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Bovine Brucellosis Seroprevalence and Potential Transmission Risk to Workers at the Port-Bouët Abattoir, Abidjan, Côte d'Ivoire

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Abstract

Brucellosis is one of the most common bacterial zoonosis in the world. It is caused by *Brucella* species and is an infectious and contagious disease transmissible to humans and to several animal species. This disease remains one of the neglected diseases in several countries and represents a real public health problem. A cross sectional study was conducted at the Port-Bouët abattoir in order to determine the seroprevalence as well as to assess the knowledge, attitudes and practices (KAPs) of workers. Three hundred and eighty-seven (387) cattle blood samples collected from January 5 to March 30 2019 were diagnosed using the Rose Bengal and indirect Enzyme-linked Immunosorbent Assay (i-ELISA) techniques. The seroprevalence was 0.52% (95% CI: 0.06265-1.8542) for i-ELISA and Rose Bengal. Regarding seroprevalence with sex, males recorded 0.3% (95 CI: 0.00776-1.6617), while females had 1.85% (95% CI: 0.04687-9.8991) for both Rose Bengal and i-ELISA tests with no statistically significant difference (X^2=2 ; df=1 ; p=0.157). Animals >3years old recorded a higher seroprevalence rate eventhough with no statistically significant difference (X^2=3 ; df=2; p=0.223). Also, our findings established that the potential risk of contracting brucellosis at the abattoir by workers is high due to the handling of animal tissues without the use of Personal Protective Equipments (PPEs). This baseline information indicates the neccesity for a more in-depth study on the traceability of animals coming to the abattoir as well as study the occurrence of brucellosis in animals and among abattoir workers following a one health approach in order to contribute to the development of a sub-regional integrated programme for the control of brucellosis. This collective approach will minimize the risk of contamination with brucellosis by workers.

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Introduction

Brucellosis caused by *Brucella* species is an infectious and contagious zoonotic disease. The most common clinical manifestation is abortion [1]. Thus, in domestic animals, it causes very significant economic losses [2]. Brucellosis is an important and notifiable disease in the animal health sector. Also, it represents a significant public health danger [3]. This disease is caused by various facultative, gram-negative intracellular bacteria belonging to the genus *Brucella* which usually infect some animal species. However, most species of the genus *Brucella* are also capable of infecting other animal species. The disease affect cattle, suidae, sheep, goats, horses, camels and dogs. It can also affect other ruminants, some marine mammals and even humans. The main species are *Brucella melitensis* (sheep, goats), *Brucella abortus* (bovids (ubiquitous) and *Brucella suis* (suidae). Transmission can be horizontal (via aborted fetuses, slaughter of infected animals, ingestion of milk or contaminated meat and airborne) or vertical *i.e.* from mother to newborn (in utero, or during the passage of the newborn through the pelvic route). Brucellosis is the most common bacterial zoonosis in the world. The number of new cases are estimated at over half a million each year. Human contamination can occur either through the cutaneous-mucous route (infection through wounds on the hands, in the oral or nasal mucosa, through contaminated hands) or through food (meat, milk and derivatives and vegetables). Roux [4] indicated that not all individuals living around an infected farm are exposed to contamination. Milkers and animal owners are the most exposed category. Likewise, veterinarians and para-veterinarians, abattoir workers, consumers of raw animal products (milk and meat) pay a heavy price to this disease.

In Côte d'Ivoire, outbreaks of bovine brucellosis have been identified mainly in the north, with infection rates ranging from 12 to 14% [5]. In addition, bayesian analysis conducted using serological data reported brucellosis prevalence rate of 8.8% in central Côte d'Ivoire [6]. The main cattle suppliers to the Abidjan cattle market are from Burkina Faso and Mali, two Sahelian countries that share borders with Côte d'Ivoire. Surveys conducted on brucellosis in these two neighbouring countries revealed the presence of brucellosis. Thus, in Mali, the report of Tounkara et al. [7], Maiga et al. [8]and Sow [9] presented prevalences of 22%, 19.7% and 1.5% respectively. In Burkina Faso, the investigations conducted on this disease by Akakpo [1], Traoré et al., [10] and Boussini et al., [11] revealed prevalence rates of 14.3%, 8% and 3.61% respectively.

