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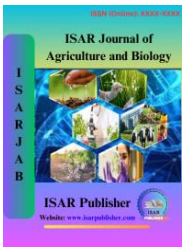

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Seroprevalence of *Brucella* antibodies in slaughtered pig and protection measures of abattoir workers in three Municipal abattoirs in Yaounde town Centre Region of Cameroon

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Abstract: Brucellosis is a neglected tropical food-borne zoonotic disease with a high prevalence in many developing countries and has a negative impact on both animal and human health. Pig brucellosis is emergent and neglected in Cameroon. However, there is a paucity of data at the national level and risk factors associated with the disease are not well known. A slaughterhouse study was conducted at three Municipal abattoirs in Yaounde to determine the seroprevalence, assess the risk practices and evaluate the usage of personal protective equipment (PPE) by abattoir workers againts *Brucella* infection. A cross-sectional study was conducted from May 2020 to January 2021. Serum samples from 384 slaughtered pigs at three municipal abattoirs in Yaounde were collected and screened for anti-brucella antibodies using Rose Bengal Plate Test (RBPT) and indirect ELISA test. Furthermore, 41 plausible exposed and vulnerable workers to brucellosis (4 veterinarians; 23 slaughteres and 15 butchers) were recruted to evaluate the perception about the usage of PPE, length of employment and their level of knowledge about zoonotic diseases. All collected data were statistical analysed using GraphPad Prism 8.1. Among the 384 serum sampled, 11(2.86%) and 23 (5.98%) were positive for *Brucella* infection to Rose Bengal Plate Test (RBPT) and indirect Enzyme Linked Immuno-Sorbent Assay (iELISA) respectively. Moreover, 10 (2.6%) sera were positive to both tests, 13 (3.38%) positive to ELISA but negative to RBPT. Only one serum (0.26%) was positive to RBPT but negative to ELISA. None of the abattoir workers used standard protective coveralls. The boots were principaly used by the slaughterers. In general, three quarter of respondents did not know brucellosis and they were not educated. This study showed that the prevalence of *Brucella* infection was low among slaughtered pigs in Yaounde abattoirs.

Keywords: Brucellosis, Seroprevalence, protection, abattoir, slaughtered Pigs, Cameroon.

Introduction

Brucellosis, also known as Malta fever or ondulate fever, is a highly contagious zoonotic disease caused by bacteria belonging to the genus *Brucella*. (Godfroid J *et al.*, 2005; Gumaa M *et al.*, 2020) Brucellosis is a notifiable disease affecting people, livestock, and wildlife globally causing high losses in livestock production and seriously threatens public health (WHO, 2018; Rebollada-Merino A *et al.*, 2022). Brucellosis represents the most common zoonotic infection, with a total of about 500.000 new human cases per year (Di Bonaventura G *et al.*, 2021). It continious to be endemic in some geographic areas, such as the Eastern Mediterranean Basin, the Middle East, Arabian Peninsula, Latin America, Southern Europe, Central Asia, the Indian subcontinent, and many African countries. Brucellosis, particularly caused by *Brucella abortus*, *Brucella melitensis*, *Brucella suis* poses serious public health problems in reproduction, and its as wel responsible for huge economic losses estimated to be about 427 million USD per year due to abortion, death, decreased milk and meat production in sub-Saharan Africa, partiularly in Low and Middle-Income Countries (LMIC) (Erume J *et al.*, 2016; McDermott J *et al.*, 2013). For instance, Kenya (203.07/100,000), Yemen (89.96/100,000), Syria (47.26/100,000), Greece (42.96/100,000) and Eritrea (21.82/100,000) are the countries with a higher incidence of brucellosis. The plausible reason is the unrestricted control, inadequate infrastructure, limited resources and lack of information on its significance and distribution (Laine C *et al.*, 2020).

Generaly, Human infection by *Brucella* is associated to acute febrile illness, severe debilitating disease that requires prolonged treatment with a combination of antibiotics, permanent disabling sequel, considerable medical expenses and loss of income due to loss of working hours (Corbel M, 2009; Pereira C *et al.*, 2020). In addition, spontaneous miscarriages and utero foetal death during the first trimesters have also been reported among pregnant women (Corbel M, 2009; Pacheco W *et al.*, 2012).

Pig brucellosis caused mainly by *Brucella suis* (Rajeswari S *et al.*, 2019) is a notifiable zoonotic disease and is widespread in Africa. The prevalence of pig brucellosis varies from 6.6% to 41.0% among countries in West and Central Africa (WHO, 2018; Akakpo A, 1987; Bayemi P *et al.*, 2008) but despite

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that, surveillance across the continent is generally poor (Pappas G *et al.,* 2005; Jindal P *et al.,* 2016). In general, pig brucellosis is associated to reproductive losses worldwide, and it is considered as an important public health problem with its zoonotic capability of causing clinical symptoms (McDermott J & Arimi S, 2002). Furthermore, due to the lack of attention and absence of adequate diagnostic facilities, the prevalence of brucellosis ranges from sporadic cases to up to 41% in some affected areas of the Sub-Saharan region (CamOHNS, 2012; Barua A*et al.,* 2017; Scolamacchia F *et al.,* 2010). The other causes of this are the lack of surveillance program, public awareness, inadequate public-sector animal health services, poor or low-income communities limited resource-settings (Ducrotoy M *et al.,* 2014; Van der GiessenJ & Priadi A, 1988). Most importantly, brucellosis is a highly contagious disease for both animals and humans as there exist cross transmission of certain *Brucella* species (Laine C *et al.,* 2020). Natural infection occurs by direct contact with infected animals or their secretions like vaginal fluids, placentae and placental fluid, aborted foetuses and foetal membranes that contains large amounts of the bacteria (Moreno E, 2014; Poester F *et al.,* 2013). Therefore, veterinarians, abattoir workers and other livestock keepers are at risk of infection. Transmission can also be through the consumption of unpasteurised milk and milk products from infected animals (Ibironke A *et al.,* 2008). Unfortunately, adequate health and safety measures are rarely observed in most developing countries thereby, increasing the chances of zoonotic transmission. Also, the fact that most animals, irrespective of where they originate, end up at the slaughter slabs or abattoirs is very important because, apart from screening live animals at the herd level, screening slaughtered pigs at the abattoirs is also crutial for the epidemiological investigation of pig brucellosis. In developping countries like Cameroon, many conditions favour the widespread nature and transmission of the disease in most of the regions (McDermott J & Arimi S, 2002). These include: uncontrolled animal movement, migrations of pastoralists in search of pasture, water, purchase of infected animals from livestock market for replacement or upgrading, anarchic development of urban livestock breeding, nature of the animal production system, inadequate sanitary measures, demographic factors, regulatory issues, climate, deforestation and wildlife interaction (Awah-Ndukum J *et al.,* 2018a; Profitós J *et al.,* 2014; Laine C *et al.,* 2022).

