

Research Article

Seroprevalence of Contagious Bovine Pleuropneumonia in Niamey Region, Niger

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• CBPP; Outbreak; c-ELISA; CFT; IBT; West-Africa, Niger

Abstract

Contagious bovine pleuropneumonia (CBPP) is a severe respiratory disease affecting cattle and buffalo caused by *Mycoplasma mycoides* subsp *mycoides* Small Colony. This disease is endemic in many countries of West Africa. This study reviews the distribution of CBPP outbreaks in West Africa from 2005 to 2017 and includes updated data from Niger where the disease is not well documented. In particular it provides a first account of the serological prevalence of CBPP in the region of Niamey in Niger. Sera from 987 cattle from this region, comprising 912 belonging to 43 herds distributed in five communes and 75 sera from a notified CBPP outbreak, were screened for CBPP using competitive enzyme-linked immune-sorbent assay test (c-ELISA) and complement fixation test (CFT), and immune blotting (IBT) for confirmation of positives.

The c-ELISA screening test indicated an estimated sero-prevalence in the Niamey region of 6.8% (62/912) at individual level, and 58.1% at herd level. The CFT appeared less sensitive detecting, only 1.6% (15/912) positive sera. Positive sera in both tests were further tested by IBT of which 18.2% (14/77) were confirmed positive. Serological testing of the CBPP outbreak revealed 73.3% (55/75) and 62.7% (47/75) positive in c-ELISA and CFT, respectively. The IBT confirmed the infection in 60.0% of the animals (45/75 cattle).

In our study the c-ELISA proved to be more sensitive than CFT. However, c-ELISA detected mainly animals in chronic stages while CFT was able to detect recent infections. Ideally, c-ELISA and CFT should be used in parallel for screening CBPP but IBT, showing a higher specificity, should be used for confirmation of the infection.

ABBREVIATIONS

CBPP: Contagious bovine pleuropneumonia; c-ELISA: Competitive enzyme-linked immunosorbent assay; CFT: Complement fixation test; IBT: Immunoblotting test; OIE: World Organization for Animal Health; ECOWAS: Economic Community of West African States; CIRAD-EMVT: Centre de Coopération Internationale en Recherche Agronomique pour le Développement. Département d'élevage et de médecine vétérinaire

INTRODUCTION

Today it is impossible to dissociate animal health, human health and the economy from agricultural production. While animal health has a repercussion on public health through the occurrence of zoonotic diseases, the impact on the economy is also very relevant mainly in those societies where agriculture and animal production are essential for subsistence. Diseases such as contagious bovine pleuropneumonia (CBPP), although not zoonotic, constitute an enormous menace for animal breeding

and continue to have great economic impact in regions where the disease is endemic, such as in some West Africa countries.

CBPP is a contagious respiratory disease of cattle caused by *Mycoplasma mycoides* subsp *mycoides* Small Colony characterized by anorexia, fever and respiratory distress such as dyspnea, polypnea, cough and nasal discharges. These clinical signs can be observed in adults whereas in young animals joint problems are the main associated problems [1]. According to the Food and Agriculture Organization of the United Nations (FAO), CBPP is transmitted by direct contact to an infected animal through inhalation of pathogen infective droplets. Airborne transmission up to 200 m is believed to be possible and shared accommodation and water holes among nomadic herds is one of the reasons for the wide distribution of CBPP in sub Saharan Africa. The incubation period for naturally infected animals ranges from three weeks to six months. When the disease is introduced for the first time in a country outbreaks can occur in which many animals are affected and develop an acute clinical form. On the other hand, it may also occur in an enzootic form, with episodes

of sporadic cases, usually sub acute, when the country or region is infected for several years. The mortality rate of new outbreaks of CBPP in Africa varies between 10-70% [2].

