American Journal of Animal and Veterinary Sciences

Original Research Paper

Sero-Prevalence of Rift Valley Fever and Brucellosis in Cattle of Northern Benin, West Africa

[1]Kadoéito Cyrille Boko, [2]Abdoulkarim Issa Ibrahim, [1,3]Yao Akpo, [1]Meriadec Evrard Alladassi, [1]Nestor Oscar Aguidissou, [1]Ange-Régis Zoclanclounon and [1]Souaïbou Farougou

[1]Research Unit on Communicable Diseases, Applied Biology Research Laboratory, Polytechnic School of Abomey-Calavi, University of Abomey-Calavi, Abomey-Calavi, Benin
[2]Central Livestock Laboratory, University Boubacar Ba of Tillabery, Niger
[3]Faculty of Agronomy Sciences, University of Parakou, Parakou, Benin

Article history
Received: 17-08-2022
Revised: 13-10-2022
Accepted: 15-10-2022

Corresponding Author:
Kadoéito Cyrille Boko
Research Unit on Communicable Diseases, University of Abomey-Calavi, Abomey-Calavi, Benin
Email: cyrilleboko@yahoo.fr

Abstract: Abortive diseases such as Brucellosis and Rift Valley Fever are among the major constraints for ruminant breeding due to the economic losses they cause for the animal keepers and the risk of human and environmental contamination. A serological study was conducted, from August to October 2021, in the commune of Malanville to assess the prevalence of the two diseases in Northern Benin. 92 blood samples were randomly collected from 7 farms that acknowledge abortion occurrence within the last 12 months. The sera resulting from collected blood samples were tested for Rift Valley Fever virus and Brucella antibodies using the competition ELISA. The results showed that 13.04 and 17.39% of the samples were positive for Rift Valley Fever and brucellosis respectively. This indicates that although there is no official reported outbreaks of Rift Valley Fever in Benin, the virus is circulating among the livestock population. Therefore to control the disease spread and protect the livestock keepers and animal source food consumers' health, all the stakeholders including animal and human health experts, researchers, policymakers, relevant ministries, and livestock keepers must develop a participatory control program of the disease for the years to come using a One Health approach.

Keywords: RVF, Brucellosis, Benin

Introduction

Livestock plays a key role in achieving sustainable food security in developing countries (Abu Hatab *et al.*, 2019). Indeed, in Benin, the domestic ruminant provides nearly 70% of local meat production and is therefore very important for the agricultural and socio-economic development of the country (Dognon *et al.*, 2018). However, the animal faces several diseases (Akakpo and N'dour, 2013), including abortive diseases such as brucellosis and Rift Valley Fever which affect significantly animal health and productivity and are also recognized to be a public health issue (Diallo *et al.*, 2020).

Brucellosis is an infectious, bacterial, causing contagious disease that affects several animal species, mainly cattle, sheep, goats, pigs, and humans (Akakpo *et al.*, 2009; CDC, 2021). *Brucella* species including *B. abortus* and *B. melitensis* can cause the disease leading to very important economic losses in livestock (abortion, stillbirths, sterility, decrease in milk production, and hindrances to trade) (Akakpo *et al.*, 2009; OIE, 2018a). In cattle, the disease is clinically characterized by abortion in females (in the last third of gestation), following bacterial colonization of the placenta, urogenital involvement, and in males, it usually evolved in the form of orchitis, hygromas, and epididymitis causing infertility (Seleem *et al.*, 2010). In Benin, Akakpo *et al.* (1984) recorded a national seroprevalence of 10% and later several pieces of research reported seroprevalence rates between 14.66 and 16.77% (Noudeke *et al.*, 2017; Vikou *et al.*, 2018).

Rift Valley Fever (RVF) is a viral infection, a contagious and hemorrhagic disease with a marked zoonotic character that affects domestic ruminants like cattle, sheep, and goats (Arsevska *et al.*, 2016), but also humans (OIE, 2018b; CDC, 2020). It is caused by an arbovirus: The Rift Valley Fever Virus (RVFV) which is transmitted to humans and animals by the bite of mosquitoes mainly of the genera *Aedes* and *Culex*

(Chevalier *et al.*, 2010; Quellec *et al.*, 2021; Léger and Lozach, 2021). It is among the major emerging and re-emerging diseases of the last decade, with numerous epidemics and epizootics essentially on the African continent (Venter, 2018). Usually occurring in epizootic form, RVF emerges following heavy rains and floods and is characterized by high rates of abortions and neonatal deaths (OIE, 2018b). Unlike brucellosis, RVF has been the subject of a few studies. Apart from the first study conducted on domestic ruminants (Gbaguidi, 1992), no other nationwide studies have been conducted. Owing to the constant and growing threat of brucellosis and RVF to public and veterinary health, the increasing emergence of mosquito-borne diseases and the circulation of the virus in countries of the sub-region such as Mali, Mauritania, Senegal, and Niger (Boushab *et al.*, 2015; Arsevska *et al.*, 2016; Hama *et al.*, 2019; Quellec *et al.*, 2021), there is an urgent need to update data for better surveillance of these abortive diseases. The objective of the present study is therefore to determine the seroprevalence of these two abortive diseases (RVF and brucellosis) in domestic ruminants in North Benin and to verify markers that testify to RVF virus circulation.