Human brucellosis is an important occupational zoonotic disease. The livestock, abattoir workers and veterinarians are more prone to infections due to the poor hygiene, poor workplace safety standards as well as ignorance favours the spread of the disease (Pereira C *et al.,* 2020; Franc K *et al.,* 2018). The main type of pathogen exposure is the contact with animal fluids, aborted foetus, placenta and viscera or accidental contact with those materials of slaughterhouse workers cutting themselves with dirty sharp blades and their frequent exposure to blood, discharges, carcasses and viscera of infected animals through cuts and wounds, as well as splashes from infected blood into their conjunctiva and inhalation of aerosols (Franc K *et al.,* 2018; McDermott J & Arimi S, 2002). In some cases of human brucellosis, the infection has been associated with laboratory-acquired infections.

In the last decades, the World Organization for Animal Health (WOAH) formerly known as OIE reported many conditions which favour the widespread nature and transmission of the disease in most of the regions, increasing the incidence of animal and human brucellosis with urbanization. Since 1996, official measures according to OIE are in place in Cameroon to control bovine brucellosis through movement control of cattle at the borders and within the country but no outbreaks have been reported to OIE (Bayemi P *et al.,* 2008). So far, two implemented program exist in Cameroon: The National Program "One Health" and a second in collaboration with the RE- SPOND project – USAID in 2014 for the prevention and control of emerging and re-emerging zoonoses and which addresses the multi-disciplinary facets of disease complexity involving livestock biosecurity, environmental conditions, veterinary and medical extension services respectively (Awah-Ndukum J *et al.,* 2018b). Despite these programs were set up, no control strategy has been developed for brucellosis and no vaccination againts *Brucella* infection is available although *Brucella* antibodies have been detected in some abattoir studies in Cameroon (CamOHNS, 2012; Awah-Ndukum J *et al.,* 2018b) However, there exist the veterinary infrastructure installed over a wide area like the ''*Centres Zootechniques et de Contrôle de Santé Vétérinaire''* (CSV) in some parts of Cameroon. Their main functions are to put in place the annual vaccinations included Cattles, goats, sheeps and pigs, veterinary health control at livestock markets, along transhumance and trade routes (Awah-Ndukum J *et al.,* 2018a; Profitós J *et al.,* 2014).

Since 1980, a number of research findings have reported the presence of *Brucella* antibodies in domestic animals including Cattle, sheep, dogs, pigs and goats in some parts of Cameroon. To the best of our knowledge, between 1982 and 2023, thirteen (13) prevalence investigations, split between four semi-intensive and seven pastoralist communities and have reported important data about brucellosis among cattle in general and humans in particular in some municipal slaughterhouses mainly in the Northern and Western parts of the country (Laine C *et al.,* 2020; Eko S *et al.,* 2023). These studies comprised two (2) in Far-North, three (3) in North, six (6) in Adamawa, one (1) in East, one (1) in Center, four (4) in North-West, three (3) in South-West, foir (4) in West, one (1) in South and one in Littoral and South-West (Domenech J *et al.,* 1980; Domenech J *et al.,* 1982; Shey-Njila O *et al.,* 2005; Bayemi P *et al.,* 2008; Scolamacchia F *et al.,* 2010; Ojong B, 2015; Bayemi P *et al.,* 2015; Kong A *et al.,* 2016; Ojong B *et al.,* 2016; Awah-Ndukum J *et al.,* 2018a; Awah-Ndukum J *et al.,* 2018b; Kamga R *et al.,* 2020; Kamga R *et al.,* 2021; Eko S *et al.,* 2023). Among these previous studies, three were conducted in abattoirs, one at a dairy farm, and one with banked sera, while all other investigations were conducted at beef farms. So far, nine of these studies targeted only cattle, one evaluated only human subjects, and one investigated both cattle and humans (Laine C *et al.,* 2020; Eko S *et al.,* 2023). The pioneer survey was done by Awah-Ndukum (2018b) who reported a bovine brucellosis seroprevalence of 3.40% (n = 590; 3.4% for RBPT, 5.93% for i-ELISA). According to this study, the prevalence of human brucellosis can range from 0.28% to 31.4% in populations of pregnant women with a history of abortion and high-risk occupational groups (HROG) that include cattle rearers, abattoir workers, and butchers (Awah-Ndukum J *et al.,* 2018b). Most recently, the first prevalence of brucellosis in small ruminants, dogs and pigs was reported for the first time in Cameroon (Kamga R *et al.,* 2020). However, the prevalence of slaughtered pigs, the main risk factors and barriers to effective protection of each abattoir workers for the disease is not yet assessed.

Despite the interesting data generated by these previous studies, no one to our knowledge has explored the precautionary protection measures and daily rick pratices of pig abattoir workers concerning *Brucella* infection. Furthermore, only one study till date in pig herds has been reported in the South region in 2022 on pig brucellosis by Kamga and collaborators (Kamga R *et al.*, 2020) but no data about abattoir pig brucellosis was reported in Cameroon (Eko S *et al.*, 2023). This could be explained by the many taboos on pig, social habits and religious persuasions, negligence in the health status of the pig brought at abattoir which contribute to the lack of knowledge about the epidemiology of *Brucella* infection, its economic impact, or the role of pigs in spreading the infection to other livestock or frequently exposed professionals. In addition, this is due to ignorance to some behaviors, such as negligence in the use of individual and collective protective measures that increases the probability of infection and the lack of access to veterinary and public health resources which makes it difficult to implement disease prevention, treatment, and control programs (Awah-Ndukum J *et al.*, 2018a; Profitós J *et al.*, 2014).

Generally, in Cameroon, slaughters, veterinarians and butchers do not take health-related precautionary measures to protect themselves (for example they are not using personal protective equipment (PPE) during procedures) that could predispose them to *Brucella* infection. However, control of disease in livestock can reduce risk for human infection by decreasing human exposure through livestock (World Organization for Animal Health, 2028). There is a need to assess its quality in terms of health hazards and ensure the safety of consumers and abattoir workers whose need for survival often outweighs the risk of infection. Therefore, the aim of this study was to create awareness around the risk of exposure to *Brucella* infection, to assess the prevalence of slaughtered pigs in the municipal abattoirs in the city of Yaounde, evaluate the practices and protection measures of abattoir workers as well as their level of knowledge about zoonotic diseases.

