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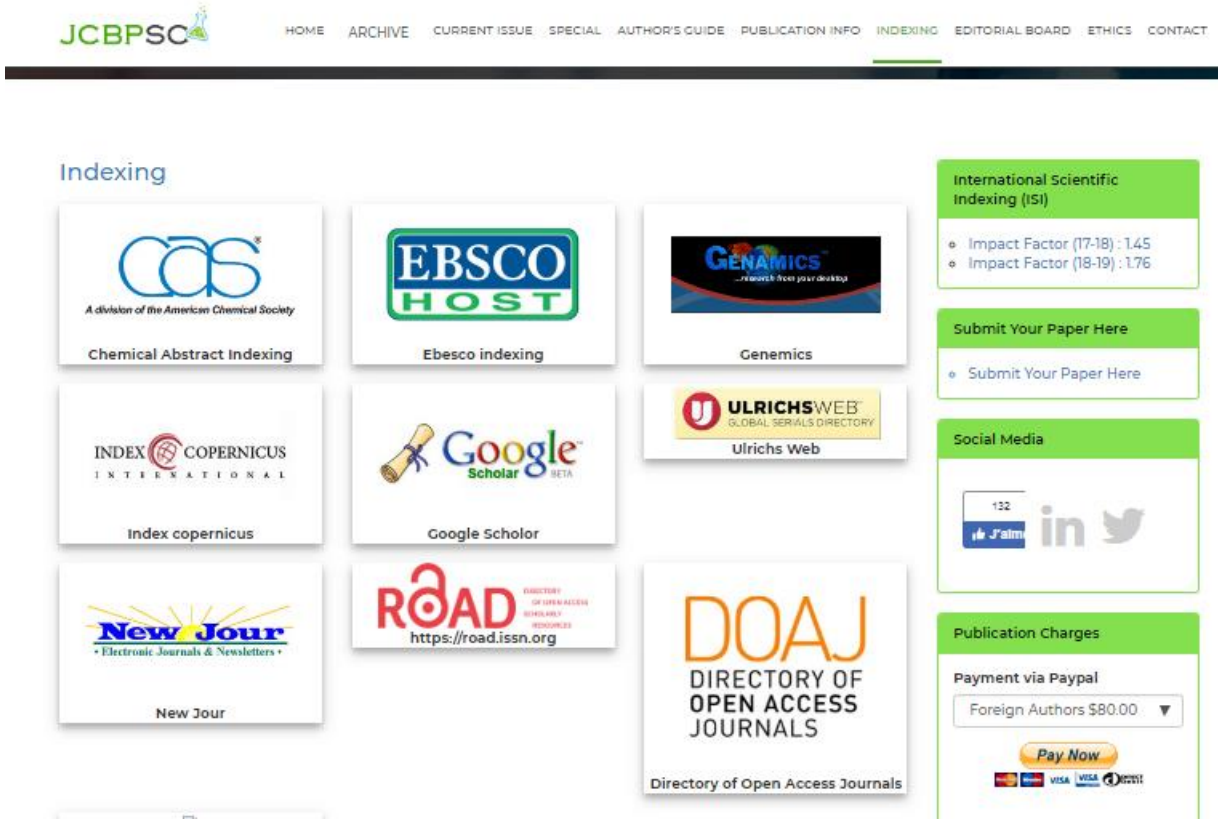

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Research Article

Serological Diagnosis of Bovine Brucellosis in the Southern Region of the Republic Of Benin

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Brucellosis is a chronic disease common to both humans and animals caused by bacteria of the genus Brucella. The damage caused by brucellosis is aggravated by the disease of humans that often leads to disability and sometimes even life. Our study focused on the serological diagnosis of the disease in cattle on private farms (in Abomey and Zogbodomey) and public in Samiondji in Benin. In fact, 444 cattle were examined. The sera collected were subjected to a fast slide agglutination test with Rose Bengal antigen (Rose Bengal), enzyme immunoassay (enzyme-linked-immuno-sorbent-assay ELISA), and milk samples - ring-proofed on milk to determine the prevalence of brucellosis. The results obtained revealed 118, 185 (out of 444 sera) and 49 (out of 115 milk samples) positive cases (prevalence of 26.6, 41.7 and 42.6%) respectively for Rose Bengal, ELISA and the test of the ring on the milk.

