm	Yes	200	2 (1.00)	1	
	No	172	6 (3.49)	3.58 (0.71 – 17.97)	0.098*
Treat abortive cases	Yes	244	7 (2.87)	3.75 (0.46 – 30.83)	0.175*
	No	128	1 (0.78)	1	
Re-breed abortive cases	Yes	130	4 (3.08)	1.89 (0.46 – 7.68)	0.291*
	No	242	4 (1.65)	1	
Experienced repeat breeding	Yes	244	7 (2.87)	3.75 (0.46 – 30.83)	0.175*
	No	128	1 (0.78)	1	
Experienced inflamed testes	Yes	150	6 (4.00)	4.58 (0.91 – 23.02)	0.050*
	No	222	2 (0.90)	1	

** fisher exact probability test, one tailed.*

Table 3: *Level of brucellosis awareness, clinical symptoms and brucellosis seroprevalence among pregnant women in Mezam Division.*

Category	Variable	Number examined	Seropositivity by iELISA n (%)	Odds ratio (95% CI)	Fisher test
Heard of brucellosis	Yes	93	2 (2.15)	7.58 (0.68 – 84.56)	0.115
	No	346	1 (0.30)	1	
Aware of zoonotic potential	Yes	71	2 (2.82)	10.64 (0.95 – 118.94)	0.069
	No	368	1 (0.27)	1	
Aware of transmission modes	Yes	71	0 (0.00)		
	No	368	3 (0.82)		
Aware of symptoms in infected pigs and persons	Yes	7	2 (28.57)	172.40 (13.36 – 224.06)	0.0007
	No	432	1 (0.23)	1	
Experienced abortion on farm	Yes	32	2 (6.25)	27.06 (2.39 – 307.13)	0.015
	No	407	1 (0.25)	1	
Use protective gloves in handling pig/pork	Yes	43	1 (2.33)	4.69 (0.42 – 52.83)	0.267
	No	396	2 (0.51)	1	
Common clinical symptoms of brucellosis	Undulant fever	191	1 (0.52)	1.26 (0.08 – 20.24)	0.690
	Appetite loss	240	1 (0.42)	1	
	Weight loss	95	2 (2.11)	5.14 (0.46 – 57.37)	0.195
	Back and / or joint pain	305	3 (0.98)	2.37 (0.25 – 22.97)	0.406
	Headaches	183	1 (0.55)	1.31 (0.08 – 27.13)	0.679
	Night sweats	160	3 (1.88)	4.57 (0.47 – 44.30)	0.178

The other factors in the study showed no significant effect (p>0.05) on human brucellosis seroprevalence, though relatively higher odds for brucella seropositivity in humans were observed for women in rural areas, that interact with pigs, heard about brucellosis, and aware of zoonotic brucellosis than women in urban areas, have no interactions with pigs, not know brucellosis and not aware of zoonotic brucellosis (Tables 3 and 4).

Discussion

The apparent seroprevalence (4.21%) for porcine brucellosis reported in the present study is similar to those reported in Cameroon (3.35%), Democratic Republic of Congo (4.42%), Ethiopia (4.50%), Bangladesh (4.80%) and India (4.33%) (Awah-Ndukum *et al.*, 2018b; Tshilenge *et al.*, 2020; Kebeta *et al.*, 2015; Rahman *et al.*, 2012; Shome *et al.*, 2022). Lower rates (1.49–2.4%) have been reported for Cameroon (Kamga *et al.*, 2020; Dinayen *et al.*, 2021; Guela *et al.*, 2025). The disparity in the rates may be associated to differences in the sample size and selection criteria adopted. In this study, live pigs reared under different production systems alongside slaughtered pigs were screened. The contributions of on-farm sampling to the better understanding of the epidemiology and control of porcine diseases has been clearly demonstrated (Fasina *et al.*, 2012). Higher rates compared to the rate in this study have also been reported by Mwebe *et al.* (2011) in Uganda (10%), Rajkhowa *et al.* (2023) in India (9%), M'Bari *et al.* (2021) in Ivory Coast (10.20%) and Ngbede *et al.* (2013) in Nigeria (30.60%).