Materials and methods

Description of study areas: The study was carried out for eight months from May 2020 to January 2021 in the city of Yaounde, Centre Region in Cameroon. The city of Yaounde (3°52′ 0’’N and 11° 31′ 0’’E) is the political capital of Cameroon, located in Mfoundi Division with 70 sub-Division (Figure 1). The population of Yaounde is estimated at 4,100,000 residents. This investigation was done in the three functional pig abattoirs of the city; one located in Yaounde II subdivision (Mbankolo Market) and the two others in Yaounde IV subdivision (Mvog-Ada and Esos Market). In the city of Yaounde, there are three livestock parks with functional pig abattoirs. These three markets are considered like the Carrefour market of pigs which comes from many regions and districts to be commercialized and serve as meat supplies for the butchers and rotisseries. Additionnaly, the city of Yaounde represents the largest pig consumption center zith 66% compared to Douala city (28%) and Dschang (6%) (Ndébi G & Ongla J, 2006).

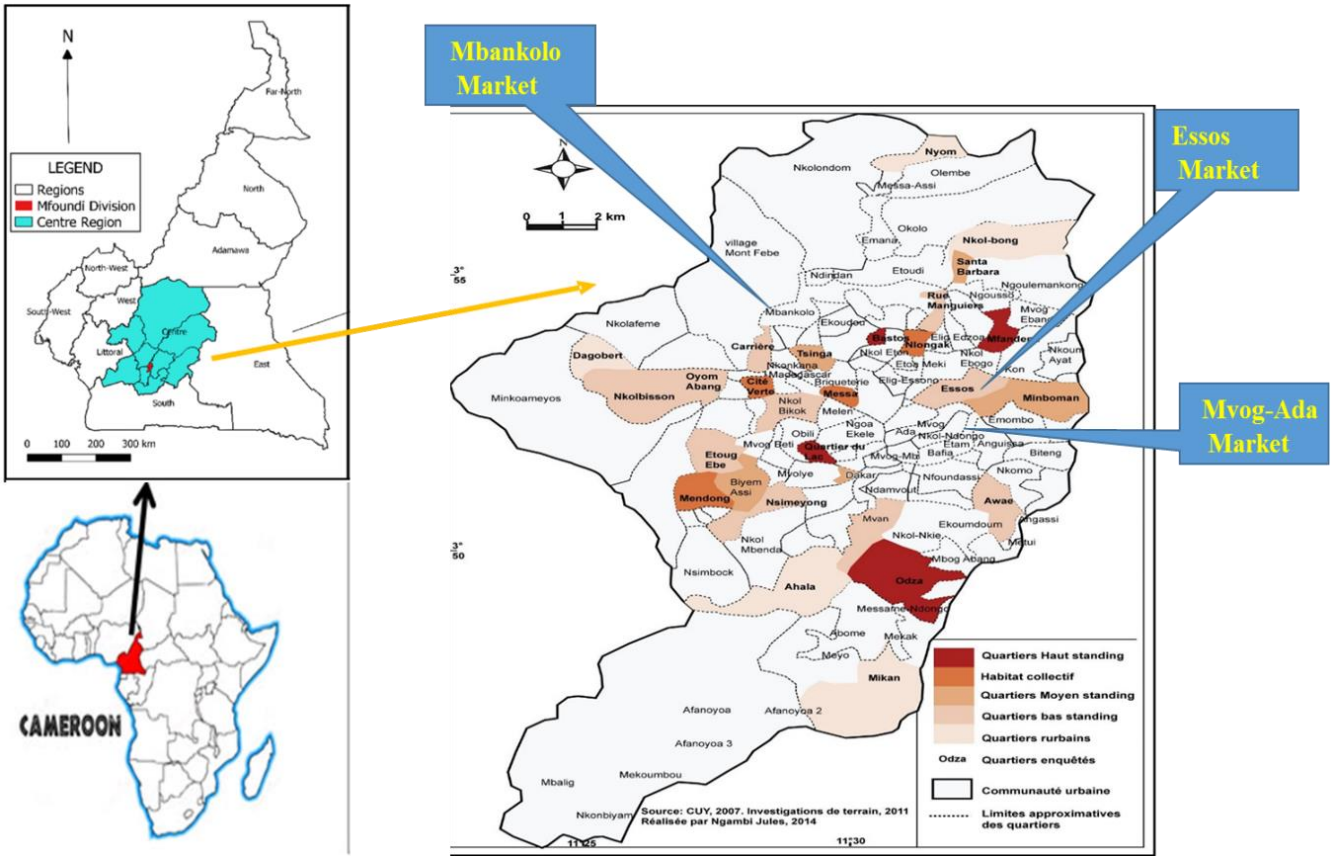

Fig 1: Map showing three abattoirs in the city of Yaounde where sampling was undertaken

Study Population/Study Design/Selection Criteria : The study population comprised pig abattoir workers including slaughters, veterinarians, and butchers who manipulate animals directly, and their products when the domestic pigs are brought in the livestock parks of the market. The study was a descriptive cross-sectional design study. To infer *Brucella* infection, a study was conducted to determine the seroprevalence of pig brucellosis among slaughtered pigs at abattoirs. Concerning the potential risk of exposure to pig-associated *Brucella* infection, an ethnographic study was undertaken to describe the interface between pig and abattoir workers.

In these municipal abattoirs, an average, 20-100 pig were slaughtered on daily basis and the abattoir workers had the highest likelihood of contact with pig and their products. Selected pigs of the study were composed of local fat White pigs, Duroc and North breed and putative biological factors believed to be associated with the epidemiology of brucellosis were recorded. These included, sex, age and breed. During our sampling, about 15–25 pigs slaughtered daily in the abattoir were randomly selected each day with the exception of Sunday.

The criteria for selection of the respondents were any individual who worked at pig abattoir markets for at least one year. The selected individual pig was subsequently enrolled after informed consent was given. Any individual selected, that was unable to participate due to health issues was excluded. The animals included in the study were both males and female's domestic pigs brought to the abattoir for sell at the time of sample collection. Piglets less than six months old and weak pigs which might be adversely affected by bleeding were excluded from the survey.

Survey study and sample size: The study was conducted from May to September 2020 and the data were collected in two steps. The first step was the observational survey which was done for two months period from May to July 2020 and consisted of identifying the usual daily practices of slaughterers, butchers and veterinarians. Furthermore, a structured questionnaire comprising both open-ended and closed-ended questions was then administered in a face-to-face interview with one respondent on abattoir workers' practices included questions about using personal protective equipment (PPE), working with open wounds on their hands, eating or smoking while working, hand washing habits, and routine disinfection of knives.