Control strategies are based on early detection of outbreaks, control of animal movements and a slaughter policy. The implementation of these strategies has led to the eradication of the disease in North America and Europe but Africa continues to be the great focus of CBPP. The clinical manifestations of CBPP in cattle range from hyperacute, acute, subacute and chronic forms. Subacute CBPP is the most common form and is a less severe form of the acute disease with only slight respiratory signs and intermittent fever. The *post mortem* pathognomonic lesions of CBPP include pleurites, hypertrophy of the lymphnodes, unilateral marbling of the lungs in acute disease, and sequestra formation in subacute to chronic cases [3]. Acute and subacute CBPP form commonly progresses into chronic CBPP, which is characterized by an apparently healthy state of the animal even though chronic lung lesions are present. These silent carriers of CBPP are infectious and thought to be an important factor in spreading the disease among cattle herds. This is why the control or eradication of the disease is hampered by the frequent occurrence of subacute or unapparent infections and the persistence of chronic carriers after the clinical phase. Disease control in Africa is mainly focused on immunization campaigns, but this is confounded by the lack of prevalence data in many African countries like Niger.

Serological diagnosis is one of the first approaches to obtaining information on the prevalence of CBPP. The OIE recommends the use in parallel of the modified Campbell and Turner's complement fixation test (CFT) and competitive enzyme-linked immunosorbent assay (c-ELISA) tests for screening and diagnosis of CBPP since none of the tests detect all positive animals during natural infection [4-7]. In addition, the highly specific and sensitive immunoblotting test (IBT), should be performed on doubtful or positive samples to confirm the infection. It is an ideal test for the evaluation of a country true prevalence of CBPP. However, IBT is a fastidious technique to perform mass-screening [5,8,9].

This is evidenced as indicated by previous reported seroprevalence rates of CBPP in Burkina Faso 2.9% and 5.4% for Mauritania, as well as 10.5% for Mali and Benin, using c-ELISA [10].

Historical distribution of CBPP

CBPP was not distinguished from other diseases of the chest until the end of the 18th century. Bourgelat (1765) distinguished pleuropneumonia from other "putrid fevers" of the lungs and described the unique aspect of exudative pleuropneumonia lesions. However, it is difficult to trace the history of the disease because of the lack of appropriate diagnostic systems available until relatively recently [11,12]. In the 19th century the awareness of the disease was marked by some attempt at vaccination, despite the lack of knowledge of the etiological agent, by inoculation of an infected lesion in healthy cattle. This technique of inoculation has also been practiced by some breeders in Africa [13].

Towards the end of the 19th century with the advent of laboratory bacteriological practices pioneered by Pasteur, the

etiological agent of pleuropneumonia was cultivated *in vitro*. Nocard and Roux in 1898, published their study on the isolation of the bovine pleuropneumonia microbe [14]. In the first quarter of the 20th century, microbiologists had attained considerable progress in the knowledge of the characteristics of the etiological agent, but they continued to consider it as a "pleuropneumonic virus". However, later during this century, bacteriologists named the pleuropneumonic microbe *Mycoplasma mycoides*, to recall the fungal morphology, the name which prevails today [12].

According to the literature, the first outbreaks of CBPP were reported in Europe between the late 18th century and early 19th century. Netherlands was the focus of several epidemics and it was believed to have infected the rest of the world including England in 1840, the United States between 1842 and 1843, Spain in 1846, and then South Africa in 1854. After being introduced into South Africa, the disease spread to West Africa [12,15].

Outbreaks distribution of CBPP in West Africa

CBPP continues to spread in many developing countries and is particularly frequent in West Africa. The inadequacy of regional control policies, the lack of resources and reduced technical capacity of governments make it difficult to eradicate this disease.

CBPP is one of the most important transboundary livestock diseases in the Sahel and West Africa. The situation of outbreaks recorded in recent years in the countries of the sub-region shows that, with the exception of Cape Verde, Guinea Bissau, Gambia and Senegal, the disease remains endemic or sporadic in most West African countries. In 1993, Gambia and Senegal reported freedom from CBPP [5].