Based on these observations, we asked the following scientific research questions : (1) what is the impact of transboundary livestock trade on the prevalence of bovine brucellosis at the Port Bouët abattoir? (2) what factors could be associated with the health risk incurred by the abattoir workers regarding bovine brucellosis? Additionally, insufficient data on the prevalence of brucellosis at the abattoir-level in Côte d'Ivoire which represents an important area for the spread of the disease to humans (breeders, abattoir workers, cattle traders etc.) prompted this present study.

Materials & methods

Study area

The present study was conducted at the Port-Bouët abattoir, located in the municipality of Port-bouët in the southern part of the city of Abidjan, between the Atlantic Ocean to the south and the Ebrié lagoon in the North. This municipality covers an area of 111.1 km^2. The climate of this area is sub-equatorial, hot and humid, characterized by two rainy seasons (from September to October and from April to July) interspersed by two dry seasons (from July to August then from November to March). The average temperature fluctuates between 25 and 33 °C with a heavy rainfall of more than 1,500 mm per year. The Abidjan cattle market that supplies animals to the abattoir covers an area of 3.2 hectares and is the largest in the country. Cattle originating from different sub-Saharan African (SSA) countries and from Côte d'Ivoire can be found here. The share of the Abidjan cattle market in the national imports is 77% for cattle and 60% for small ruminants [12]. The small ruminant (sheep and goats) section of the market has 600 pens and an abattoir covering an area of 1.8 hectares.

Sample size determination and blood collection

A sampling frame was constructed to list all the registered cattle at the abattoir. The total number of cattle to be sampled was calculated by assuming that the prevalence of the disease at the abattoir is 5% at 95% confidence interval (CI) with 5% desired precision by using the following formula :

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

where

N = sample size,

Pexp = expected prevalence, and

d = absolute precision [13].

From the above formula, the estimated sample number was 384, but sampling was conducted on 387 cattle. Blood of these animals was collected via the jugular vein into collection tubes [14]. The blood collection tubes were immediately placed in a cooler and sent to the Bingerville Central Veterinary Laboratory (LCVB) where the sera were prepared and stored frozen (-20 ° C) prior to serological analyzes.

Laboratory tests

Two serological tests were used in this study- the Rose Bengal (RB) and the indirect Enzyme Linked Immunosorbent Assay (i-ELISA) tests. All samples were double-tested (RB and i-ELISA). The Rose Bengal and the ELISA kits were manufactured by ID.VET Innovative Diagnostics, France. The RB test principle is based on rapid Ab-Ag agglutination. It detects IgG antibodies [15]. On the other hand, the i-ELISA test, is a semi-quantitative antibody detection method that works with the same principle of agglutination as the RB. The advantage of ELISA test is its high sensitivity, which is superior to that of immunofluorescence techniques. They show very low amounts of antibodies, and they are well suited for conducting epidemiological surveys [16]. Thus, the joint application of these two methods in the present study was considered inorder to overcome their respective sensitivity weaknesses as proposed by Adamou [17] and Nielsen [18].

The buffered antigen test or rose bengal test

The RB test allows for the detection of specific antibodies towards *Brucella abortus* (in cattle), *Brucella melitensis* (in small ruminants) and *Brucella suis* (in pigs). The Rose Bengal Antigen test (ID vet, France) was used as a rapid test to screen for antibody to *Brucella* species with a published sensitivity of 87.2% [19] and specificity of 99.6% [20]. Because of the success of this

test, it is widely used and prescribed by the OIE [21]. The test serum (0.03 ml) was mixed with an equal volume of RBT antigen on a glass slide to produce a zone of approximately 2 cm in diameter. The mixture was agitated gently for 4 min at ambient temperature and then observed for agglutination. Tests were considered positive when any visible reaction or agglutination were observed.

The indirect ELISA test

The "ID Screen® Brucellosis Serum Indirect Multispecies" i-ELISA kit (ID vet France, kit reference BRUS-MS-5P) was used to test sera. The kit detects antibodies towards different species of smooth lipopolysaccharide (S-LPS) expressing *Brucella*, such as *B. abortus*, *B. mellitensis*, and *B. suis*. The sensitivity and specificity of this test were 96.8 and 96.3%, respectively, according to the Bayesian estimation approach [22]. All the testing procedures were performed according to the protocols provided by the manufacturer. The test plates were read under the ELISA reader ("Multiskan™ FC Microplate Photometer") at an optical density (OD) of 450 nm within 15 min.

