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STUDY OF THE SEROPREVALENCE OF SWINE BRUCELLOSIS IN SELECTED CAMEROON REGIONS

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SUMMARY
Brucellosis is a contagious disease of farm animals due to bacteria of the genus *Brucella.* Six species (*B. abortus, B. melitensis, B. suis, B. ovis, B. canis, B. neotomae*) are incriminated in the natural infection of several animal species like cattle, goats, pigs , rodents, carnivores and other mammals (OIE, 2007 ; Boukary *and al.*, 2010). It can also reach other ruminants, some marine mammals and humans. It is therefore a notifiable zoonosis. This study on brucellosis in pigs was carried out from August 2015 to January 2016 in farms in the Far North region and in the slaughter areas of pigs in the city of Douala. The general objective of this work is to contribute to a better knowledge of the epidemiology of swine brucellosis in Cameroon. Blood samples were taken from 1081 pigs at least 6 months old and were then analyzed with Rose Bengal and indirect ELISA for antibodies against the *Brucella* bacterium. The serological analysis of the sera made it possible to screen 20 sera out of 1081 samples taken with proportions of infected pigs of 6.03%; 3.67%; 2.39% and 0% respectively for the North-West, Littoral, West and Far North of Cameroon. The most affected age group of pigs is between 12 and 24 months with a prevalence of 1.20% (95% CI: [0.55 - 1.85]) (P <0.05). The Duroc, Landrace and Pietrain hybrids are the most affected with respective prevalences of 0.56%, 0.65% and 0.46%.

KEYWORDS: brucellosis, seroprevalence, pigs, Far North, Douala, Cameroon.

INTRODUCTION
Swine brucellosis is caused by *Brucella suis* and occasionally by *Brucella abortus* and *Brucella melitensis* (OIE 2008). It is distributed worldwide in pig farming countries and has already been diagnosed in several African countries including Guinea Conakry, Nigeria, Uganda, Central African Republic, Democratic Republic of Congo and Chad (Nielsen, 2000; CFSPH,2009; Onunkwo *and al.*, 2011 ; Akakpo and Ndour 2013). The prevalence of 0%, 0.06%, 4% and 30% was obtained in Gabon, South-East Nigeria, Chad and Guinea-Conakry respectively (Onunkwo *and al.*, 2011, Akakpo *and al.* Ndour, 2013). Cameroon shares with some of these countries not only long borders, but also many trade exchanges. These exchanges are considered to be one of the main means of spreading brucellosis worldwide brucellosis worldwide (Nielsen 2000, MINEPIA 2009, FM / GLOBE 2010). Several other potential sources of infection exist mainly the breeding of breeders between farms, the exchange of breeding equipment between that of pigs and that of sick domestic ruminants (cattle, goats) and to a lesser extent the proximity of domestic pigs with wildlife (Ndébi *and al.*, 2009; CFSPH, 2009; Bronner *and al.*, 2010; Marcé and Garin Bastuji, 2011). *and al.*, 2010, Marcé and Garin Bastuji, 2011). At the limit of current knowledge, the seroprevalence of swine brucellosis has not yet been the subject of any study in Cameroon. Yet in addition to its impact on animal health, it causes public health problems (Akakpo and Ndour, 2013). This is what was recommended in the work of Shey-Njila *and al.* (2006) carried out at the slaughterhouse of Dschang on the serological survey of bovine brucellosis in Cameroon. Seroprevalences of 10%, 8.34% and 10.5% were reported on bovine brucellosis respectively by Shey-Njila *and al.* (2006), Bayémi (2009) and Bayang (2014). In the light of all the above, swine brucellosis is a neglected disease like many others without investigation in Cameroon despite the many socioeconomic and health consequences (Garin-Bastuji *and al.*, 2000, Cadmus, 2006). OIE, 2008; CFSPH, 2009; Kaoud *and al.*, 2010). The general objective of this work is to contribute to a better knowledges of the epidemiology of swine brucellosis in Cameroon. Cameroon. More specifically, it aims:

- To determine the specificity and sensitivity to anti-*brucella* antibodies of each test used;
- To determine the seroprevalence of anti-*Brucella* antibodies in pigs according to age, sex and existing breeding system;
- To determine the seroprevalence of anti-*Brucella* antibodies in pigs in certain regions.

MATERIAL AND METHODS

Framework of study

This study was carried out in pig farms in the Far North region and in the pig slaughter areas of the city of Douala. This is a cross-sectional study that took place over a period of 6 months (from August 2015 to January 2016).