Key words: Brucellosis, prevalence, cattle, serological diagnosis, Benin.

INTRODUCTION

Brucellosis is a major, contagious zoonosis that affects all mammals, including humans. The disease is transmissible to many animal species [1](Noudeke et al., 2017) and is of great importance for public health because it causes not only very significant economic losses in breeding but also represents a public health hazard. Negligible. The prevalence of bovine brucellosis in the African continent is 50%

and above [2](Anagonou SIN et al., 2013, [3]Tialla D. et al., 2014, [4]Ayoola MC et al., 2017, [5]Sagamiko FD et al., 2018; [6]Vikou R. et al., 2018) and that of sheep - 12.5%.

- Estimated at 36.36 p. 100.
- The actual prevalence of bovine brucellosis in our study was
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Brucellosis is caused by bacteria of the genus Brucella. All domestic species and many wild animals are susceptible. In cattle, yaks, buffaloes, camels, horses the disease is mainly due to Brucella abortus, in sheep and goats - by Brucella melitensis, in pigs and reindeer - by Brucella suis, in dogs - by Brucella canis, at lambs, ewes and sheep - by Brucella ovis etc. Recently, Brucella inopinata has been isolated from humans, Brucella. pinnipedialis and Brucella ceti - in aquatic mammals and Brucella microti - in small rodents[7](De figueiredo et al., 2015).

The pathogens are very stable in the environment, can be stored in dairy products for 15-60 days, in meat for up to 20 days, in wool and on the skin for 3-4 months, in water during 5 months in the soil in summer for 40-70 days and in winter for 4.5 months [8](Sanogo M. et al., 2013). From the epidemiological point of view raw milk products are more hazardous (cheese, milk)[9] (Muma J.B. et al., 2012).

In animals and humans, brucellosis leads to reproductive and miscellaneous clinical and pathological sequelae. Economic losses due to brucellosis are associated with abortions, stillbirths, births of unsustainable offspring, decreased productivity of milk and use of animals for field work. Along with these significant economic damages, the disease also presents a social threat.

The important point in the complex of anti-brucella measures is the diagnosis of the disease, based on the results of laboratory methods (serologies and bacteriologies) taking into account the epizootic data and the clinical symptoms of the disease.

The most reliable method for diagnosing brucellosis is the isolation of the causative agent. However, serological methods have proved useful in the study of brucellosis in developing countries because they are simple, cost-effective and reproducible[10](Baddour M.M., 2012, [11]Del Pozo J.S.G., 2014). According to A.R. Boukaryet al.[12] (2011) Determination of the actual prevalence in sub-Saharan Africa of infectious diseases of socio-economic importance and impact on public health, is difficult because of lack of precision, regional collaboration, low representativeness of samples tested or the inadequacy between the results and the objectives of the studies. In Benin, it should be noted that there is very little precise information on the disease, which is linked to the imperfections of the existing diagnostic methods, the complexity of implementation and the inaccessibility of many activities to the farms. Individual.

The purpose of this study is to determine the current prevalence of brucellosis in southern Benin in order to improve the diagnosis of the disease.

MATERIALS AND METHODS

Period and scope of study: The studies were conducted from May 2017 to September 2017 in collaboration with the Veterinary Diagnostic Laboratory of Bohicon and the breeders. Three livestock

farms in the Zou department of southern Benin, two of which were private (in Abomey and Zogbodomey) and one public (from the Beninese state of Samiondji) were indicted. A total of 444 animals, aged from 5 months to 7 years, of which 99 in Abomey, 126 in Zogbodomey and 219 in Samiondji were included in the work. The number of cows is 255, heifers - 128 and that of the bulls - 61 of indigenous breeds - zebus, Borgou and lagoon.