Assessment of the KAPs of abattoir workers on Brucellosis

A descriptive cross-sectional survey with simple random sampling made it possible to interview 100 individuals at the Port-Bouët abattoir. This method consisted of randomly selecting workers from all available workstations of the abattoir. In addition, the survey sheet was designed using the Sphinx software. The questionnaire focused on assessing the general principles of hygiene, the experience of workers, time of contact with animals or blood, the use of PPEs and the consumption of milk from animals.

Data processing and statistical analysis:

The data collected was entered in the Excel spreadsheet version 2013. The same spreadsheet was used to calculate the prevalence using the following formula:

Apparent prevalence = (Number of positive animals / Number of animals sampled) x100

Animals that tested positive for one of the two diagnostic tests were considered seropositive.

Statistical analysis was conducted using the JASP 0.13.0.0 statistical software package [40]. The Chi-square test was used to compare the apparent prevalence of the study variables (sex, age and origin of the animals). The significant level of the test was stated at $p<0.05$.

Ethical considerations

The study was conducted under the strict supervision and agreement of the Directorate of Veterinary Services (DSV), the Directorate of the abattoir and Hygiene of the District of Abidjan. In addition, prior consent was obtained from anyone agreeing to be interviewed (verbal informed consent). They were told they could withdraw from the study at any time. The handling of animals and samples was carried out in accordance with the standards and respect of animal welfare.

Results

Bovine brucellosis seroprevalence

Out of the 387 cattle samples tested for brucellosis, only two (2) (0.52% (95% CI: 0.06265-1.8542)) tested positive with the Rose Bengal test and i-ELISA. These positive samples were from Zebu originating from Mali. Regarding the anti-*Brucella* antibodies prevalence with breed of cattle, it was found that the zebu had more of such antibodies than their taurine counterparts eventhough with no statistically significant difference (X^2=2 ; df=1 ; p=0.157). Animals above three (3) years old (0.54%: 95 CI: 0.06535-1.93369) were highly detected with the anti-*Brucella* antibodies than those below three (3) years old (0%) eventhough with no statistically significant difference (X^2=3 ; df=2 ; p=0.223) (Table 1).The anti-*Brucella* antibody prevalence rate was much higher in females (1.85% (95% CI: 0.04687-9.8991)) than with their male (0.3% (95% CI: 0.00776-1.6617)) counterparts eventhough with no statistically significant difference (X^2=2 ; df=1 ; p=0.157).

Table 1: Prevalence of brucellosis with breed, sex and age cohort of cattle.

Parameters	Category	N(%)	RB-test	i-ELISA	X^2	df	P-value
Breed							
	Zebu	362 (95.54)	2 (0.52)	2 (0.52)	2.000	1	0.157
	Taurine	25 (6.46)	0 (0)	0 (0)			
Age (years)							
	<3	5 (1.29)	0 (0)	0 (0)	3.000	2	0.223
	≥ 3 ≤ 6	195 (50.39)	2 (0.52)	2 (0.52)			
	>6	187 (48.32)	0 (0)	0 (0)			
Sex	Female	54 (13.95)	1 (1.85)	1 (1.85)	2.000	1	0.157
	Male	333 (86.05)	1 (0.3)	1 (0.3)			

Origin of animals slaughtered at the abattoir

Most of the animals slaughtered at the Port-Bouët abattoir originated from Mali (189 (49%)), followed by Burkina Faso (166 (43%)), then Côte d'Ivoire (29 (7%)) and lastly from Niger (3(1%)) (Figure 1). It was noticed that the two positive cases for brucellossis at the abattoir were diagnosed in cattle from Mali.

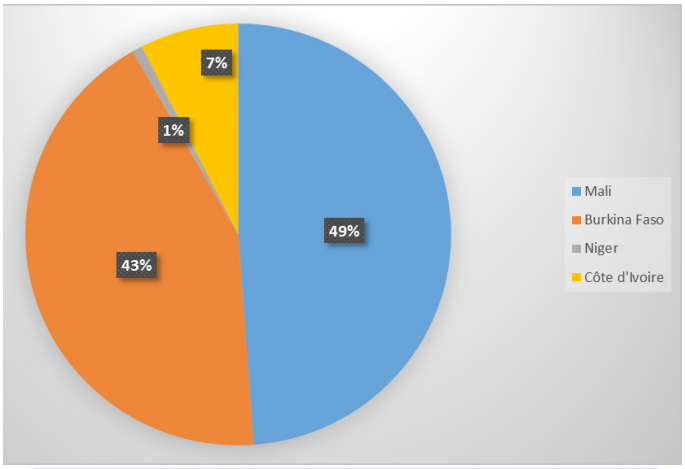

Figure 1: Proportion of animals slaughtered at the Port-Bouët abattoir based on their origin.