The second step was the collection of slaughtered pig samples was done within a three months period from July to September 2020. Blood sample was collected every working day (from Monday to Friday) except public holiday. The sample size was calculated using the formula for simple random sampling technique as described by Thrusfield (Thrusfield M, 2007) and with an estimated *Brucella* infection rate of roughly 50% considering an infinite population (no recent census published data on the pig brucellosis prevalence in Cameroon during sampling), a precision level of 1% and a 95 % confidence interval. For this study, an expected prevalence of 50% was used to estimate the sampling size as follows:

$$\boldsymbol{n} = \frac{\mathrm{z}^2.p(1-p)}{d^2}$$

Where n= the minimum sample size required for very large population, Z= the critical value for a given confidence interval, P= expected proportion of the event to be studied, d= margin of error (the margin of error is 0.05). Accordingly, the minimum sample was 384 with 95% confidence interval and 5% margin of error.

Interview procedure among abattoir worker: The principal investigator (primary author) was the only one responsible for collection of all data for the study.

Each abattoir was visited only working day (except the weekeng and the holydays) and abattoir workers were interviewed. For each abattoir, a meeting was organized with the workers to inform them for the study and their participation. Briefly, the principal investigator (first author) explained the objectives of the study and they were told that their participation in the study was voluntary and thet their personal information would not be disclosed.

Afetr the meeting, the collection data was done using pre-tested questionnaires comprising of open-ended and closed-ended questions in a face-to-face interview with one respondent. Briefly, a structured questionnaire was administrated at all voluntary participants. The questionnary comprised 4 sections including the socio-demographic characteristics of abattoir workers; their perception of the use of PPE; the awareness and the level of knowledge of zoonotic diseases like brucellosis; the main abattoir workers' practices as potential pathways for *Brucella* infection.

Blood collection from the slaughtered pig and serum preparation: The pig blood samples were collected according to the World Organization for Animal Health (OIE) recommendations before slaughter and previously described by Alton and collaborators (Alton G *et al.*, 1988). For each slaughtered pig, approximately 3–5 ml of venous blood was collected after slaughtering at the jugular by venipuncture into 5ml labeled sterile vacutainer tubes (BD vacutate, UK), without anticoagulant a permanent marker recording the case number of the respective study subject and the name of the abattoirs. The blood samples were allowed to clot at room temperature in order to get clear serum and to minimize hemolysis of blood and kept in slanted position on ice for serum formation. Blood samples were kept in an icebox and transported immediately to LANAVET Annex Yaounde. The serum was separated after spinning at 2,500 rpm for 10 minutes at room temperature and the serum was transferred to a labeled vial and stored in a deep freezer (-20 °C) until laboratory analysis. The metadata for each sample included breed, sex, age, abattoir and date were noted.

Serological examination: Two types of serological tests were achieved in parallel, screening and confirmatory tests for the detection of antibodies against *Brucella* in the pig serum samples by the Rose Bengal plate Test (RBPT) and i-ELISA respectively. Both serological assays were done at the LANAVET.

Rose Bengal plate test: RBPT (ID.Vet, Innovative Diagnostics) was used as a screening test for the serum samples collected for the presence of agglutinins. The test was performed according to Alton and collaborators (Alton G *et al.*, 1988), also described by others studies (World Organisation for Animal Health, 2009; Gupte S & Kaur T, 2015). Before performing the test, both sera and RBPT

antigen were aloquoted and brought to room temperature as recommended by the manufacturer. Following, equal volumes (30 µL) of test sera and RBPT were then mixed thoroughly using a toothpick. The mixture was agitated sogftly for 4 minutes with the hand. The reaction was immediately assessed after 4 minutes and interpretation of the results was made according to the presence and degree of agglutination. The reaction was considered as positive if agglutination (reaction between serum and antigen) was observed and negative if there was no agglutination (no reaction between serum and antigen) (World Organisation for Animal Health, 2009; Miassangoumouka J *et al.*, 2019).

Detection of brucellosis antibodies by indirect ELISA test: The indirect-ELISA (i-ELISA) test was carried out using the commercial kit which was sourced from IDvet Innovative Diagnostics, Grabels, France; a multi-specie indirect ELISA diagnostic kit designed for the detection of antibodies directed against *Brucella abortus* (bovine), *Brucella melitensis* (ovine and caprine) and *Brucella suis* (swine) in serum. The test was performed according to the manufacturer's instructions in polystyrene plate of 96-wells pre-coated with purified *Brucella spp*. antigens and essentially as described previously (Kamga R *et al.* 2020; Ukwueze K *et al.* 2020). Before each i-ELISA test, reagents and serum samples were brought to room temperature as recommended by the manufacturer (22 ± 4°C).

For the achievement, 100 µl of diluted buffer was added to each well then, ten microlitres of both positive and negative control were introduced into two different wells of the polystyrene plate and 10 µl of each serum sample was introduced in the remaining wells and each plate was sealed and homogenized. The plate was incubated at room temperature for 45 min and washed three times with PBS-Tween. Next, 100 µl of multispecies horseradish peroxidase (HRP) conjugate was added to each well and the plate was subsequently incubated for 30 min at room temperature. After, the plate was washed three times to eliminate the excess conjugate, then, 100 µl of the substrate solution (tetramethylbenzidine in substrate buffer containing H_2O_2) was added to each well and the plate was incubated in the dark for 15 min at room temperature. The last step was the addition of 100 µl of 1 N-hydrochloric acid (HCl) as stop solution. Each plate was introduced in a micro plate photometer (Bio Tek ELX800 absorbance reader) to measure at 450 nm the optical density in each well. For each sample tested, S/P% was calculated as previously described (Ukwueze K *et al.* 2020; Khan A *et al.* 2019) using the formula:

$$\frac{\boldsymbol{S}}{\boldsymbol{P}}\% = \frac{(ODsample - ODnc)}{(ODpc - ODnc)} \text{x} 100$$

Where OD_{sample}, OD_{nc}, and ODpc are the readings of optical densities 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 ODpc >0.350 and ODpc/ODnc >3 indicated that the tests were working properly.

Ethical statement: Permission for the study was obtained from the required authorities and Local Ethical Committees in the Centre Region, Cameroon. Briefly, the protocol for field studies and collection of animal materials was approved by the Ethical Committee of the University of Yaounde I, the Regional delegation of Livestock, Fisheries and Animal Industries, the Veterinary National Laboratory (LANAVET) and the faculty of sciences of the University of Yaounde I. The purpose of the study was explained (with the assistance of the local veterinary practitioners, and research assistant) to personnel at the abattoir.