Inclusion criteria

As part of this study, the frame was developed from the directory of pig farms identified by CARPA (Center for Support to Research and Pastoralism) in the departments of Diamaré, Mayo-Danay and Mayo-Kani. Only farms with at least 5 pigs and whose breeders gave verbal consent for participation in the study were chosen. Depending on the size of the pig herd, a maximum of 3 to 10 adult pigs were taken. In each farm, the choice of animals was made on the basis of information provided by the breeders (sex, age, race, physiological state). Each time, the assessment of the morphology of the animals allowed us to decide which animals to take. The origin of these animals was confirmed by the veterinary health certificates presented in the slaughter areas by the traders or simply from the information provided by the traders for the pigs that were presented without the health certificates.

Criteria of non-inclusion or exclusion

Weaned pigs were not selected in this study because they show only a very weak serological response to the different serological tests after infection with *Brucella spp*. farms with fewer than 5 pigs were excluded

Sample size determination

The sample size was calculated according to the LORENTZ formula.

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

Where n is the sample size, Z is the significance threshold, p is prevalence, α is the accepted error, For a 95% confidence level, α = 5% and Z = 1.96, and p = 50%,

The minimum size is: 384 Samples

In this study, two batches of sera were studied. The first batch was taken from pigs in the Far North. The second batch of serum was obtained in the pig slaughter areas in Douala. For this purpose, the samples were taken from male and female pigs of reproductive age.

Collection and conservation of serum

The blood samples were taken by puncturing the auricular vein using a needle mounted on a needle holder after restraint of the animal in lateral decubitus. The blood was then collected in a dry tube and centrifuged at 3500 rpm for 5 minutes to obtain the serum. The serum that is the biological material for this study was taken using a micropipette and kept in the well labeled eppendorf tube for each animal. These sera were transported in a cooler containing dry ice to the freezer to be stored at -20 ° C prior to serological testing.

Serological analyzes

The serological tests were carried out at the microbiology laboratory of IRAD (Research Institute for Agricultural Development), regional agency of Adamaoua located in Wakwa. All the sera were successively subjected to two serological tests, namely: the buffered antigen or Rose Bengal test and the indirect ELISA.

Buffered antigen or Rose Bengal test

This is a serological test used for the detection of brucellosis. The one used in this study is a suspension of Brucella abortus biovar 1 (Weybridge strain 99) inactivated by heat and phenol, stained with Rose Bengal and diluted in acidic buffer produced by the laboratory IDvet® France. The principle is based on the rapid seroagglutination reaction on the slide due to the formation of the antibody-antigen complex. It was carried out following the protocol of the World Organization for Animal Health (OIE, 2008).

Indirect ELISA

The confirmatory test used is that defined by the OIE, namely the indirect ELISA test (OIE, 2013). The indirect ELISA kit used is a "multi species" kit indicated for the detection of antibodies against *Brucella abortus, Brucella melitensis and Brucella suis*. The principle of this enzyme immunoassay technique is to fix a specific antibody on the antigen of interest then to reveal the presence of this antibody by a second antibody, anti IgG multi species coupled to peroxidase (HRP). This makes it possible to visualize an antigen-antibody reaction by means of a color reaction.

Statistical analysis

The data collected was encoded on an Excel spreadsheet (Microsoft, USA, 2010). Prevalence confidence intervals were calculated with 5% error using the following formula (Thrusfield, 2007): p_- 1.96√p (1_p) ÷ n; p + 1,96√p (1_p) n. The prevalence retained in this combined study of two RB / i-ELISA tests was calculated using the Bayesian approach using the conditional probability with the WinBugs14 software. The Chi-square independence test was used to identify the variables statistically associated with seropositivity and the threshold of significance was set at $p < 0.05$. A research certificate was obtained before the beginning of our study. We also obtained from various officials of the structures concerned an authorization to conduct our research. This study was carried out with the consent of the breeders.

RESULTS

Seroprevalence of anti-*Brucella* antibodies in pigs

In this study, it appears that the prevalence of anti-*Brucella* antibodies is very low with the Rose Bengal test. Only two sera out of 1081 are positive (0.19%, 95% CI: [0 - 0.0045]). With the indirect ELISA confirmatory test, 20 sera out of 1081 are positive (1.85%, 95% CI: [1.05 - 2.65]). Rose Bengal-positive sera were confirmed in the i-ELISA test. However, 18 sera were found to be positive for i-ELISA (1.67%, 95% CI: [0.91-2.43]) and negative for RB.

Table I: Combined results of different serological tests carried out.

Serological tests	Numbers (n=1081)	Proportion (%)	(IC : 95%)
RB (+)	02	0,19	[0 - 0,45]
i-ELISA (+)	20	1,85	[1,05 - 2,65]
RB (+) and i-ELISA (+)	02	0,19	[0 - 0,45]
RB (-) and i-ELISA (+)	18	1,67	[0,91 - 2,43]

+: positive; -: negative; n: total number.