There was a considerable decrease in the incidence of the disease following annual vaccination against rinderpest virus with the bivalent vaccine (Rinderpest/CBPP) in the 1980s. However, with the eradication of rinderpest, and the cessation of vaccination, there was an upsurge of CBPP in countries declared free and an increase in incidence in endemic countries [16].

Starting in 2005 to 2017, from the 15 Economic Community of West African States (ECOWAS), 13 were infected with the disease. In Liberia the disease was suspected in 2016 but has not yet been confirmed. Cape Verde has remained unscathed perhaps due to the fact that the country is an island and the reduction in animal trade would hamper disease transmission.

According to OIE (Organization International des Epizooties), the number of reported CBPP outbreaks remained relatively high in the south part of West Africa between 2005 and 2017. This would explain the re-emergence of CBPP in uninfected countries like Senegal and Gambia. It is known that CBPP reappeared in Gambia because of unregulated trade of cattle between Mali and Mauritania where the disease remains a problem. The infection then rapidly spread to neighboring areas before reaching Tambacounda in Senegal [17]. The distribution of the outbreaks varied between the countries. From 2005-2017 the average number of outbreaks in West-Africa was 8.5~9.0 while the median was 2 outbreaks (95% CI, 6.02 - 11.00; Figure 1), with Benin, Burkina Faso, Ivory Coast, Ghana, Nigeria and Togo showing the higher number (Table 1). The estimated average of

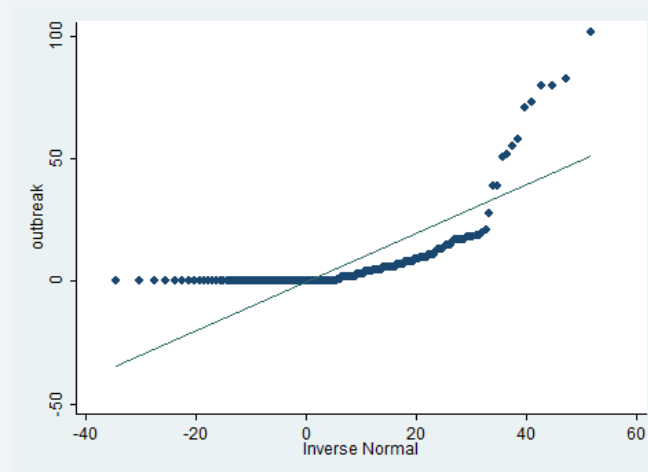


Figure 1 Graph of normality test of distribution of CBPP outbreaks on STATA 11.

Table 1: CBPP outbreaks in West Africa countries reported to OIE from 2005 to 2017.

Country	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017
Benin	7	6	11	8	3	4	10	18	10	5	2	7	ND
Burkina Faso	10	6	14	16	13	21	5	4	11	2	3	13	17
Cape Verde	0	0	0	0	0	0	0	0	0	0	0	0	0
Ivory Coast	5	+	0	1	8	18	18	11	15	39	6	11	ND
Gambia	0	0	0	0	0	0	0	1	0	0	0	15	0
Ghana	8	19	6	55	52	58	73	71	51	102	80	83	80
Guinea	+	+	0	0	0	0	0	+	2	+	0	0	3
Guinea Bissau	0	0	0	0	0	0	0	0	0	0	0	0?	2
Liberia	0	0	0	0	0	0	0	0	0	0	0	?()	0
Mali	17	8	5	5	6	3	4	3	3	5	3	4	6
Niger	+	+	+	+	7	2	6	2	7	0	0	6	4
Nigeria	2	2	2	28	39	17	17	6	10	2	+	4	9
Senegal	0	0	0	0	0	0	0	1	1	2	8	5	19
Sierra Leone	0	0	0	0	0	0	0	0	0	0	0	0	0
Togo	17	12	7	20	6	15	9	9	13	8	7	10	ND

Abbreviations: ND: Not Determined;+: Presence of disease with quantitative data but with an unknown number of outbreaks; 0: Absence of disease; 0?: Disease absent but suspected; ?(): Disease suspected but not confirmed and limited to one or more zones.