The higher seroprevalence rate observed in Mifi division (10.70%) compared to the Mezam division (4.80%) is characteristic of studies (5 – 15%) in high-endemic areas like Latin America and South-east Asia (Diaz, 2013). Pig farms in the Mifi division (unlike the more rural settings characteristic of most farms in the Mezam division) are usually located within peri-urban to urban settings and kept predominantly under the semi-intensive system requiring moderate to high levels of biosecurity to limit disease outbreaks (Dieste-Pérez *et al.*, 2015). However, the human and animal population densities is higher in Mifi division than in Mezam division, as well as volume and motility of pigs within, into and out to the markets and slaughterhouses is higher in Mifi than in Mezam division (MINEPIA, 2021). Therefore, there is increased risks to the emergence of isolated and localized disease clusters or hot spots in Mifi than in Mezam division. High human and animal

population densities can result in high pollution of natural resources (water bodies, soil, and air) which favour the transmission of infectious diseases among pig herds have been previous reported (Onunkwo *et al.*, 2011; Korennoy *et al.* 2014).

The relatively higher odds for brucellosis infection was observed with large pig herds compared to small and medium scale herds similar to the findings of Rajala (2016) and Robi *et al.* (2023). The contact levels between animals and chances of transmission of brucella increases in herds with higher density, especially in the presence of aborted and other contaminating materials. Strengthening biosecurity measures within large pig herds can greatly limit pathogen spread among pigs.

Though not significantly associated with porcine brucella seropositivity in this study, the higher risk for brucella seropositivity seen in males has previously been reported in Nigeria (31.20%) (Ngbede *et al.*, 2013), Ivory Coast (12.10%) (M'Bari *et al.*, 2021), Democratic Republic of Congo (8.06%) (Tshilenge *et al.*, 2020), and India (6.08%) (Shome *et al.*, 2022). Also, the observation that younger pigs aged ≤ 12 months old were at a much higher risk for brucella infections ($p<0.05$) has also been reported in Cameroon (Awah-Ndukum *et al.*, 2018b). Other studies in Ethiopia (5.9%), Ivory Coast (10.70%) and Nigeria (30.10%) equally found higher seroprevalence rates

Table 4: *Life style, socio-demographic characteristics and brucellosis seroprevalence among pregnant women in Mezam Division.*

Category	Variable	Number examined	Seropositivity by iELISA n (%)	Odds ratio (95%CI)	Fisher test
Residence	Rural	77	2 (2.60)	9.63 (0.86 – 107.54)	0.081
	Urban	362	1 (0.28)	1	
Marital status	Married	353	3 (0.85)		
	Not married	86	0 (0.00)		
Age (years)	15–29 years	268	0 (0.00)		
	30–44 years	169	3 (1.78)		
	45–60 years	2	0 (0.00)		
Educational level	Primary	45	0 (0.00)		
	Secondary	229	2 (0.87)	1.44 (0.13 – 16.07)	0.621
	Tertiary	165	1 (0.61)	1	
Pregnancy stage	1st trimester	95	0 (0.00)		
	2nd trimester	188	1 (0.53)	1	
	3rd trimester	156	2 (1.28)	2.43 (0.22 – 27.04)	0.430
Parity (number of births)	0 birth	122	0 (0.00)		
	1 – 3 births	238	2 (0.84)	1	
	4 – 5 births	57	1 (1.75)	0.47 (0.042 – 4.33)	0.476
	> 5 births	22	0 (0.00)		
History of miscarriage and or neonatal death	Yes	111	3 (2.70)		
	No	328	0 (0.00)		
Interacts with pigs	Yes	64	2 (3.13)	12.06 (1.08 – 135.07)	0.057
	No	375	1 (0.27)	1	
Milking of ruminants	Yes	38	1 (2.63)	5.39 (0.48 – 60.88)	0.238
	No	401	2 (0.50)	1	
Consume fresh milk and fresh milk by-products	Yes	279	3 (1.08)		
	No	160	0 (0.00)		
Eat raw / smoked meat	Yes	244	2 (0.82)	1.60 (0.14 – 17.18)	0.584
	No	195	1 (0.51)	1	
Eat bush meat	Yes	116	2 (1.72)	5.56 (0.51 – 62.90)	0.172
	No	323	1 (0.31)	1	

for brucellosis among younger pigs of age ≤ 12 months old compared to adult pigs (>12 months old) (Kebeta *et al*., 2015; M'Bari *et al*., 2021; Ngbede *et al*., 2013). However, the finding is contrary to investigations that recorded higher infection rates among older pigs than young pigs in Bangladesh (8.10%) (Rahman *et al*., 2012), Cameroon (1.74%) (Dinayen *et al*., 2021) and Democratic Republic of Congo (5.54%) (Tshilenge *et al*., 2020).