Data analysis: All human and animal datas were statistically and graphically analyzed using GraphPad Prism 8.1. Frequencies were generated and Odds ratio, 95% confidence interval, Chi-square or/and Fisher's exact tests were computed to see the degree of association of the risk factors with *Brucella* seropositivity. The difference was considered significant if the p-value was lower than 0, 5.

Results and discussion

Results of observationnal survey: The investigation which lasted two months allowed us to make various observations as to the practices and the use of adequate PPE by slaughterhouse workers. In all these three pig abattoirs, the slaughter system and infrastructurres are still traditional; there are no appropriate preventive measures (the abattoir workers worked in the environment that may be become contaminated at every time by the *Brucella* pathogen) and they handled potential infected animals/carcasses and aborted fetuses or placentas (Figure 2A and 2B).

Concerning the use of PPE by the abattoir workers, our investigation revealed that the main protective equipment of both slaughters and butchers workers during their activity was the simple clothes such as trousers, Tee-shirts and slipper (not designated). As shown in figure 2, the slaughterers and butchers for example used old and worn Tee-shirts during the evisceration processing of slaughtered pigs (Figure 2B). So far, the blood of pigs slaughterered finds itself in contact with the skin and the clothes of the slaughterers; moreover, they clean them only in the evening for some and never for the others. It should be noted that the majority used just boots only as personal protection but none of them used gloves because of a perceived inconvenience arising from its use. Concerning the butchers, the majority of them wore slippers or bare-foot either (Figure 2A).

A B

Fig 2. : Slaughters and butchers working with a few and inadequate protection equipment

(A) Butcher with a clothing defected and deviscering a slaughtered pig for selling

(B) Slaughter slaughtering pig having boots, an ordinary clothing not designated and no gloves

As shown in figure 3, the veterinarians and para-veterinarians did not use adequate PPE. They used just lab jackets like body protection; they had no hand protection (i.e. gloves), no eye protection, no respiratory protection, no closed footwear, and no face shield. No process was performed judiciously to minimize spills, splashes, and aerosols as recommended by OIE and they were at some time in direct contact with animal fluids. The veterinarians and para-veterinarians used the coverall frequently, at some time they used boots but they used gloves rarely (Figure 3).

Fig 3. Veterinarians inspecting a suspected slaughtered animal presenting skin symptoms

Results of the interviews

Socio-Demographic Characteristics of Respondents at different sites

The participant characteristics are presented in Table 1. A total of 71 individuals were approached and copies of questionnaires were administrated, but 41 copies were completely and correctly filled with a response rate of (51, 89%). The majority of the respondents were selected from the Mbankolo abattoir (n=19; 46, 34%). The percentage of men and women who participated in the study was 92, 68% and 7, 31% respectively. Most of the respondents were not married or separated (70, 26%), having either no education or a primary level of education (60, 97%). Only 11 (26, 82%) had attained a secondary education and 5 (12, 19%) had not attended tertiary school. The mean age of the respondents ranged between 20-35 years with most of them 23 (56, 09%) being slaughters.

Table 1 : Demographic profile of the participants (N = 41)

Characteristics	Variables	Frequency (n)	pourcentage (%)
Collection Site	Mbankolo	19	46.34
	Mvog-Ada	13	31.70
	Essos	9	21.95
Sex	Male	38	92.68
	Female	3	7.31
Workers category	Veterinarians	04	9.75
	Slaughters	23	56.09
	Butcher	14	34.14
Age	<20 years	1	2.43
	20-35 years	27	65.85
	35-50 years	11	26.82
	>50 years	2	4.87
Matrimonial statut	Single	14	34.14
	Married	27	65.85
Education	Primary School	25	60.97
	Secondary school	5	12.19
	Tertiary education	11	26.82
Years of work experience	≤3	6	14.63
	4-10	21	51.21
	≥10	14	34.14

The perceived practice of respondents on the use of PPE was positively correlated with the knowledge on zoonosis. On the other hand, there were no significant correlations between perceived practices of respondents on the use of PPE on age of the respondents and number of years in operation. These findings suggest that practice of respondents on the use of PPE to protect against brucellosis may not be associated with age and number of years respondents had spent in operation.

Awareness and level of knowledge of Brucellosis among Respondents

As shown in Table 2 most of respondents (n=4) were not aware about brucellosis (9, 75%) and the majority of them, got their information about brucellosis from MINEPIA trainings of MINEPIA or the veterinary students who came at the abattoir for their research. While a majority of the respondents indicated that brucellosis can not affect or is not dangerous to human health. These results suggested that, subjects who had more knowledge about brucellosis had spent 4-10 years in operation.

Table 2 : Abattoir worker Practices among Respondents in the three municipal abattoirs

Topics	Category	Yes n(%)
Perception of the use of PPE	Strongly Agree	6 (14.63)
	Agree	17 (26.82)
	Undecided Disagree	7 (17.073)
	Strongly disagree	11 (26.82)
Awareness and level of knowledge of Brucellosis	Good	2 (4.87)
	Moderate	2 (4.87)
	Poor	37 (90.24)
Slaughtering surface should be washed with clean water regularly	Strongly Agree	8 (19.51)
	Agree	11 (26.82)
	Undecided Disagree	17 (26.82)
	Strongly disagree	5 (12.19)

This study recorded high-risk practices at high rates among pig abattoir workers. In addition, all workers worked with open hand wounds (100%) and 6 smoked while working (14.63%). Finally, only four workers (veterinarians, 9.75%) would disinfect their knives daily using lab jackets.

Table 3 : Abattoir worker Practices among Respondents according the attituds during their work in the three municipal abattoirs

Topics	Category	Yes n(%)	No n(%)
Abattoir workers' practices	Using PPE	0 (0)	41 (100)
	Get injured during working	35 (85.36)	6 (14.64)
	Working with open/cut hand-wound	41 (100)	0 (0)
	Eating while working	32 (78.05)	9 (21.95)
	Smoking while working	27 (65.85)	14 (34.85)
	Washing hands before eating	41 (100)	0 (0)
	Washing hands before smoking	6 (14.63)	35 (85.36)

With respect to the table 3, eight (n=8, 19.51%) of the respondents either strongly agreed and 11(26.82%) agreed that slaughtering surfaces should be washed with clean water regularly and daily. The majority n=17 (41.46%) disagreed about the washing of the slaughtering surfaces regularly and daily, that its is not necessary but one time daily is sufficient. While 5 (12.195%) strongly disagreed, it disturbed their work and could be done after the work in the evening.