Materials and Methods

Study Area and Design

The study was conducted in the commune of Malanville located in the North of Benin (Fig. 1). The commune of Malanville is bordered to the North by the Republic of Niger (Niger River), to the South by the communes of Kandi and Ségbana, to the West by the commune of Karimama and to the East by the Federal Republic of Nigeria.

This zone is situated between latitudes 11°24 and 12°00 N. It is called the Sudanian Zone (SZ) which is characterized by ferruginous soils with savanna and dry woodlands. The climate is a dry tropical type with annual precipitation varying from 900 to 1 000 mm. The humidity index varies from 1.9 to 1.4. The vegetation is strongly influenced by the dry Harmattan wind. The Significant families are Leguminosae (24%), Rubiaceae (8%), Combretaceae (7%). The herd structure is often average in cows, suckler cows, calves, heifers, bulls, young bulls, oxen, and steers (Houessou *et al.*, 2019).

The dominant rearing modes of cattle in Benin are transhumant and sedentary modes. The animals are housed in the open air in night parks around the dwellings of the camp attached to a stake (Fig. 2). Animal feeding is reduced to natural pasture, supplemented by crop residues and cooking salt. Animals are watered in streams and reservoirs. Random natural mating is used by all breeders generally. Animal health monitoring is limited to two vaccinations (contagious bovine pleuropneumonia and bovine pasteurellosis) and is carried out by livestock management officers (Worogo *et al.*, 2019).

The choice of this commune is based on the fact that it is a border area that receives transhuman animals from Niger every year, a border country that has experienced RVF epizootic in the past.

A cross-sectional seroprevalence study was carried out from August to October 2021 concerning animals that had aborted less than one year before the collection date to assess the distribution of brucellosis and Rift Valley Fever in cattle herds of the commune of Malanville of Benin (Fig. 3).

Sampling

Sera samples were collected from cattle belonging to herds where abortion was reported within a year by the farmers. These samples were collected from August to October 2021. The target populations were selected randomly in seven (7) herds. A total of ninety-two (92) samples were collected, of which eighty-two (82) were females and ten (10) were males (Table 1). The blood samples collected were centrifuged (Centrifuge Eppendorf 5804, supplied by Eppendorf, Hamburg, Germany) to obtain the sera. These sera were stored at -20°C. Collected sera were stored on dry ice and transported to the Central Livestock Laboratory of Niamey (LABOCEL) in Niger for serological analysis.

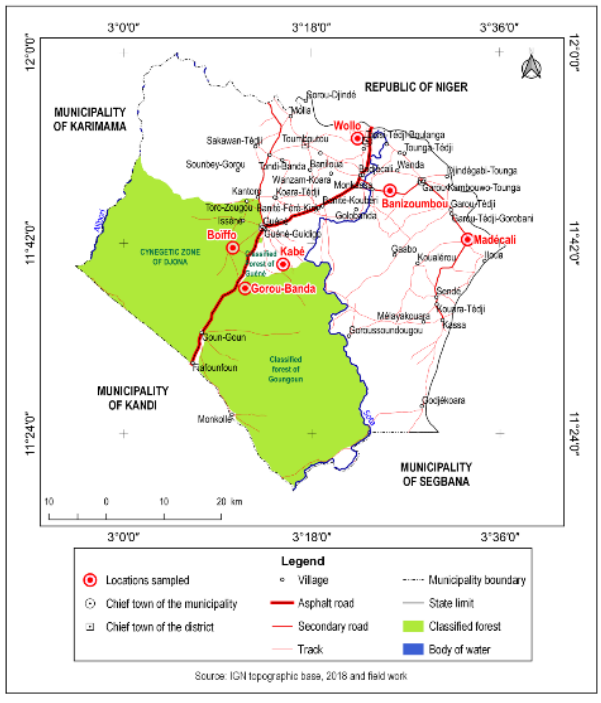

Fig. 1: Study area (source: IGN Topographic base, 2018 and field work)

Serological Analysis

RVF Antibodies Determination

Sera obtained were assessed for the presence of the antibodies against the Rift Valley Fever virus Nucleoprotein (NP) using the competition ELISA (ELISA ID Screen Rift Valley Fever Competition Multi-Species kit) as described by Hama *et al.* (2019). Optical densities were recorded on a Multiskan FC spectrophotometer at 450 nm wavelength. The values obtained from the test plates were exported to Microsoft Excel software, where they were processed by the c-ELISA RVF kit ID vet reading template. The Percentage of Inhibition (PI) was calculated using the following formula:

$$PI = (DO_{Ech} / DO_{CN}) \times 100$$

Sera tests with PI values ≤ 40 are considered positive and sera tests with PI values > 50 are considered negative for RVF antibodies.