In Cameroon, most farmers breed pigs in the first quarter of the year following massive sales that accompany end of year festivities. Though not a major factor of brucellosis in pigs in the present study, the practice of borrowing boars is common (57.00%) in the country and the rise in disease incidence during this period may be due to such practices. Farmers need to be educated on the risk associated with the common use of breeding boars to limit the spread of brucellosis. Again, the very high temperatures and low rainfall levels that characterizes the dry season in this region causes natural water sources (streams and springs) available for agriculture to shrink (Molua, 2006; Tume, 2021), adversely affecting the efficacy of environmental waste management within communities. Consequently, runoffs from the onset of the rainy season have the potentials to contaminate communal water bodies available for livestock watering, thereby aiding in spreading diseases over long distances. The source of brucella infections in pigs has been associated to contaminated stagnant lake used for watering trade cattle and goats grazing in Nigeria (Onunkwo *et al*., 2011). Understanding the role of such natural resources and treatment before use on farms in the epidemiological dynamics of porcine brucellosis in Cameroon is vital for control against the disease. The decline in disease incidence with the onset of the dry season probably stems from the lack of naïve susceptible pigs needed for the continued transmission of the infection following mass slaughtering during end of year festivities in the region (Eko *et al*., 2022).

The poor handling of afterbirths and carcasses by simply throwing away into the environment though with no significant effect on brucellosis in this study, showed much higher risk for infection. The indiscriminate discharge of infective afterbirths, aborted, materials and carcasses in the environment can easily contaminate natural water bodies like streams, a principal source for the watering of pigs in this region. Contaminated and infected abortive materials can be mechanically transported by scavenging rodents to neighboring farms leading to the disease outbreak (Fasina *et al*., 2012). Proper disposal of farms effluents by deep burial and incineration are useful in controlling the spread of brucellosis between pig farms.

The sharing of farm tools between farms in this study was found to be a major factor for brucella spread among pig herds, especially as the level of biosecurity on such farms is very low. The washing and disinfection of farm equipment and tools was shown to be negatively associated with African swine fever infection on farms in Nigeria (Fasina *et al*., 2012). The inability of low in-come farmers to acquire most farm tools (spades, shovels, axes, wheel barrows, trucks) can lead to borrowing from neighboring farms. The return of heavily contaminated borrowed tools without proper disinfection constitutes a very serious source of infection to naïve pigs. Such practices could be discouraged due to the inability of the predominantly smallholder farms to maintain adequate levels of biosecurity (Kouam *et al*., 2020). Although not identified as a potential risk for brucella seropositivity, poor and inappropriate use of farm wears were more likely to lead to increase in brucella seropositive compared to farms where farm wears were properly used. Changing farm wear lowers the risks of transmission of contagious diseases among pigs since pathogens may attach on clothing and footwear (Amass *et al*., 2003; Ribbens *et al*., 2007).

The relatively higher incidence of abortions (53.76%) recorded in this study is indicative of the presence of pathogens causing reproductive failure in pigs in the region. Abortion in sows is among the most important signs of brucella infections in pig herds, although under field conditions it has been considered a minor component of disease presentation (Olsen and Tatum, 2017). In most of such cases, early embryonic deaths between days 17 and 21 occurs, with such early abortions rarely noticed by farm owners (Megid *et al*., 2010). The non-significant risks for brucellosis observed in non-abortive pigs compared to abortive pigs could probably not be mutually exclusive of females returning to heat after early abortions that went unnoticed, and thus considered as non-abortive pigs. This finding contrast those of Rahman *et al*. (2012) and Shome *et al*. (2019) who found abortion in pigs to be strongly associated with brucella infections.

Farms with an experience of females returning to heat after service to a fertile male in this study had a more than 3.00-fold higher risk for brucella infection compared to those that did not. The phenomenon of repeat breeding often arises from conception failure because of chronic inflammation following brucella colonization of the reproductive organs of female animals. Much lower proportions have been reported in pigs in India (Shome *et al.*, 2019).