CBPP outbreaks per year in the different countries is summarized in Figure 2. Burkina Faso, Ivory Coast, Nigeria and Togo had a mean of 10 outbreaks, while Ghana had more than 50 outbreaks [18].

The prevalence of CBPP is the number of infections (old and new) that occur in a given cattle population at a given time and, although few studies are available, prevalence of CBPP differ according to the cattle production system so different values are reported for different West African countries. In 2006, Tambi and colleagues citing different sources, refer to an estimated prevalence of 0.29% and 0.51% in Nigeria, 2.9% for Burkina Faso, 5.4% for Mauritania and 10.5% for Mali [10].

Situation of CBPP in Niger

In Niger, livestock production is practiced by nearly 87% of

the active population either as a main or as a secondary activity after agriculture. Breeding is the dominant form of income for rural and urban households, helping to build resilience in response to particular crises and social events.

CBPP continues to be the first concern for livestock production in Niger and the epidemiological situation is under-documented. The prevalence is poorly known due to the scarcity of diagnosis for the disease and the existing data are essentially limited to the morbidity and mortality rates in annual number of outbreaks reported by the veterinary services. This data is insufficient to estimate the prevalence of the disease although has endemic country, Niger continues to record the sporadic outbreaks of CBPP.

Recently, a serological survey using c-ELISA was performed in

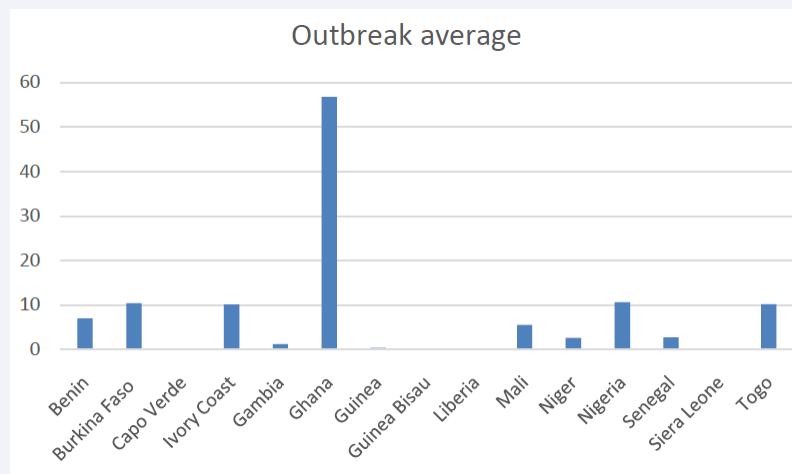


Figure 2 Average number of CBPP outbreaks in West African countries 2005-2017, (OIE country reports).

Niger revealing an overall seroprevalence of 4.15% at individual level, with higher seroprevalences values obtained in the regions of Zinder, Differ and Tahoua [19]. To update the situation, a study on the seroprevalence of CBPP in cattle was conducted in the region of Niamey, Niger.

MATERIALS AND METHODS

Study area

The study was conducted in the region of Niamey, located in the southwestern part of Niger (between latitudes 13 ° 33 'and 13 ° 24' South and meridians 2 ° and 29 ° 15 'East), that includes five Urban Communes covering 552.27 km², from which 297 km² is the City (Figure 3). The region contains agricultural production areas in the surrounding village lands and perurban pastoral areas (corridors, pastoral enclaves, water points) allowing the breeding of large herds.