Knowledge, Attitudes and Practices Analysis (KAP) of the Risk factors for the transmission of brucellosis among abattoir workers

Sex of respondents

Ninety-seven (97%) of the population surveyed were men while only 3% consisted of women (Table 2).

Task of respondents

The respondents worked at various posts of the abattoir. Thus, 17% of them worked in more than two posts while 83% worked in only one post. Among the 83% surveyed working in atleast one post, 15% consisted of butchers, 10% animal transporters, 9% cattle dealers, 8% slaughterers, 8% cattle dealer assists, 7% cleaners, 7 % inspectors. Finally, 6% of our interviewees were working in one section while 13% worked in multiple sections. Of the 13% respondents working in different sections of the abattoir, 30.8% consisted of meat roasters, 15.4% retailers, 7.7% skin burners, 15.4% were agents of the Abattoir Department of Food Hygiene (DAHA), 23% consisted of sellers of roasted meat "Choukouya" and 7.8% sellers of skin (Table 2).

Working experience at the abattoir

Regarding the professional experience of the respondents, 31% had between 5 and 10 years of experience, 27% claimed to have worked between 10 and 20 years, 22% had between 1 and 5 years while 17% said they have been in the office for more than 20 years. Only 3% of respondents have been at the abattoir for less than 1 year (Table 2).

Contact of workers with live animals, blood and carcasses

From our interview, we noticed that 59% of workers came in contact with live animals while 41% said the contrary. They claimed to be in contact with the carcases (53%) and 47% claimed not to have been in contact with carcases. 60% of workers were exposed to the blood of slaughtered animals compared to 40% that did not. Fifty-seven percent (58%) of workers claimed to work more than 12 hours a day; 15% between 6 and 10 hours/day; 10% between 10 and 12 hours/day; 8% between 2 and 6 hours/day and only 9% worked less than 2 hours/day (Table 2). The average working time at the abattoir was 13 hours/day.

Some practices of abattoir workers

Out of the 100 individuals surveyed, 72 (72%) claimed to eat with their working attires while 28% said they changed their working clothings before eating. Regular washing of working attires is very crucial in implementing good hygienic practices. Indeed, of the 100 individuals interviewed, more than 58% said they washed their working attires every day whereas 30% said they did it after every two days, 7% washed their outfits once a week and 4% cleaned it two times a week.

During working periods, 29% of respondents responded positively to the wearing of gloves while 71% did not wear them. It was noticed that 86.5% of the target population washed their hands with soap and water before eating their meals whereas 13.5% washed their hands only with running water. The results of our survey confirmed that 75% of workers continue to work even with open wounds while 25% said that the presence of an open wound prohibited them from performing well. From the dietary exposure analysis, we found that 61% of respondents claimed to consume milk from their animals while 39% did not. Among those who consumed milk from their animals, 37.7% consumed boiled milk and 26.23% consumed raw milk and 36.07% consumed both raw and boiled milk.

Workers' knowledge on the existence of zoonoses and PPEs

The present survey revealed that 84% of our study population claimed to be aware of the existence of zoonotic diseases, but 16% did not know that such diseases exist. Of the 100 workers surveyed, only 83% found PPEs to be useful, while 17% said that wearing PPEs was not important in performing their tasks (Table 2).

Table 2: Knowledge, Attitudes, and Practices (KAPs) on brucellosis transmission among abattoir workers.

Parameters	Category	Number	%	X2	df	P-value
Sex	Male	97	97	2.000	1	0.157
	Female	3	3			
Professional experience (years)	>1	3	3	20.000	16	0.220
	1- 5	22	22			
	5-10	31	31			
	10-20	27	27			
	>20	17	17			
Number of tasks conducted	1	83	83	2.000	1	0.157
	>1	17	17			
Type of activity conducted at the abattoir	Butchers	15	18.07	54.000	48	0.256
	Animal transporters	10	12.05			
	Livestock traders	9	10.85			
	Cleaners	7	8.43			
	Inspectors	7	8.43			
	Slaughterers	8	9.64			
	Livestock trader assists	8	9.64			
	Meat vendors	6	7.23			
	Others	13	15.66			