Brucella infections in boars may manifest as lowered conception rates and birth of fewer live pigs per litter in sows bred to such males (Olsen and Tatum, 2017). The agent often localizes in the testicles and accessory sexual organs of infected males leading infrequently to testicular hypertrophy or abscesses (Megid *et al.*, 2010). Pigs showing inflamed testes in this study had significant effects on brucella seropositivity, with about 5.00-fold odds for infection in affected males compared to normal males. Much higher rates have been reported for orchitic pigs in India (Shome *et al.*, 2019).

The apparent seroprevalence for brucellosis among pregnant women (0.68%) in this study showed that the disease is endemic in the region at a low level. This finding is similar to reports from Cameroon (0.28%), Gambia (0.17%) and Togo (0.10%) (Awah-Ndukum *et al.*, 2018a; Germeraad *et al.*, 2016; Dean *et al.*, 2013). Low prevalence rates of porcine brucellosis (< 5 %) characteristic of low-endemic areas like sub-Saharan Africa have been reported (Corbel, 2006; Olsen *et al.*, 2012). Most probably, pigs in this area serve as reservoirs and source for the human infections. The result of the present study is lower compared to those involving persons in high risk professions (abattoir workers, veterinarians, laboratory workers) and in areas with high incidence in resident animal populations (Kamga *et al.*, 2020; Ducrotoy *et al.*, 2017; Ntivuguruzwa *et al.*, 2025; Makala *et al.*, 2020; Nguna *et al.*, 2019). The study highlights the potential risks of human interactions with pigs in porcine brucellosis endemic areas. Emphasis on the adoption of safer practices by persons exercising in pig related activities (farming, pork processing, and veterinarian among others) should be considered in the development of future strategies against brucellosis in the region.

The non-significant association between seropositivity to human brucellosis and place of residence observed in this study has also been reported in Yemen (Al-Shamahy *et al.*, 2000). However, pregnant women residing in rural areas were more than 9 times at higher risk for brucellosis compared to urban dwellers, an observation equally shared by other studies (Tumwine *et al.*, 2015; Sofian *et al.*, 2008; Shafqat *et al.*, 2025). Close interactions between natural brucella hosts and humans in remote areas favour the transmission of zoonoses including brucellosis (Corbel, 2006). The finding is contrary to other reports associating human brucellosis to urban settings (Abdullah *et al.*, 2018; Verma and Prakash, 2025).

Educational level did not seem to influence the human brucella seropositivity in this study, contrary to reports in Uganda (Tumwine *et al.*, 2015) where educational was a factor of the disease. This finding contrast those in Angola (Mufinda *et al.*, 2017) and Pakistan (Shafqat *et al.*, 2025) were uneducated/ illiterate livestock professionals and pregnant women were found to have significantly higher levels of infection.

Pregnant women in their 3rd trimester showed higher odds for brucella seropositivity compared to those in earlier stages of pregnancy, though with no significant effects on human brucellosis seroprevalence. Similar to the findings of this study are reports in Yemen, Jordan, Tanzania and Turkey which found no statistical significant differences between history of miscarriages, parity, and stage of pregnancy in humans and brucella infections (Abdullah *et al.*, 2018; Abo-Shehada and Abo-Halaweh, 2011; Makala *et al.*, 2020; Kirmizi *et al.*, 2022). However, this result is contrary to a report in Pakistan which found 1st and 2nd trimesters of gestation as a significant risk factor for human brucellosis (Shafqat *et al.*, 2025).

Although not found to be significantly associated with human brucellosis in this study, pregnant women who interacted with pigs and engaged in milking ruminants were more likely to be seropositive for brucella compared to those who did not. The risk of human infections from the handling of brucella infected pigs and pork was believed to be greater when compared to that associated with the handling of seropositive natural hosts of other *Brucellae* (Olsen and Tatum, 2017). These observations align with reports in Cameroon, Uganda and Yemen (Awah-Ndukum *et al.*, 2018a; Tumwine *et al.*, 2015; Al-Haddad *et al.*, 2013), while contrasting with other reports in Rwanda, Tanzania, Somalia and Pakistan

(Rujeni and Mbanzamihigo, 2014; Cash-Goldwasser *et al*., 2018; Yosef and Ismail, 2025; Shafqat *et al*., 2025; Ntivuguruzwa *et al*., 2025).