Serological studies were carried out with diagnostic kits for brucellosis in animals: the ring test on milk, Rose Bengal and ELISA, manufactured by "Symbiotique" (France).

Sampling: Blood samples from cattle for serological testing were collected from all farms on the farms examined (444 samples) and milk from 115 cows. For this it was used: dry tubes (hermetic, not leaving air), VENOJECT needles, support, pens, and containers. The blood samples contained in the tubes were held for 1 hour in an incubator and 16-18 hours at a temperature of 4-10oC. Serum samples in volume of 5 ml were emptied into labeled test tubes. For each batch of samples of the serum was carried out the inventory (the list of information). The sera were stored under refrigerator conditions at -20 ° C and together with the milk samples were transported to the Veterinary Diagnostic Laboratory of Bohicon for analysis.

Agglutination reaction with Bengal Rose antigen: The materials used are: Eppendorf pipettes, porcelain serology plates, glass tubes, glasses + distilled water, shaker (mixer), stopwatch, gloves, cleaning papers, towels, reagents (the test sera, negative sera, positive sera), sterile distilled water for self-agglutination of the antigen, antigen: the French Bengal ose (InstitutMérieux).

The pink bengal antigen is a concentrated suspension of heat-inactivated Brucella abortus (Weybridge 99 strain) and phenol (0.5%) in buffer solution and stained with pink rose bengal. The pink bengal antigen is used in blood serum tests on bovine brucellosis, sheep, goats, camels, horses, deer and other species in the plaque reaction.

In each study the identical antigen test of the positive and negative serum samples was performed as the reaction controls.

For the actual analysis, we took 30 µl of the test serum in each hole of the plate. The same amount of antigen is added next to the test serum in the same hole. Using a glass test tube, we gently mixed the serum and the antigen. We stirred the plate for 4 minutes on the shaker, and the result was read immediately.

The result is positive when agglutination is noted, even if small, and negative - in the absence of agglutination

Ring test on milk (EAL): The test milk, Brucella antigen for EAL (heat-inactivated Brucella suspension (strain B. abortus 19) stained with hematoxylin in blue), dry brucella serum (a blood serum) were used. Lyophilized bulls-producers hyperimmunized by the culture of the vaccine strain B. abortus 19).

Before the test, the milk was thoroughly mixed with stirring to evenly distribute the cream. The reaction was performed in Florinsky serological tubes which are numbered according to the milk sample list. We introduced 2 ml of milk into the tubes. After each sample the pipette was washed twice with hot water. The 0.1 ml volume antigen was added to each tube with the milk sample. After adding the antigen to every ten samples, we vigorously shake the support with the tubes to distribute the preparation evenly in the milk.

The supports with the milk samples to be tested and those controls were placed in the thermostat or in a water bath at the temperature of 37-380C for 1 hour and then they were kept for 30 min. at room temperature.

The results of the reaction were "+" visually evaluated in 30-40 minutes after removing the supports from the water bath (thermostat). All milk samples, which gave an annular reaction with an estimate of 3 and 2 crosses, are considered positive

Enzyme immunoassay (EIA, ELISA): The enzyme immunoassay of the serum samples was carried out in the veterinary laboratories of the cities of Bohicon and Parakou. For the work we used 96-hole plates, the diluted antigen (1/1000), 2 positive and 2 negative controls (100 µl in each control hole), the samples to be tested (100 µl in each hole), antibodies for platelet sensitization, detecting antibodies, conjugate (100 µl in each hole) and solution to stop the reaction (50 µl). To carry out the analysis we carried out by order platelet sensitization, their washing with the buffer solution, the addition of the sample to be tested, the addition of the detecting antibodies, the conjugate and the stopping of the reaction.