Anti-Brucella Antibodies Determination

Sera were tested for anti-Brucella antibodies using the competition ELISA. The ID.vet/ID Screen Brucellosis serum indirect Multi-species was used following Vikou *et al.* (2018) protocols. The sera were thawed and the reagents were brought to room temperature (5-21°C) and homogenized. Test samples and controls were dispensed into the wells and diluted at 1: 20 (v/v). The wells were sensitized with *Brucella abortus* LPS. Anti-Brucella antibodies, if present, form an antigen-antibody complex (Ag-Ab). A peroxidase-labeled multispecies conjugate (HRP) was distributed in the wells. It binds to the anti-Brucella antibodies, forming an Ag-Ab-HRP-conjugate complex. After the removal of the excess conjugated by washing, the reaction was revealed by a revelation solution (TMB). The resulting staining was related to the specific antibodies present in the test sample. In the presence of antibodies in the sample, a blue coloration developed and turns yellow after blocking, and in the absence of antibodies in the sample, no coloration appears. The reading was taken at a wavelength of 450 nm. For brucellosis data, the percentage (S/P %) was calculated for each sample using the following formula:

$$S / P\% = \left(DO_{Ech} - DO_{Cp} / DO_{Cn} - DO_{Cp}\right) \times 100$$

Samples with an S/P% ≤ 110% are considered negative and those with S/P% ≥ 120% are considered positive.

Statistical Analysis

All data obtained were coded and entered into Microsoft Office Excel and analyzed using R software version 4.1.3. All the results were drawn out through a descriptive study of data. The overall prevalence of RVF antibodies and brucellosis was estimated by dividing the number of positive sera by the number of sera tested. The sex-specific prevalence for each disease was estimated and compared by pairing it with the z-test on R software. The confidence level for this test was 5%.

Fig. 2: A cattle herd (Banizounbou, Malanville, North of Benin)

Fig. 3: Blood sampling collection from cattle jugular vein (Banizounbou, Malanville, North of Benin)

Table 1: Number of collected samples

District	Villages	Number of samples	Female	Male
Guéné	Cabé	15	13	2
	Boiffo	15	14	1
Madécali	Madécali	17	15	2
Malanville	Walo	15	13	2
	Banizounbou	15	14	1
Toumboutou	Gorou-Banda	15	13	2
Total		92	82	10

Table 2: Seroprevalence of RVF and brucellosis

	Number of analyzed sera	Number of positive sera	Prevalence (%)
RVF	92	12	13.04
Brucellosis	92	16	17,39.00

Table 3: Seroprevalence of RVF and Brucellosis by gender

Diseases	Sex	Number of tested sera	Number of positive sera	Prevalence
RVF	Males	10	0	0
	Females	82	12	14,63
Brucellosis	Males	10	0	0
	Females	82	16	19,51

Results

The results showed that out of the 92 sera tested, 12 were positive for RVF (13.04%) and 16 positive for brucellosis (17.39%), however, the difference between the disease prevalence was not significant. Moreover, no male animal tested positive for both diseases (Table 2).

Serological results for RVF and brucellosis concerning sex showed that positive cases were observed only in females with a prevalence of 14.63 and 19.51% for RVF and brucellosis respectively (Table 3). However, there was no significant difference between the prevalence obtained concerning sex for RVF and brucellosis.

Discussion

Bacteria of the genus Brucella cause brucellosis. The disease is widespread worldwide and humans can be infected by ingesting contaminated and undercooked meat, raw milk, or their by-products. However, these animal source food are usually consumed in Benin. *Brucella abortus* frequently infects cattle. The rate of infection was increasing in recent years in developing countries due to the lack of rigor in farm management (Khan and Zahoor, 2018).

Previous scholars have reported the presence of brucellosis in cattle in Benin. Reported a prevalence of 10% and Koutinhouin (2003) reported a prevalence of 15% from a study conducted from 2002 to 2005. Later on Noudeke *et al.* (2017); Vikou *et al.* (2018) reported a prevalence of 16.77% and 14.66% of cattle brucellosis respectively. This indicates the maintenance of the microorganism in the country is probably due to poor control strategies although it's a zoonosis.