Abattoir seroprevalence of pig brucellosis

The overall seroprevalence of *Brucella* infection at the abattoir by Rose Bengal Plate Test (RBPT) was 2.86% (11/384); while the seroprevalence of *Brucella* infection was 5.98% (23/384) by indirect Enzyme-Linked Immuno-sorbent Assay (i-ELISA). Most 10 (2, 6%) sera were positive in both tests; additionally, 13 (3.38%) were positive in ELISA but negative in RBPT. Only one serum (0.26%) was positive in RBPT but negative in ELISA (Table 4).

Table 4: Results combined results of Rose Bengal Plate test and Enzyme Linked Immunosorbent Assay of seropositivity of Brucellosis among slaughtered pigs

Serological tests	RBPT		i-ELISA		RBPT+i-ELISA		
Results	+ n(%)	- n(%)	+ n(%)	- n(%)	R+ & E- n(%)	R- & E + n(%)	R+ & E+ n(%)
Nber of case	11 (2.86)	373 (97.14)	23 (5.98)	361 (94.01)	1 (9.09)	13 (4348)	10 (90.91)

(-): negative; (+): positive; (R) : Rose Bengal Test ; (E) : ELISA test; Nber: Number

Among the 384 sera sampled, most 170 (42.27%), 162 (42.18%), and 52 (13.54%) were collected from the pig abattoirs located in Mbankolo (Yaounde II Sub-division), Mvog-Ada and Essos (Yaounde IV Sub-division) market respectively (Table 5). The majority of the pig came from different parts of Cameroon. Most 233 (69.7%) serum samples were females while 151(39.32%) were males; and 237 (35.67%) were N eima, followed by Duroc 141 (36.71%) and the last were local North breed 6 (1.56%) the least. Moreover, the majority, 206 (53.64%) of the slaughtered pigs sampled were adults, the old were 120 (31.25%) followed by the young representing 58 (15.10%) of the pig population.

Table 5: Results of descriptive and univariate analysis between potential individual slaughtered pig risk factors and the serological status of brucellosis in pigs sampled at the three Municipal abattoirs in the city of Yaounde

	RBTP				i-ELISA			
Variables	**Positive** N (%)	**Negative** N (%)	^{2}	**-value**	**Positive** N (%)	**Negative** N (%)	^{2}	**-value**
COLLECTION SITE								
Mbankolo	4 (3.35)	166 (97.63)	.368	.831	10 (5.88)	160 (94.11)	.53	.464
Mvog-Ada	5 (3.08)	157 (96.91)			8 (4.93)	154 (95.06)		
Essos	2 (3.84)	50 (96.15)			5 (9.61)	47 (90.38)		
SEX								

Males	7 (4.64)	144 (95.36)	.806	.093	10 (6.62)	141 (93.38)	.17	.673
Females	4 (1.72)	229 (98.28)			13 (5.58)	220 (94.42)		
BREED								
Neyma	6 (2.53)	231 (97.46)	.203	.122	13 (5.485)	224 (94.51)	.36	.506
Duroc	4 (2.83)	137 (97.16)			9 (6.382)	132 (93.61)		
North	1 (16.67)	5 (83.33)			1 (16.67)	5 (83.33)		
AGE								
Young (1-2Yrs)	3 (5.17)	55 (94.82)	.310	.191	7 (12.06)	51 (87.93)	.77	.092
Medium (2-4Yrs)	3 (1.45)	203 (98.54)			9 (4.36)	197 (95.63)		
Old (≥4 Yrs)	5 (4.16)	115 (95.83)			7 (5.83)	113 (94.16)		

X^2 : Chi-squared

The study revealed that, the seroprevalence of *Brucella* infection by RBPT in slaughtered pigs at Mbankolo, Mvog-Ada and Essos abattoir market were 4 (3.35%), 5 (3.08%) and 2(3.84%), respectively (Table 6). The test showed that the highest seroprevalence (4.64%) was recorded for the male pigs while the lowest (1.72%) the female pigs. Moreso, more of the North breed (16.67%) were seropositive followed by Duroc breed (2.83%) and lastly by Neyma breed (2.53%). In addition, the highest percentage seroprevalence (12.068%) in the RBPT was among the young pigs (1-2 years), followed by the adults (≥4years) (5.83%). The Young adults (2-4 years) had the last prevalence (4.36%).

Furthermore, among the 11 seropositive animals to RBPT, 7 (63.64%) were males while the lowest 4 (36.36%) females. Also, more Duroc breed (36.36%) were seropositive than North breed (9.09%); while the Neyma breed recorded the highest percentage (54.54%). The highest percentage infected adult (45,.46%) were above 4 years of age. However, the other ranges (1-2 and 2-4) revealed the same percentage (27.27%).

Table 6: Results of multivariable logistic regression between potential risk factors and the serological status of brucellosis in slaughtered pig according the RBPT results

Variable	**Categories**	**Positive to RBPT**					
		NT	**Nb+ (%)**	**Odd Ratio**	**95% CI**	***X^2***	***p*-value**
Collection Site	Mbankolo[1]	170	4(3.35)	/	/	/	/
	Mvog-Ada	162	5(3.08)	0, 756	[0.2289 - 2.567]	0.169	0.680
	Essos	52	2(3.84)	0, 6024	[0.1374 – 3.249]	0.337	0.561
Sex	Males[1]	153	7 (4.64)	/	/	/	/
	Females	233	4 (1.72)	2, 783	[0.871 - 8.603]	2.806	0.093
Breed	Neima[1]	237	6 (2.53)	/	/	/	/
	Duroc	141	4 (2.83)	0, 8896	[0.2352 – 2.836]	0.031	0.858
	North	6	1 (16.67)	0, 1299	[0,0186 - 1.765]	4.203	0.040[Yes]
Age	Young[1] (1-2 Yrs)	58	3 (5.17)	/	/	/	/
	Medium (2-4 Yrs)	206	3 (1.45)	3, 691	[0838 - 16.056]	2.814	0.093
	Old (≥4 Yrs)	120	5 (4.16)	1, 255	[0.322 - 4.982]	0.092	0.761

[1] Fixed categories for comparing serological status amongst pig; [Yes] $p < 0.05$: significant difference in serological status as compared to the fixedcategory for each variable.