The combined prevalences of the two RB / i-EILISA tests determined with the Bayesian approach is shown in the conditional probability contingency table below. The prevalence retained in this study by combining RB and i-ELISA is 0.238% (95% CI: [0.012 - 0.674%]) (WinBugs14).

Prevalences by sex, age and race

With regard to the age of the animals, the most sensitive age group is between 12 and 24 months with a prevalence of 1.20%. Similarly, the Duroc, Landrace and Pietrain hybrids are the most affected with respective prevalences of 0.56%, 0.65% and 0.46%.

Table III: Distribution of prevalences by sex, age and breed of pigs.

Variables	Number of animals tested	Number of animals Positive to i-ELISA	Prevalences (%)	(IC : 95%)
Gender				
Females	734	15	1,39	[0,69 - 2,09]
males	347	05	0,46	[0,06 - 0,86]
Ages (Month)				
< 12	475	04	0,37	[0,01 -0,73]
[12-24]	424	13	1,20	[0,55-1,85]
≥ 24	182	03	0,28	[0 - 0,60]
Breeds *				
Hybrid Bershire	66	01	0,09	[0-0,27]
Hybrid Duroc	60	06	0,56	[0,12-1,00]
Hybrid Landrace	225	07	0,65	[0,37-0,93]
Hybrid Largewhite	28	01	0,09	[0 - 0,27]
Hybrid Pietrain	259	05	0,46	[0,06 - 0,86]
Local breed of the Far North	442	00	0,00	//////
	1081	**20**	**1,85**	**[1,05 - 2,65]**

* : Significant differences (P < 0,05).

Prevalences according to livestock systems and regions of origin

Three regions have positive cases, including West, North West and Littoral. The proportions of infected pigs are higher in the North West (6.03%) followed by Littoral (3.67%) and West (2.39%). There is no significant difference between the different rearing systems (P> 0.05). Between the prevalence found in the West and that found in the Littoral, there is no significant difference (P> 0.05). Similarly, there is no significant difference between the prevalence of Northwestern and Western (P> 0.05).

Table IV: Distribution of prevalences according to rearing systems and regions of origin of pigs.

Variables	Number of animals tested	Number of animals Positive to i-ELISA	Prevalences (%)	(IC : 95%)
Breeding system				
Extensive breeding	730	12	1,11	[0,49-1,73]
Semi-intensive breeding	351	08	0,74	[0,23-1,25]
Region of origin of pigs				
Far North	456	00	0,00	///////
Littoral	109	04	0,37	[0,01-0,73]
North West	116	07	0,65	[0,37-0,93]
West	376	09	0,83	[0,29-1,37]
South	24	00	0,00	///////
TOTAL	**1081**	**20**	**1,85**	**[1,05 - 2,65]**

DISCUSSIONS

The purpose of this study is to determine the seroprevalence of anti-*Brucella* antibodies in pigs in certain regions of Cameroon. The serological survey carried out by the buffered antigen test (EAT) and by the indirect ELISA test made it possible to detect anti-*Brucella* antibodies in pigs with prevalences of 0.19% and 1.85% respectively. A similar result where the indirect ELISA test had a higher prevalence than Rose Bengal was found by Shey-Njila *and al.* (2006) after a serological survey of bovine brucellosis at the Dschang slaughterhouse. The prevalences obtained in the study recall the difference in sensitivity and specificity of the two serological tests used (Nielson *and al*., 2000, OVF 2005, Shey-Njila *and al.*, 2006, Nielsen and Yu, 2010, Boukary *and al.*, 2014). Therefore, taking into account the sensitivities and specificities of the above serological tests, the Bayesian approach allowed us to have a general prevalence of 0.238% (95% CI: [0.0122 - 0.674 %]). EAT can diagnose infections at the acute stage of the disease including IgM immunoglobulin and IgG that appear first and are detected from the 10th day after the clinical onset of the disease. In addition the indirect ELISA helps to diagnose the infection during the different stages of its evolution including the main classes of antibodies (IgM, IgG and IgA). It thus makes it possible to diagnose infection in the acute phase (sepsis phase), in the focusing phase and in the chronic phase (Tounkara, 1994). In this respect, sera that have been tested positive for indirect ELISA and negative for EAT may be sera taken from animals whose infection was in the focusing phase or in the chronic phase. However, one can not differentiate by the nature of the antibodies the evolution phase of the disease (Maurin, 2005).