The evaluation of the reaction was conducted visually and using the spectrophotometer. It should be noted that the positive samples have acquired a green color, while the negatives have not colored.

During the instrumental evaluation - the holes with the samples to be tested are placed on the spectrophotometer connected to the computer, containing all the data of each serum (the control sera and the sera to be analyzed). The software used is EDI version 2.3.1.

The computer automatically displays the optical densities (OD) of the sera. It calculates the positivity threshold of the sample and displays the state of each serum analyzed.

Statistical analyzes: The overall prevalence for each diagnostic test was calculated by relating the total number of positive cases to the total number. Thus, the prevalence of brucellosis by farm, category of animals and sex was calculated.

RESULTS

The prevalence of test brucellosis in the flocks examined in our studies is shown in **Figure 1.**

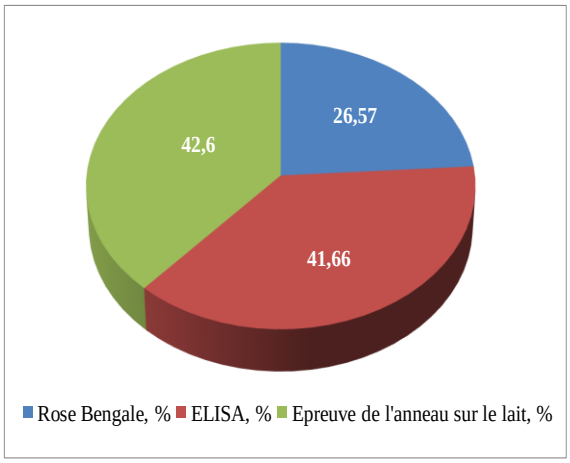

Figure 1: prevalence of brucellosis obtained by test in the farms examined

As the figure shows, the Rose Bengal for the diagnosis of brucellosis in farms detected the disease 1.57 and 1.6 less than the ELISA and ring test on milk - 26.57% , 41.66% and 42.60 respectively (p = 0.003).

The prevalence of Rose Bengal disease by sex and locality is shown in **Table 1.**

Table 1: Distribution of brucellosis prevalence with Rose Bengal by livestock, sex and location

Breeders	ROSE BENGALE								
	Number of sera			Results				Overallprevalence	
				Mâle		Femelle			
	Total	Mâle	Femelle	Abs.	%	Abs.	%	Abs.	%
Abomey	99	23	76	0	0	0	0	0	0
Zogbodomey	126	37	89	3	8,10	9	10,11	12	9,52
Samiondji	219	51	168	20	39,21	86	51,19	106	48,40*
TOTAL	444	111	333	23	20,72	95	28,52	118	26,57*

The disease is widespread in public (state) breeding compared to private farms. Thus while no case of the disease was detected in Abomey, its prevalence in Samiondji was 5 times higher than in Zogbodomey. It should be noted that the prevalence of the disease also varies according to the sex of the animals. The data in the table show that females suffer from the disease 1.37 times more often than males, and this ratio has no statistically significant difference between locations - 1.24 and 1.30 at Zogbodomey and Samiondji respectively (p = 0.02).

The prevalence of the disease with ELISA by sex and locality is presented in **Table 2.**

Table 2: Distribution of brucellosis prevalence with ELISA by livestock, sex and location

Breeders	ELISA								
	Number of sera			Results				Overallprevalence	
				Mâle		Femelle			
	Total	Mâle	Femelle	Abs.	%	Abs.	%	Abs.	%
Abomey	99	23	76	0	0	0	0	0	0
Zogbodomey	126	37	89	4	10,81	12	13,48	16	12,69
Samiondji	219	51	168	31	60,78	127	75,59	158	72,14*
TOTAL	444	111	333	35	31,53	139	41,74	185	41,66

The results obtained by the ELISA show as well as Rose Bengal the absence of the disease in Abomey. While the prevalence of brucellosis in Zogbodomey was 12.69%, in Samiondji she was diagnosed 5.68 times more. The ELISA also showed a prevalence of 1.32 times more in females than

in males without a statistically significant difference according to localities - 1.24 to Zogbodomey and Samiondji(p = 0.03).