Abbreviations: NT, Numbers tested ; Nb+ (%), Number of positive cases ; 95% CI, 95% confidence interval ;

As shown in Table 5, the highest 10 (5.88%) seroprevalence by ELISA test was recorded at Mbankolo, followed by Mvog-Ada 8 (4.93%) and the lowest 5 (9.61%) at Essos abattoir. Generally, the breed-specific result showed that the highest seroprevalence occurred among the North breed pigs (16.67%), followed by Duroc breed pigs (6.38%) and the last was Neyma breed pigs (5,485%). The highest age pecific seroprevalence was recorded in the young (1- 2 years) (12.06%) followed by adults (≥4years) (5.83%) and lowest in the young adults (2-4) (4.36%). Also, the sex–specific result showed higher seroprevalence among the males (6.62%) than the females (5.58%) (Table 5).

In addition, among the 23 seropositive animals to ELISA test, the females revealed a higher seroprevalence (56.52%) compared to males (43.47%) (Table7). The age specific prevalence was highest among young adults (39.13%) compared to the youngs and adults with the same seroprevalence rate of (30.43%). Duroc breeds (36.36 %) were more seropositive than North breeds (9.09%); while the Neyma breed recorded the highest percentage (54.54%). The highest percentage of infected adult (45.46%) were above 4 years of age. However, the others range (1-2 and 2-4) revealed the same percentage (27.27%).

Moreso, more Neyma breed (56.52%) were seropositive than Duroc breed (39.13%); while the North breed recorded the last percentage (4.34%).

Table 7: Results of multivariable logistic regression between potential risk factors and the serological status of brucellosis in slaughtered pig according the ELISA results

Variable	Categories	Positive to i-ELISA					
		NT	Nb+ (%)	Odd Ratio	95% CI	X^2	*p*-value
Abattoir	Mbankolo[1]	170	10 (5.88)	/	/	/	/
	Mvog-Ada	162	8 (4.93)	1.203	[0.451 - 3.065]	0.144	0.704
	Essos	52	5 (9.61)	0.587	[0.204 - 1.609]	0.88	0.348
Sex	Males[1]	153	10 (6.62)	/	/	/	/
	Females	233	13 (5.58)	1.2	[0.5196 – 2.819]	0.177	0.673
Breed	Neima[1]	237	13 (5.48)	/	/	/	/
	Duroc	141	9 (6.38)	0.851	[0.348 - 2.112]	0.13	0.718
	North	6	1 (16.67)	0.29	[0.033 – 3.662]	1.348	0.245
Age	Young[1] (1-2 Yrs)	58	7 (12.06)	/	/	/	/
	Medium (2-4 Yrs)	206	9 (4.36)	3.004	[1.139 - 8.036]	4.713	0.029[Yes]
	Old (≥4 Years)	120	7 (5.83)	2.216	[0.810 – 6.012]	2.098	0.147

[1] Fixed category for comparing serological status amongst pig. [Yes] $p < 0.05$: significant difference in serological status as compared to the Fixed category for each variable.

Statistically, none of the intrinsic factors such as sex, breed and age score were significantly associated with *Brucella* infection both by RBPT and i-ELISA (Table 5); There was no statistically significant difference (p>0.05).

Discussion

To the best of our knowledge, this is the first study to investigate the occurrence of brucellosis in slaughtered pigs in Cameroon or the Central African countries and with main goal to evaluate the knowledge of the risks and protection measures of abattoir workers.

Despite the endemicity of *Brucella* infection among humans and livestock in Cameroon being evident for many years (Laine C *et al.*, 2020; Eko S *et al.*, 2023 brucellosis among slaughtered pigs has not been reported to our knowledge. Many published studies highlighted the detection of *Brucella* antibody in cattle, sheep, goats, and dogs mainly and these findings were mainly split between semi-intensive and pastoralist communities (Laine C *et al.*, 2020). The present study confirmed that *Brucella* infection was present in slaughtered pigs for consumption at Mbankolo, Mvog-Ada and Essos abattoir market in Yaounde Centre Region of Cameroon.

Concerning the observation of the practices and protection measures of abattoir workers in different site, we examined the risks practices, the exposure potentials and the use of personal protection by abattoir workers in all three abattoirs setting in Yaounde. Our findings revealed that the main protection among abattoir workers was boots as wel as PPE and the principal reason is that they are obligated to have it. In fact, it was obligatory to have the boots to work there. If not, they would be penalised by the abattoir association leaders against defaulters or by the veterinary services in the place. However, many of them perceived inconvenience arising from its use and wore slippers or bare-foot when they were not controlled. Moreso, we observed that the majority of the respondents would not be deterred from handling meat and other animal products despite having cuts or wounds on their hands. This could be explained by ignorance and poor knowledge about the risk of exposure to zoonotic diseases which could be contracted through unguarded contact with blood, meat and other by-products during meat processing (Profitós J *et al.,* 2024; Laine C *et al.,* 2022). In addition, most of the respondents posited that the cost of PPE was not a barrier to its usage. Concerning protection barriers in the abattoir environment, the majority of workers practiced better protection because the management of slaughterhouses imposed on workers' personal protective boots and dresses for those who had cuts on their skin to avoid contamination through the wounds. Focusing on knowledge of *Brucella* infection, three quarter of respondents did not know brucellosis. The plausible reason is associated to their level education, briefly, a high proportion of participants were not educated. This situation about inadequate knowledge can increase the prevalence of the disease in humans. Our findings concerning the control measures for pig brucellosis shown that the four veterinarians had few knowledge about brucellosis and then, they did not practice the good hygiene to avoid skin contamination and no special precautions were taken when they made the sanitary document for the traders. The principal reason is that they were not appropriately trained and not qualified as competent to perform the tasks assigned.

The slaughtered pigs investigated in our study at Mbankolo, Mvog-Ada and Essos abattoir market were likely having close contact with abattoir workers (slaughters, veterinarians and butchers workers) who did not take health-related precautionary measures to protect themselves.

In this study, two serological tests (RBPT and i-ELISA) were adopted and used to confirm the seroprevalence of brucellosis in the slaughtered pigs using serum samples.

The RBPT and i-ELISA tests are the standard and common serological tests recommended for epidemiological study on brucellosis (Leuenberger R *et al*., 2007). In fact, OIE demonstrated that combination of RBPT for screening of infected herds and the iELISA for identifying infected individuals was considered to be quite appropriate and effective diagnostic tool for large-scale serological survey of brucellosis. RBPT is selected as a screening test based on low cost, easy performance and high sensitivity, especially in endemic areas like Cameroon (Alton G *et al.,* 1988). The RBPT is an affordable, quick, simple and efficient screening test and is used as a diagnostic test for screening individual animals and herds, as well as in humans. This test was found efficient in diagnosis of the acute human brucellosis and still is used in the diagnosis of chronic cases. Although rapid and excellent for screening, this test is not reliable for vaccinated animals, because it can generate false positives due to its high sensitivity (Alton G *et al.,* 1988; Saavedra M *et al.,* 2019). It is conventional that RBPT have little specificity in animals and humans that are already immunized with strain 19 of *Brucella* (Alton G *et al.,* 1988). Therefore, a positive blood sample should be confirmed by definitive test (Saavedra M *et al.,* 2019).