Sampling procedures

To evaluate the seroprevalence of CBPP in the region of Niamey, 43 non vaccinated herds from Communes 1, 2, 4 and 5 were randomly selected. Herds from Commune 3 were not included in this study because cattle were already vaccinated against CBPP (using T1sr). A total of 912 sera samples were collected during three months (December 2015 and February 2016). Twenty animals were sampled from each herd, ten of which have been CBPP symptomatic (*e.g.* with fever or respiratory signs such as coughing, labored breathing, loss in appetite and body condition, and decreased milk production) in the last two years prior to sampling. Additionally, 75 sera samples from a CBPP outbreak in a herd from Commune 4, were also included in the study. This outbreak was reported in the beginning of 2016 and more than a half of the cattle presented with clinical signs of CBPP. At necropsy a single slaughtered animal showed pneumonic necrotic lesions and lung marbled appearance characteristics of CBPP. All serum samples were stored in a Micronic system, kept at -20°C until they were sent to the National Institute of Agrarian and Veterinary Research (Instituto Nacional de Investigação Agrária e Veterinária, INIAV, Portugal) for serological analysis.

The study did not require ethics committee approval in accordance with local legislation.

Serological tests

All sera were tested using competitive enzyme-linked immunosorbent assay test (c-ELISA; CIRAD-EMVT, France) [4] and complement fixation Test (CFT) [20]. Those samples found positive in at least one of the tests were further checked by immunoblotting (IBT) [21] for confirmation. Competitive ELISA was performed as described by the manufacturers. The optical density (OD) was measured at 450 nm in a spectrophotometer (Dy nex Technologies, USA) and the inhibition percentage (PI) value for each serum calculated. A cut-off point of 58% inhibition was used to determine positivity or negativity comparing to a CBPP positive control. CFT was performed according to the modified method of Campbell and Turner (1953) described in OIE Manual, chapter 2.4.9 (2014) [5,20]. Briefly, serum samples were diluted 1/10 to 1/320 and mixed with a suspension of whole cell *M. mycoides* subsp. *mycoides* Small Colony as antigen in a microplate. Commercially available complement from guinea-pig serum (Virion\Serion, Germany) was added followed by 30 min incubation at 37°C. Then, sensitized sheep red blood cells (SRBCs) consisting of sheep red blood cells and rabbit hyperimmune serum of sheep red blood cells (hemolysin; Virion\Serion, Germany) were added to the microplate and incubated for another 30 min at 37°C. Microplates were centrifuge at 125 g for 5 min in order to sediment the SRBCs. Results were read as percentage of observed complement fixation. A positive result corresponds to 100% inhibition of hemolysis at 1/10 (++++ at 1/10 dilution) and results down to 25% (+ at 1/10) are doubtful. IBT is an immunoenzymatic test that has been developed to confirm doubtful/positive CFT or c-ELISA results. The IBT was performed according to Gonçalves et al. [21], and the OIE manual [5]. Briefly, western blotting nitrocellulose membrane strips prepared with separated proteins of *M. mycoides* subsp. *mycoides* Small Colony strain B345/93 (Portugal), were incubated with serum samples diluted at 1/3 and positive control serum (1/100 dilution). Reactive bands were visualized using Pierce™goat anti-bovine IgG secondary antibody (Thermo Scientific, USA) and substrate BCIP/NTB (5-bromo-4-chloro-3-indolyl phosphate