Direct contact with fluids and tissues during parturition and abortion cases may constitute a source of infection to humans as large numbers of brucellae are released with these materials (Olsen and Tatum, 2017). In this study, pregnant women who had experienced an abortion on their farm were at a much higher risk for brucellosis as compared to those who had not. This finding is similar to reports in Tanzania, Rwanda, Angola and Somalia (John *et al*., 2010; Ntivuguruzwa *et al*., 2025; Mufinda *et al*., 2017; Yosef and Ismail, 2025) but contrary to reports in Uganda and Tanzania (Nguna *et al*., 2019; Makala *et al*., 2020). Protecting humans against direct contact (proper usage of protective equipment) with tissues and fluids when working with pigs or any of their by-products, and the proper disposal of afterbirths or abortive materials are important in reducing the risk of transferring brucellosis from pigs to humans. Culinary behaviors such as drinking fresh milk and its byproducts, as well as eating raw, smoked, or bush meat, were not determined to be significant risk factors for human brucellosis in this study. Nonetheless, pregnant women who ate bush meat were more than five times more likely to be brucella-positive than those who did not. Similar findings have been reported in Botswana and Uganda, where anti-brucella antibodies were identified in various wild ungulates (buffalo, giraffe, etc.) (Alexander *et al*., 2012; Aruho *et al*., 2021).

Low levels of brucellosis awareness among pregnant women in this study has also been reported in sub-Saharan Africa (Awah-Ndukum *et al*., 2018a; Mufinda *et al*., 2017; Tumwine *et al*., 2015; Ntivuguruzwa *et al*., 2025). Good knowledge of brucellosis among pig farmers is essential to limit human transmissions from pigs. In the present study, persons who had knowledge of the symptoms in infected pigs and humans showed higher risk for the infection compared to those that did not. However, there was also a high probability of negligence in the adoption of adequate biosafety precautions to limit human infections among pig farmers, and higher risk for brucellosis in pregnant women who reported using protective gloves in handling pigs and pork in this study. The clinical symptoms for human brucellosis had no significant effects on brucella seropositivity, though pregnant women who experienced weight loss and night sweats were at higher risks. Higher brucella seropositive levels have been associated to malnourished and underweight states in pregnant women in Pakistan (Shafqat *et al*., 2025) and other clinical symptoms (Dean *et al*., 2012; Shafqat *et al*., 2025; Verma and Prakash, 2025).

Conclusion

Brucellosis is endemic but neglected in pigs and humans in the Western Highlands Regions of Cameroon. The disease incidence in pigs was significantly influenced by the location of the farm, age of the animal, season, practice of sharing farm tools between farms, and persistent presence of male pigs with inflamed testicles on farms. There was evident potentials for the occurrence of zoonotic brucellosis in vulnerable persons such as pregnant women in contact with pigs, though they were aware of the occurrence of brucellosis in pigs and humans and there is little or no surveillance and control policy for brucellosis in Cameroon. Continuous education of the public on the critical adoption of "One Health approach" is vital to improve knowledge of the epidemiology and control of brucellosis in livestock, including pigs, and humans in Cameroon.

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Novelty Statement

This study has provided the prevalence of brucellosis of pigs and vulnerable persons and the first assessment of the public health significance of zoonotic brucellosis in humans in the Western Highlands of Cameroon.

Author's Contribution

HNC and JAN conceived, designed and coordinated the study. HNC and JAN contributed equally and were the principal investigators. HNC, AJGN, ATN, and JAN designed data collections tools, methodology and implementation. HNC, AJGN and JAN contributed reagents, materials and analysis tools. HNC, ATN and JAN supervised the field investigation and laboratory work as well as data entry. HNC, AJGN, ATN, and JAN were involved in data validation, statistical analysis and interpretation. HNC drafted the original manuscript, which was reviewed and edited by JAN. All authors participated in preparation and critical reviewed of the manuscript. All authors have read and approved the final version of the manuscript.

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The authors declare that they have not used generative AI and AI-assisted technologies in the drafting, reviewing and editing process of this article before submission.

Conflict of interest
The authors have declared no conflict of interest.

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