The prevalences of the disease with the ring test on milk by sex and locality are presented in Table 3.

The milk ring test, unlike the other tests, diagnosed the disease in one cow in Abomey (3.57%). The highest prevalence, as in the case of Rose Bengal and ELISA was obtained in Samiondji and is 5 times higher than in Zogbodomey - 84.31% and 16.66% respectively (p = 0.015). The overall prevalence of the ring test on milk in cows is not statistically different from that of ELISA in all animals on farms - 42.60% and 41.66% respectively, while it is 1.6 times higher than Rose Bengal.

Table 3: Brucellosis prevalence distribution with the ring test on milk by farm, sex and location

Breeders	THE TEST OF THE RING ON MILK		
	Number of milksamples (cows)	Results	
		Abs.	%
Abomey	28	1	3,57
Zogbodomey	36	6	16,66
Samiondji	51	43	84,31*
TOTAL	115	49	42,60

DISCUSSION

Despite the fact that isolation of the causative agent is the most reliable method for diagnosing brucellosis, nowadays, serological methods have proved useful in the study of brucellosis in developing countries because they are simple, profitable and reproducible. In our studies, the prevalence of brucellosis was determined by Rose Bengal, ELISA and the ring test on milk. The enzyme immunoassay ELISA is known as a very sensitive serological test detecting both acute and latent infections, recent, old and chronic. The overall prevalences obtained in our studies by Rose Bengal, ELISA and the ring test on milk (26.57%, 41.66% and 42.60% respectively). Females are more susceptible to the disease than males with a prevalence of 1.37 times higher for Rose Bengal and 1.32 times for ELISA - 28.58% versus 20.72% and 41.74%, respectively. 31.53% respectively. These results are lower than those obtained in 2013 [2](Anagonou SIN et al., 2013) - 47.50%, 58% and 60.2% respectively for the Rose Bengal, the ELISA and the test of the ring on milk allow us to admit that work has been done for the fight against the disease although the greatest measures remain to be taken in this direction. Our ELISA results are not in line with those obtained by our colleagues in their respective studies - 15.21% reported by B. Koutinhouinet al.[13] (2003), 16.77% by N.D. Noudeke et al.[1] (2017) and 14.66% by R. Vikou et al.[6](2018). In our opinion this high prevalence in our studies and this big difference with the results of the colleagues would be related to the fact that we had just included in our research 3 farms of which 2 privates and an audience. The low prevalence in private farms can be explained by the small number of animals in general, the respect and implementation of certain measures to control the disease. A large number of animals are concentrated on public farms,

which together with the inefficiency of the complex of disease control measures due to lack of control over the main provisions contributes to a higher rate of prevalence in past cers.

CONCLUSION

This study allowed us to obtain the overall prevalence of 26.57%, 41.66% and 42.60% by Rose Bengal, ELISA and the ring test on milk respectively and a score of slight decrease in the prevalence of brucellosis in farms compared to our 2013 results. The ELISA and the milk ring test thus demonstrated a similar and stronger sensitivity than that of the bengal rose both in in cows - 41.74% and 42.60 respectively. The test of the ring on the milk proves to be simpler and practical in the conditions of the ground. It would be desirable to supplement the results with the causative agent isolation method in order to interpret the results obtained by the serological tests.