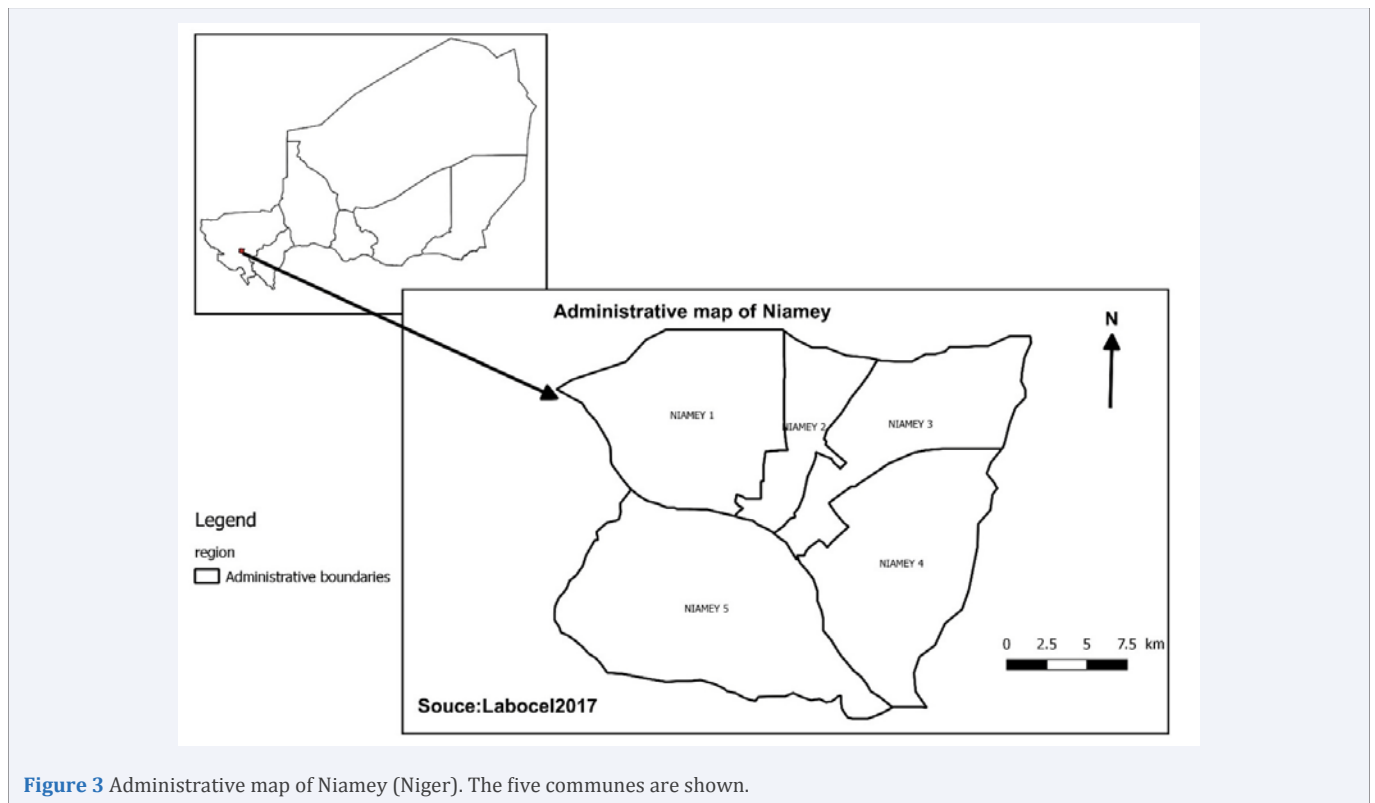


Figure 3 Administrative map of Niamey (Niger). The five communes are shown.

combined with nitroterazolium blue chloride, Sigma-Aldrich). Positive sera should show a specific pattern of reactive bands at 110, 98, 95, 60/62, and 48 kDa.

Data analysis

The results were recorded as positive or negative and compiled in an Excel database. The c-ELISA results were validated by CIRAD Excel worksheet and STATA software (Stata Corp. 2009. Stata Statistical Software: Release 11.0) was used for data analysis. A positive herd was defined as any herd that had at least one animal positive to c-ELISA and/or CFT. The CBPP individual seroprevalence was estimated by comparing the number of positive sera (c-ELISA or CFT) to the number of sera tested. A confidence interval (CI) of 95% and a significant level of 0.05 (5% level) were used. The map was developed using the Q-GIS (version 2.18.14) software.