Pig brucellosis is a zoonotic disease and is widely prevalent in many pig-rearing countries (Pappas G *et al.,* 2005; Jindal P *et al.,* 2016). Our study showed an overall prevalence of 2.86% (11/384) and 5.98% (23/384) with the RBPT and i-ELISA, respectively among the pigs slaughtered. This was higher than 1.87% (3/160) reported by Kamga and collaborators in 2020 among domestical pig (Kamga R *et al.,* 2021) and much higher than 0.28 % recorded in Uganda (Erume J *et al.,* 2016) and 0.6% in Nigeria (Nwanta *et al.,* 2011). However, the seroprevalence obtained in this study was lower than 4.83% by i-ELISA and 10.8% by c-ELISA recorded among pigs slaughtered in Cairo and Giza Governorates in Egypt (Khan *et al.,* 2019). This is much lower than 22.3% and 14.9% recorded, and 41% and 54.5% by Serum Agglutination Test in Indonesia in West Java and East Java respectively (Van der Giessen J & Priadi A, 1988). Beside, this seroprevalence was slower than the 30,13% (86/231) recorded among pigs slaughtered in Makurdi, Benue State, North-Central Nigeria (Ngbede *et al.,* 2013). The difference in prevalence between our study and other previous studies could be partly due to the methodology used, in other previous studies only samples positive based on RBPT were tested with ELISA while our study tested all the samples with both RBPT and iELISA. Additionally, this difference can be explained by the sampling frame based on the number of animals seen at the time of sampling and may not depict the actual sampling. The low prevalence of *Brucella* antibodies in slaughtered pigs could be explained by their large-scale slaughtering for meat consumption, a phenomenon that reduces the number of life-infected animals. Another reason could be the involvement of these animals in the intensive production systems in which they are not often in contact with infected animals or contaminated products.

The high prevalence obtained in our study by RBPT is not reliable for vaccinated animals, because no program known for vaccination against *Brucella* infection is available in the country; furthermore, it can generate false positives due to its high sensitivity (Khan A *et al.,* 2019). Only one pig serum (0.26%) was positive by RBPT but negative by ELISA. It is conventional that RBPT has little specificity in animals and humans that are already immunized with strain 19 *Brucella* as demonstrated previously (Alton G *et al.,* 1988; Saavedra M *et al.,* 2019). Therefore, a positive blood sample should be confirmed by a definitive test. In fact, in most countries, the RBPT is mostly used as a screening test for brucellosis diagnostic for its high sensitivity in adition it is important to complement highly specific tests due to the false–positive reactions which probably arise from cross-reactions with other bacteria and mainly with *Yersinia enterocolitica O:9, Eschrichia coli O:157*; and some *Salmonella species*, which could lead to false positive results. Moreover, the pig complement interacts with other animal species and the environment, and so, pig serum may sometimes contain nonspecific antibodies, probably IgM, that reduce the specificity of conventional tests. Just as RBPT, 23 serum samples of pigs slaughtered were positive by i-

ELISA (5.98%) and was higher than 1.87% (3/160) reported previously (Nwanta J *et al.*, 2011; Erume J *et al.*, 2016; Kamga R *et al.*, 2021). As there has never been a history of vaccination in Cameroon, seropositivity in all cases is due to natural infection of pigs by *Brucella*. In the same way, the i-ELISA test is selected due to its high specificity to discriminate between false positive cross–reactions and *Brucella* infections (Khan A *et al.*, 2019; Saavedra M *et al.*, 2019). Although i-ELISA showed higher specificity, OIE demonstrated that a combination of RBPT for screening of infected herds and the i-ELISA for identifying infected individuals was considered to be a quite appropriate and effective diagnostic tool for large-scale serological survey for brucellosis.

The proof of the existence of pig brucellosis in Cameroon may now raise awareness and can help to tailor control strategies to improve human health.

Limites: The main limitation of this study was that the bacteriological isolation of *Brucella spp*. was not performed; this could have helped to confirm the species of *Brucella* circulating among the pig population screened and provided a better insight into the epidemiology of the disease. However, earlier studies in Cameroon and other developing and developed countries, have found serological investigation useful for large-scale studies similar to this one.

Conclusion

To the best of our knowledge, this is the first study reporting the presence of anti-*Brucella* antibodies in serum collected from pigs slaughtered in Cameroon. This study, although performed focusing on only one division in the city of Yaounde, confirmed that pig brucellosis is emergent in Cameroon. As the investigated pigs in this study were apparently healthy and admitted for slaughtering, we believe that pigs can be carriers of brucellosis and present a risk to humans or may act as a dead-end host, unlikely to be involved in the transmission. Further investigation is needed to assess the prevalence of *Brucella* species particularly *Brucella suis* in pigs to explore the ways of cross-contamination and the risk for consumers. This study revealed the presence of *Brucella* antibodies detected in serum sample from slaughtered pigs in the abattoir and so, confirmed the endemicity of *Brucella* infection among domestic pigs in Cameroon. In addition, brucellosis constitutes a potential danger to abattoir workers and others who are at risk. Efforts to prevent *Brucella* infection should focus on education of abattoir workers, veterinarians, butchers and local public health workers. For this, there is a need to establish control strategy for brucellosis among pigs slaughtered for meat consumption by the MINEPIA and calls for surveillance of brucellosis among veterinarians, abattoir workers, traders and other livestock keepers who are at risk of infection. The program 'One Health collaborative approach in tackling the *Brucella* problem is hereby recommended for the management of health security of animal and human population by implementaion of adequate health and safety measures with the Ministry of public Health.

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Authors' contributions

Olivier Bela Ndongo provided the materials for the study, wrote the project and the protocol of all laboratory analysis, performed the sampling in differents site, the laboratory analysis and wrote the first and final draft of the manuscript

Maurice Boda conceived the idea, managed the project and manuscript writing, coordinated and read the final draft of the manuscript.

Annita Diobe Munge participated financially to buy the materials, assisted the principal investigator in sampling and transport of the samples, participated in the preparation of the first and final version of the manuscript (to write and read the first draft of the manuscript) and helped to carry out the statistical analysis.

Ascension Maximillienne Nyegue participated to the conception of the idea and supervised all the work (the project and manuscript writing, the coordination and the reading of the final draft of the manuscript).

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