Contact with live animals	contact	59	59	2.000	1	0.157
	No contact	41	41			
Contact with carcases	contact	53	53	2.000	1	0.157
	No contact	47	47			
Contact with blood	contact	60	60	2.000	1	0.157
	No contact	40	40			
Duration of working time (hours/ day)	<2	9	9	20.000	16	0.220
	2-6	8	8			
	6-10	15	15			
	10-12	10	10			
	>12	59	59			
Eating on duty	With working attire	72	72	2.000	1	0.157
	Not with working attire	28	28			
Frequency of washing of working attires	1 time/day	58	58	12.000	9	0.213
	1 time/2days	30	30			
	1 time/week	8	8			
	2 times/week	4	4			
Working gloves	Absence	29	29	2.000	1	0.157
	Presence	71	71			
Washing of hands before meal	Water and soap	86	86	2.000	1	0.157
	Water only	14	14			
Working with open sores	Yes	75	75	2.000	1	0.157
	No	25	25			
Consumption of the milk of animals of the abattoir	Yes	61	61	2.000	1	0.157
	No	39	39			
Nature of the milk consumed	Raw	16	26.23	6.000	4	0.199
	Boiled	23	37.70			
	Raw and boiled	22	36.07			
Use of PPEs	Yes	83	83	2.000	1	0.157
	No	17	17			

Discussion

Two serological diagnostic methods were used in this study to establish the existence of *Brucella* species infection. These methods were the RB and the i-ELISA tests. The results of our diagnostic tests revealed positivity concordances between the two serological tests. This observation is similar to that made by Koutinhouinet al. [23]. All the seropositive animals detected with the RB test were also positive with the i-ELISA test. However, this finding was different from that of Adamou [17] in Niger, Sanogo [6] in Côte d'Ivoire and Amona [24] in the Republic of Congo who reported variable results between the RB and i-ELISA tests.

The apparent prevalence of bovine brucellosis in this study was 0.52%.This result can be justified on the one hand, by the duration of this study that was carried out within a short period (from 01 to 28 February 2019) and on the other hand due to the method of cattle breeding (extensive breeding). Indeed, studies have shown that the method of animal breeding and the season have an impact on the prevalence of this disease because a hot and dry climate destroys *Brucella*. Also, since in the extensive animal breeding system, animals are kept for longer periods in fresh pastures and in a humid environment, this could have an influence in the detection of the disease [1,25].

The apparent prevalence of bovine brucellosis obtained in this study was superior to that obtained by Kouamé et al., [26] in abattoirs in Senegal but lower than 9.6% at the Dschang abattoir in the west region of Cameroon [27] and 8.7% - 12% at the Dakar abattaoir in Senegal [14]. These results are significantly lower than those previously reported in farms throughout the Ivorian territory by Angba et al. [5] and Pilo-Moron et al. [28] whose prevalences were within the range of 12-14% and 10.8% respectively. The same is true for the results of the study carried out in the center of Côte d'Ivoire by Sanogo et al., [6] where the prevalence obtained was 8.8% and in the north of Côte d'Ivoire by Kanouté et al. [29] with prevalence rate of 4.6%. These discrepancies in the prevalences of brucellosis in Côte d'Ivoire could be explained by the differences in sampling (size, procedure, etc), the type of animals, the epidemiological context, the tests used and the fact that the Ivorian live cattle market is mainly supplied by two Sahelian countries which have a common border with Côte d'Ivoire [12]. The apparent prevalence of this study is also lower than the prevalence from studies carried out in countries where animals originated from before reaching Côte d'Ivoire. Thus, in Mali, the survey of Tounkara et al. [7], Maiga et al. [8] and Sow [9] indicated prevalences of 22%, 19.7% and 0.98% respectively. The investigations carried out on this disease by Akakpo [1], Traoré et al. [10] and Boussini et al. [11] revealed prevalences of 14.3%; 8% and 3.61% respectively.

These prevalences are higher than that obtained by Kouamé et al. [26] in an abattoir in Senegal.