RESULTS AND DISCUSSION

This work presents the first study on the seroprevalence of CBPP in the region of Niamey using c-ELISA and CFT as first line tests for infection screening. The study included 912 sera randomly selected from Communes 1, 2, 4 and 5 and 75 sera belonging to a defined cattle CBPP outbreak. Concerning the seroprevalence study, the c-ELISA results showed an individual CBPP seroprevalence of 6.8% (62 sera out of 912 were positive; 95% CI, 5.34–8.4), with no significant difference between the communes (Table 2), while herd seroprevalence was 58.14% (95% CI, 42.13% – 72.99%). Regarding CFT data, 1.64% of the analyzed cattle sera were positive (15/912; 95% CI, 0.8–2.5) but significantly different ($P=0.005$) results were obtained for different communes, with percentages varying from 0.0%, 0.97%

(95% CI, 0.45– 2.11) and 1.19% (95% CI, 0.03– 6.46) to 4.76% (95% CI, 2.08–9.17), for communes 2, 5, 1 and 4, respectively (Table 3). Herd prevalence estimation using CFT was 18.6% (95% CI, 8.39–33.40).

The seroprevalence results obtained in this work were higher than those found by Bloch and Diallo [22], that used CFT to analyze 400 sera from unvaccinated cattle belonging to herds from several regions in the country; their study gave a prevalence level of 3.7%; 15 out of 400. However, our results were lower than those found by Sidibé et al. (2012) [7] and Sery et al., (2015) in Central Niger Delta (Mali), who obtained levels of 14.1% and 18.11% [23], respectively, using c-ELISA test.

Competitive ELISA was more sensitive than CFT detecting 62 positive sera while CFT only detected 15 positive sera ($\chi^2=9.502$; $P=0.025$). These results were in agreement with previous studies performed by Le Golf and Thiaucourt (1998), and Muuka and colleagues (2011) [4,6]. However, we noted a higher sensitivity of CFT compared to c-ELISA as described by Marobela-Raborokgwe and colleagues (2003) [24]. From the 912 sera tested only four were both positive in c-ELISA and in CFT, revealing a reduced concordance between the two tests. The 77 positive sera in c-ELISA and/or CFT were further tested by IBT and 18.2% (14/77) were confirmed positive. From the 14 IBT positive sera, 64.3 % (9/14) were CFT positive; in contrast, only 35.7 % (5/14) of sera were both IBT and c-ELISA positive. The data analysis showed a statistically significant difference ($P<0.0001$) between IBT and c-ELISA or CFT results (Table 4,5). In fact, previous studies demonstrated that IBT shows a higher specificity than c-ELISA or CFT enabling the detection of false positive reactions [25].

Table 2: Individual seroprevalence of CBPP using c-ELISA in different communes of Niamey, Niger.

Communes	Number of Sample	Number of positive sera	Prevalence %	95% CI	P-value
Niamey 1	84	8	9.52	4.20–17.91	0.172
Niamey 2	44	0	0	---	
Niamey 4	168	9	5.36	2.48–9.93	
Niamey 5	616	45	7.31	5.50–9.64	

Abbreviations: CI: Confidence Interval

Table 3: Individual serological prevalence using CFT of CBPP in different township of Niamey, Niger.

Communes	Number of Sample	Number of positive sera	Prevalence %	95% CI	P-value
Niamey 1	84	1	1.19	0.03–6.46	0.05
Niamey 2	44	0	0	---	
Niamey 4	168	8	4.76	2.08–9.17	
Niamey 5	616	6	0.97	0.45–2.11	

Abbreviations: CI: Confidence Interval

Table 4: Comparison of seroprevalence at animal and herd-level using CFT and c-ELISA.

Tests	Sample size	Prevalence %	Std Err	95% CI	P-value
Animal-level.					
c-ELISA	912	6.80	0.008	0.0516–0.0843	<0.0001
CFT	912	1.64	0.004	0.008–0.025	
Difference	---	5.16	0.009	0.033–0.070	
Herd-level					
c-ELISA	43	58.14	0.075	0.434–0.729	<0.0002
CFT	43	18.60	0.059	0.067–0.302	
Difference	---	39.54	0.096	0.207–0.583	

Abbreviations: CI: Confidence Interval

Table 5: Comparison of cross-tabulation of IBT with CFT and c-ELISA.