REFERENCES

1. N.D.Noudeke, L.G.Aplogan, F.Dossa, I.Youssao, S.Farougou, Monthly variations of the prevalence of bovine brucellosis in Benin. Adv. Anim. Vet. Sci. – 2017. – 5(1): 23-29. DOI: http://dx.doi.org/ 10.14737/journal.aavs/2017/5.1.23.29.
2. S.I.N.Anagonou, L'amélioration des mesures de lutte contre la brucellose dans les conditions d'élevage transhumant en République du Bénin: thèse de dissertation PhD. – M., 2013. – 132 p.
3. D.Tialla, P.Koné, M.C.Kadja, A.Kamga-Waladjo, Prévalence de la brucellose bovine et comportements à risques associés à cette zoonose dans la zone périurbaine de Dakar au Sénégal. Revue d'élevage et de medecine vétérinaire des pays tropicaux. – 2014. – 67(2). – P.67-72.
4. M.C.Ayoola, V.O.Akinseye, E.Cadmus, E.Awosanya, O.A.Popoola, O.O.Akinyemi, L., Taylor A.Perrett, J.Stack, I.Moriyon, S.I.Cadmus,Prevalence of bovine brucellosis in slaughtered cattle and barriers to better protection of abattoir workers in Ibadan, South-Western Nigeria. Pan. Afr. Med. J. – 2017. – 28.68. DOI: [10.11604/pamj.2017.28.68.10925].
5. F.D.Sagamikoab, J.B.Mumaa, E.D.Karimuriboca, A.M.Mwanzaa, C.Sindatod, B.M.Hang'ombea,Sero-prevalence of bovine brucellosis and associated risk factors in Mbeya region, Southern highlands of Tanzania. https://doi.org/10.1016/j.actatropica.2017.11.022.
6. R.Vikou, L.G.Aplogan, C.Ahanhanzo, L.Baba-Moussa, A.B.Gbangboche, Prévalence de la brucellose et de la tuberculose chez les bovins au Bénin. Int. J. Biol. Chem. Sci. – 2018. – 12(1). – P.120-128.
7. P.De figueiredo, A.F.Thomas, R.F.Allison, A.R.Carlos, A.L.Garry, Pathogenesis and immunobiology of brucellosis review of Brucella-Host interactions. Am. J. Pathol. – 2015. – 185. – P.1505-1517. DOI: http://dx.doi.org/10.1016/ j.ajpath.2015.03.003.
8. M.Sanogo,E.Abatih, E.Thys, D.Fretin, D.Berkvens, C.Saegerman, Importance of identification and typing of Brucellae from West African cattle: a review. Vet. Microbiol. – 2013. – 164(3-4) – P.202-211.
9. J.B.Muma,G.S.Pandey, M.Munyeme, C.Mumba, E.Mkandawire, H.M.Chimana, Brucellosis among smallholder cattle farmers in Zambia: public health significance. Trop. Anim. Health Prod. – 2012. – 44(4). – P.915-920.
10. M.M.Baddour Diagnosis of brucellosis in humans: a review. Journal of Veterinary Advances. – 2012. – 2(4). – P.149-156.

11. J.S.G.Del Pozo, S.L.Ortun, E.Navarro, J.Solera Detection of IgM antibrucella antibody in the absence of IgGs: a challenge for the clinical interpretation of brucella serology. PLoS. Negl. Trop. Dis. – 2014. – 8(12):e3390. https://doi.org/10.1371/journal.pntd.0003390.

12. A.R.Boukary, E.Thys, S.Mamadou, L.Rigouts, F.Matthys, S.G.Vias Franck, D.Gamatie, A.Yenikoye, C.Saegerman, La tuberculose à Mycobacterium bovisenAfriquesubsaharienne. Ann. Méd. Vét. – 2011. – 155. – P.23-37.

13. B.Koutinhouin, A.K.I.Youssao, A.E.Houehou, P.M.Agbadje, Prévalence de la brucellose bovine dans les élevages traditionnels encadrés par le Projet Pour Le Développement De l'Elevage (PDE) Au Bénin. Revue. Méd. Vét. – 2003. – 154(4). – P. 271-276. DOI: https://doi.org/10.5897/JVMAH2017.0567

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