The apparent prevalence of bovine brucellosis was higher in females (1.85%) than in males (0.3%). This finding is similar to that of Chantal and Thomas [14], Kubuaforet al. [30], Traoré et al [10], Faye et al. [31] and Amona [24]. This trend of seroprevalence with sex can be explained by the fact that females are often kept for longer periods for production activities such as milk, reproduction, etc, thus they are much more exposed to infections than their male counterparts. Also, females that are taken to the abattoir usually reports fertility problems and suffer from infections. Elderly animals (> 3years) recorded a higher seroprevalence than their younger counterparts. An increase in seropositivity with age has already been demonstrated (Traoré et al. [10]; Faye et al. [31]; Chimana et al. [32]; Kubuaforet al. [30]. This observation could be justified by the fact that the number of younger animals (0 to 3 years) was lower. As a result, the likelihood of having many positive cases with the youner cohort was lower. In addition, adults often stay in the herd for a longer period which leads to their longer exposure to the risk of contamination and accumulation of antibodies over such a period. The trend seems logical because the older the animal, the more likely they are to be infected and become source of contamination for younger ones [23]. Asmare et al. [33] reported that brucellosis is primarily a disease of sexually matured animals and sensitivity to testing increases with sexual maturity and gestation due to the influence of sex hormones. Additionally, the limitation of sampling methods where a simple random sampling approach was adopted could have influenced the detection of this disease. Also, insufficient statistical data on the characteristics of the animals at the abattoir made it impossible to carry out a stratified sampling. Applying such a sampling approach could have improved the representativeness of our sampling, since the animals came from different countries.

Based on the risk of exposure for abattoir workers to brucellosis, the transmission of brucellosis occurs through direct contact with a sick animal or its tissues, carcases, but also with contaminated environment [34]. Among the workers interviewed, 59% were in contact with live animals, 53% were in contact with carcases, 60% of workers were exposed to the blood of slaughtered animals, more than 40% of workers did not carry out daily washing of their working attires and 75% worked with open sores. The contact time between workers and these dangerous contamination sources was very long (13h/day). This exposure of workers to blood, secretions and tissues for a long time theoretically increases the risk of exposure to infection. The risk of transmission of bovine brucellosis to humans exists although the prevalence of bovine brucellosis in this study is low (0.52%). This could be explained by the very rudimentary slaughtering and waste treatment system (killings), the poor organization of work by workers of the abattoir and the non-compliance with hygiene measures.

Regarding the use of PPEs and the practices of abattoir workers, 72% said they ate with their working attires and 14% said they washed their hands only with running water without soap before eating. 71% of workers did not wear gloves, 17% said wearing PPEs during work was not important. Thus, observations regarding the use of PPEs by abattoir workers showed that none of them used protective clothing, but only the inspectors and butchers wore PPEs. This observation is similar to that made by several authors [35-37], who reported that the risk of contracting brucellosis by abattoir workers was high due to their handling of tissues and live animals without using PPEs. The insufficient use of PPEs by these workers puts them at serious risk of contracting brucellosis and other zoonotic diseases in such an unsanitary and highly contaminated environment [38,39].

For the food exposure risk of abattoir workers, 61% of them claimed to consume milk from their animals. Of these, 37.7% consumed boiled milk, 26.23% consumed raw milk and 36.07% consumed both raw and boiled milk. Consumption of raw milk is an important factor for the transmission of brucellosis and other zoonoses. Any sick animal is susceptible to transmitting a pathogen through milk or meat. In particular, animals suffering from tuberculosis or brucellosis shed infectious agents in milk such as *Mycobacterium* and *Brucella* respectively. The present observation is different from that of Sow [9] in Mali who reported that 70.3% of the milk were consumed in raw and fermented forms, 20.8% in fermented form and 7% in raw form.

Conclusion

The objective of this work was to determine the prevalence of bovine brucellosis at the Port-Bouët abattoir through two serological tests (RB and i-ELISA) and to determine the risk factors for its transmission to workers. The results of the two serological tests showed that brucellosis is present with a low prevalence (0.52%) in cattle of the abattoir. The practices and dietary exposure of abattoir workers are factors involved in the transmission of brucellosis to humans. The lack of awareness among abattoir workers on the zoonotic implications of brucellosis is a key finding in this study. This is also reflected in their attitudes and the need to use PPEs. This study highlights the importance of improving hygienic conditions in abattoirs. Indeed, the presence of *Brucella* and poor hygiene practices could expose workers to brucellosis. It is therefore important to carry out an integrated study (humans and animals) which would enable the consistent evaluation of the risk and propose appropriate measures for the surveillance and control of bovine brucellosis at the abattoir level.

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