Tests	IBT+	IBT-	Total	P-value
c-ELISA +	5	57	62	<0.0001
CFT+	9	6	15	
Total	14	63	77	

Abbreviations: c-ELISA: competitive enzyme-linked immunosorbent assay; CFT: complement fixation test; IBT: immunoblotting test

Regarding the 75 sera from the 2016 outbreak, 73.3% (55/75) and 62.7% (47/75) were positive in c-ELISA and CFT, respectively. The IBT results confirmed the infection in 45 out of the 75 cattle (60.0%), where characteristic clinical signs were also seen. There was 95.5% (43/45) concordance between c-ELISA and IBT and 84.4% (38/45) between CFT and IBT. Although no bacteriological analysis for agent isolation was performed, the gross pathological lesions observed, highly suggestive of CBPP, and the serological results confirmed the CBPP outbreak.

The OIE recommends the use of CFT and c-ELISA in parallel for the testing of CBPP. Generally, c-ELISA is the most used test to assess accurately the CBPP status of a region due to its facility of execution, easy interpretation and validity of the components; but it must be stressed that in fact neither ELISA

or CFT can independently detect all the positive animals in a herd during an outbreak [6,7]. How well either test performs depends on the evolution and stage of the disease, and the type of immunoglobulins present at the moment of sera collection.

Considering the endemic status of the region, these results were expected since c-ELISA detects mainly cattle in chronic stages of the disease while CFT is able to detect early stages of the infection [4,5,26]. Ideally, c-ELISA and CFT should be used in parallel for confirmation of CBPP, as the sensitivity and specificity of the two tests depends on the stage of the disease [6,7,24–27]. Still, although more laborious, a confirmation of the doubtful and positive results by IBT is necessary for ultimate diagnosis of the infection [5].

The serological study presented here gave an overview on the seroprevalence of the CBPP in the region of Niamey and was the first step to initiate a surveillance program in Niger. In fact, in the framework of the revitalization of livestock, the Laboratoire Central de l'Élevage (LABOCEL) with the collaboration of the project "Projet Regional d'Appui au Pastoralisme au Sahel (PRAPS)", supported by the World Bank, a National program was started to evaluate the serological status of cattle concerning CBPP in order to provide measures for the eradication of the disease in Niger. Therefore, in 2017, a nationwide seroprevalence study was performed in 1590 cattle sera from six regions, showing an individual CBPP seroprevalence of 4.15% using c-ELISA [19].

In West Africa, the Fulani tribe, practiced for a long time the traditional vaccination of cattle with a lung lesions macerated, by subcutaneously inoculation on the nose. In Europe during the 19th century, this inoculation was done at the tail of the animal [12,28]. The ethno-veterinary Fulani pastoralists' practices in Nigeria are a good example of vaccine prophylaxis in Africa. The lung tissues from infected dead cattle with CBPP were soaked in fresh milk and briefly placed on the nose of healthy cattle or wrapped in a rag and hung on a tree very close to the herd site. Also ground dried infected lungs have also been used to vaccinate herds [13].

CBPP is one of the diseases for which the OIE has established a formal status recognition procedure. The OIE Terrestrial Animal Health Code describes the steps that a country must follow to be officially recognized by the OIE as being free from CBPP. Control strategies are mostly based on early detection of the outbreaks, control of animal movements, vaccination of animals and a stamping-out policy. The application of these strategies would be possible only if we have accurate data on the prevalence and distribution of the disease. Given the endemic nature of CBPP in West Africa, the main strategy is to perform mass annual vaccination and sero-monitoring.

CONCLUSION

CBPP continues to be endemic in West Africa with the frequent occurrence of sporadic outbreaks. The disease data in Niger was essentially based on outbreak information. The serological survey in Niamey region using c-ELISA revealed an individual seroprevalence of 6.8%. The c-ELISA was found to be more sensitive than CFT confirming the endemic status of this area. In order to eradicate this disease, a synergy of forces is being set up in West Africa through regional projects, including vaccination with T_{1/44} strain and serological-monitoring.

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