This study shows that pigs in the southern part of the country are tested positive and the prevalence remains low however, it is zero in the Far North. Pigs from the western, northwestern and coastal regions are the most infected. However, the proportions of infected pigs are higher in the North west (6.03%), followed by Littoral (3.67%) and West (2.59%). This result can be justified by the fact that the prevalence increases from dry regions to humid regions on the one hand (Boukary *and al.*, 2014). And the temperature that often reaches 40°C in the Far North would be responsible for the destruction of the *Brucella* genus bacteria from other parts (Hubálek *and al*., 2002, Houwé, 2011). Rainfall can reach 2000 mm per year in the North West region against 1000 mm per year in that of the Far North (Houwé, 2011, MNMSA, 2013). In addition, the humidity observed in the Littoral, North West and West regions would be favorable for the conservation of *Brucella* sp. (Diaz, 2013).

The absence of positive cases in the Far North may be justified since the dominant pork rearing system is an extensive system in which the serological incidence of the disease is generally low (Almeida, 1983). According to Leon and Ferri (2003), infected animals can also lose their anti-*Brucella* antibody titre after latent infection without seroconversion. In addition, the absence of positive cases in this part of the country may be due to sampling bias. However, the seronegativity attributed to the extensive breeding system of the Far North can be discussed because it is mainly thanks to the industrialization of pig farms in Europe and the implementation of prophylactic measures that porcine brucellosis has eradicated (Cvetnic *and al.*, 2009).

The most affected hog age group is between 12 and 24 months of age and the prevalence is 1.2% ($P < 0.05$). This is justified by the fact that pubescent pigs are more sensitive than young people because of the complete development of their genitalia (ENVF, 2004). Adult animals can remain infected throughout their lives while young people often recover from their infection and develop only a discrete and transient serologic response (ENVF, 2004). Other authors believe that age is not a determinant of receptivity for *Brucella* in pigs (Diaz, 2013).

The most affected breeds are the pietrain hybrid, the Landrace hybrid and the Duroc hybrid ($P < 0.05$). Indeed, this result is contrary to the idea that certain breeds of pigs in this case the animals from the cross between the Duroc and the red Jersey may be less sensitive to the experimental infection by *B.suis* (Diaz, 2013). Compared to other studies, the prevalence of porcine brucellosis is generally low (Onunkwo *and al.*, 2011; Diaz, 2013) and sera were analyzed mainly with the Rose Bengal test. The prevalence of 0.19% (95% CI: [0 - 0.45]) found in the present study with Rose Bengal is not very far from that found by Onunkwo *and al.*, (2011) in the literature. South-East Nigeria (Anambra, Enugu and Ebonyi States). This prevalence of 0.6% is related to a number of risk factors such as age of animals, drinking water distributed to pigs, grazing and the proximity of pig farms with those of cattle and small ruminants. Since porcine brucellosis is a major zoonosis (OIE, 2008), human infections are likely to occur in breeding areas where positive sera are derived. Thus, the risks of human infections are high in these farming areas (North-West, Littoral, West) unlike the Far North region where the risks are zero due to the absence of positive cases. Brucellosis is endemic in Nigeria in humans and in various animal species with prevalences ranging from 0.20% to 79.70% (Cadmus *and al*, 2006). Brucellosis can occur in pigs in areas where it is endemic in domestic ruminants (Radostits 1995, Young 1995, Onunkwo et al. Paradoxically Cadmus *and al.*, (2006) obtained a 0% prevalence on a sample of 200 pigs taken from a slaughterhouse and analyzed with Rose Bengal in Ibadan, in southwestern Nigeria, while at the same time - *Brucella* were detected in cattle (5.82%) and goats (0.86%) in the same abattoir. This result is similar to that obtained in Far North Cameroon, since bovine brucellosis, which is a favorable factor for porcine brucellosis (Corbel 2006, CFSPH 2009, Diaz 2013), is

endemic in the region in cattle (Bayang, 2014). For the other countries of Central Africa where anti-*Brucella* antibodies were detected, the prevalences found in this study (0.19% with Rose Bengal and 1.85% with indirect ELISA) is high compared to the one found in Gabon (0%) and low compared to Chad (4%) (Akakpo and Ndour, 2013).

CONCLUSION

Porcine brucellosis is a major zoonosis with economic and health implications that are not negligible. Porcine sera were analyzed for this purpose. At the end of the serological analyzes carried out on 1081 sera of pigs, the anti-*Brucella* antibodies were detected with a prevalence of 0,238%. In sum, this study has found that the bacterium *Brucella* kind circulates in pigs in Cameroon. However, given the absence of traceability in the pig sector, it would be biased to extrapolate the results obtained to the entire national pig population and even less to the herd of a region.

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