In the sub-region, of Mali, a prevalence of 22% has been reported by Traore *et al.* (2020). Boukary *et al.* (2013) indicated 11.2 and 17.2% in Niger.

Despite the awareness campaigns, the prevalence of this disease remains high on farms. The present study revealed a 17% infection rate. Since it is a chronic disease and the clinical symptoms are not visible, abortions are often the basic signs of suspicion of the disease's occurrence. However, many other microorganisms can cause abortions. It's therefore unreliable to provide a diagnosis just on such a basis. Human infections can lead to economic losses, poor health, and work incapacity.

Given that transhumance remains a customary activity despite the efforts made at the sub-regional level such as projects to build watering points and promote the installation of fodder plots, it is clear that the State authorities must develop long-term (five to ten years) strategies, coupled with rigorous zero surveillance to ensure better protection of the population that consume meat, milk, and its by-products daily.

The evaluation of the seropositivity of Rift Valley Fever and brucellosis was determined using the indirect ELISA test. This enzyme-linked immunosorbent assay is known to be a highly sensitive serological test capable to demonstrate recent, old, and chronic infections (OIE, 2018a). The choice of the study area (Malanville commune) is justified by the fact that it is a high-risk area given the frequent movement of transhumant animals from Niger every year, a border country that experienced RVF epizootics/epidemics in 2016 (Doutchi *et al.*, 2017). In Benin, apart from brucellosis which has been the subject of several studies, no official data exist on Rift Valley Fever. Thus, the overall seroprevalence obtained (13.04%) indicated the presence of the RVF virus in this area. It is important to

emphasize that this study involved the detection of IgG, thus highlighting the possible contact of animals with the virus. The prevalence regarding sex showed higher infection rates in females. The results were similar to those reported by Hama *et al.* (2019) in Niger and could be explained by the structure of the herds, females being generally more than males and having a longer reproduction career (Hanzen *et al.*, 2016). These results allowed us to suspect the presence of a maintenance reservoir of the virus in this area and the possibility of experiencing epizootic outbreaks related to transhumance in this border area.

This study already indicates that there is evidence of virus maintenance in livestock in this border township between Benin and Niger. There are several indications that the virus is still circulating in this locality. The Autochthonous Ecosystem and the Niger River forming a bridge between Benin and Niger contributed to the movement of mosquitoes that play a major role in the transmission of this virus to animals. Researchers established a link between *Aedes* mosquitoes, the winter period, and the emergence of RVF (Ndione *et al.*, 2005). This observation raises the need for entomological research in the study area to shed more light on the probable role of this vegetation in the maintenance of the infection.

In addition, a study targeting farmers, herders, butchers, and hospitalized patients in Mauritania recorded a mortality rate of ten (10) out of eleven (11) people who tested positive for RVF (Boushab *et al.*, 2015). They also revealed that the consumption of raw meat and unpasteurized milk was a risk factor. From these observations, probably, the farmers and surrounding populations living where the samples were collected may have already been infected. This result suggests the finding must be taken into account to establish disease control at the human level in Benin. The use of a more advanced diagnostic tool such as the polymerase chain reaction could provide more details on the circulation of this virus in the country.

Conclusion

This study revealed the existence of Rift Valley Fever and brucellosis in cattle herds in the commune of Malanville with an overall seroprevalence of 13.04 and 17.39% respectively found in female animals only. These results confirm the circulation of the RVF virus in the country which probably comes from the neighboring country Niger which has experienced epizootic outbreaks. The persistence of brucellosis and the detection of the Rift Valley Fever virus indicated that the situation is worrisome and calls for the establishment of a regional surveillance system between the two countries for better control and eradication of these diseases.

It is also important to investigate the development of IgM and confirm the results by identifying the pathogens from cattle at slaughterhouses and abortions on farms by the molecular biology technique.

Acknowledgment

The authors thank the farmers from whom the samples were taken and the Central Livestock Laboratory in Niamey where the analyses were performed.

Funding Information

This study was not funded by any institution.

Author's Contributions

Kadoéito Cyrille Boko: Wrote the protocol, performed the serum collection, and wrote the first draft.

Abdoulkarim Issa Ibrahim: Performed the laboratory analysis.

Miriadec Alladassi; Nestor Oscar Aguidissou and Ange-Régis Zoclanclounon: Participated in the serum collection.

Yao Akpo, Benoit Koutinhouin and Souaïbou Farougou: Critically reviewed the manuscript

All authors contributed to and commented on subsequent revisions of the manuscript. All authors approved the final manuscript.

Ethics

The corresponding author confirms that all of the other authors have read and approved the manuscript and that no ethical issues are involved.

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