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Using sero-positivity to assess geospatial burden of contagious bovine pleuropneumonia on pastoral cattle herds of north-central Nigeria

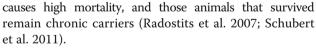
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Abstract

Contagious bovine pleuropneumonia (CBPP) is an infectious disease of cattle endemic in sub-Saharan Africa. A cross-sectional sero-geospatial survey was conducted to assess the CBPP sero-geospatial burdens among estimate at-risk sero-positive cattle in nomadic and sedentary pastoral cattle production systems of north-central Nigeria, between January and August 2013. A total of 765 cattle in 125 nomadic herds and 375 cattle in 125 sedentary herds were sampled. Sera were analysed using c-ELISA. OpenEpi version 2.3 was used for statistical analyses. Geo-coordinates of herds were taken using Garmin GPS, while ArCGIS 9.3 was used to map geospatial data of sero-positive cattle and herds in the agro-geographical zones. In nomadic production, 16.2 % (95 % CI 13.7 to 19.0.) cattle were sero-positive in 47.2 % (95 % CI 38.2 to 56.3) herds. And the sedentary system had 9.6 % (95 % CI 6.9 to 12.0) sero-positive cattle in 27.2 % (95 % CI 19.6 to 35.9) herds. Agro-geographical zone A was more likely (OR 3.42; 95 % CI 1.90, 6.15) to have significant impacts on cattle-level sero-geospatial burden than Agro-geographical zone B. Also, Agro-geographical zone C was more likely (OR 5.14; 95 % CI 2.91, 9.08) to have significant impacts on cattle-level sero-geospatial burden than Agro-geographical zone B. The developed GIS CBPP risk maps showed various densities of its burdens in the agro-zones. The visualized proportional circle maps presented GIS usefulness in the active surveillance of CBPP, and if used in conjunction with sero-diagnosis, the maps would aid policymakers with practical imageries for livestock disease control decisions in pastoral cattle herds.

Background

Contagious bovine pleuropneumonia (CBPP) is an important infectious and contagious disease of cattle in sub-Saharan Africa, caused by *Mycoplasma mycoides* subsp. *mycoides* (*Mmm*) (Manso-Silván et al. 2009; Tardy et al. 2011). It is characterized by sero-fibrinous interlobular oedema and hepatization giving a marbled appearance to the lung in acute and sub-acute cases and capsulated lesions (sequestra) in the lungs of chronically infected cattle (Radostits et al. 2007; Schnee et al. 2011; Tardy et al. 2011). The disease is transmitted by direct contact between infected and susceptible cattle (Tambi et al. 2006; Vilei and Frey 2010). Once it is introduced into a naïve cattle herd, it



CBPP is regarded as the most serious infectious disease affecting cattle in sub-Saharan Africa (Amanfu 2009; Marobela-Raborokgwe 2011) and impacts animal health and poverty of livestock-dependent people, especially the livestock pastoralists, through decreased animal productivity, reduced food supply for households, and the cost of control measures (Windsor 2000; Tambi et al. 2006; Jiuqing et al. 2011). The disease constitutes a barrier to trade in many African countries due to reduction in the value of livestock and the income of many value chain stakeholders (Nicholas et al. 2008; Jores et al. 2013).

The advancement of science and technologies for Global Positioning Systems (GPS) and Geographical Information Systems (GIS), a computerized system that



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combines spatial and descriptive data for mapping and analysis, has enabled expanded identification of areas with increased risks and formulation of hypotheses about diseases (Moore and Carpenter 1999; Lai et al. 2008; Pfeiffer et al. 2008; Tatem et al. 2012). However, this expansion in developing countries, such as Nigeria, has not been exploited in the development of spatial datasets on infectious animal diseases (Jebara 2007; Thrusfield 2009; Molla and Delil 2015).

The decline in CBPP reports on outbreaks and impacts in Nigeria and other affected African countries was due to the absence of science-based evidence for disease burden and distribution, which does not augur well for the implementation of internationally coordinated control programmes as it underpins control and preventive actions (Amanfu 2009). Also, cattle managed under pastoral extensive systems are consistently at risk of contracting contagious infectious diseases, including CBPP, due to continuous mixing of herds at grazing and watering points (Alhaji 2011).

Currently, there is a paucity of documented evidencebased information on the geospatial burden of CBPP in pastoral cattle herds under different climatic conditions in Nigeria. Availability of such science-based information on burden and geographical factors would assist in the development of surveillance and control strategies for the disease in pastoralists' settlements in Nigeria. This study was, therefore, aimed to assess geospatial burden of CBPP among nomadic and sedentary pastoral cattle populations of Niger State, north-central Nigeria, through serology, GPS, and GIS techniques. Our null hypothesis was that agro-geographical zones cannot impact on sero-geospatial burden of CBPP in pastoral cattle herds of the State; and also, there was no relationship between the two pastoral cattle production systems. The herds were under zero vaccination status before the survey because the last CBPP vaccination campaign in the State was conducted in November 2011. The T1/44 vaccine used in Nigeria has limited efficacy as the immunity conferred is of short duration and vaccination must be repeated annually (Thiaucourt et al. 2000). For vaccination to be effective, it must be repeated initially at short intervals of six months and thereafter annually over three to five years (FAO 2002; FAO 2004).

Study area

Niger State is located geographically in the southern Guinea Savannah ecological zone, in the north-central geopolitical area of Nigeria, between latitudes 8° 20′ N and 11° 30′ N and longitudes 3° 30′ E and 7° 20′ E. It is one of the 36 states of Nigeria and covers a land area of about 76,363 km² (29,484 square miles) or about 9.8 % of Nigeria's total land area, making it the largest in terms of land mass in the country (www.nigerstate.gov.ng). Accordingly, Niger State has an estimated cattle population of about 2.4 million cattle in 2012, mainly Bunaji, Rahaji, and Bokoloji breeds, in nomadic and sedentary pastoral management systems (MLFD 2013).

The study was conducted in the three existing agrogeographical zones of the State (Figure 1), which have variable climatic conditions. These are Agro-geographical zone A or Southern zone with eight local government areas (LGAs) and many river bodies and fadamas (low land water logged muddy areas, with small streams, used mainly for rice farming during rainy season and for cattle grazing during dry season); Agro-geographical zone B or Eastern zone with nine LGAs, many mountains, trees, and few rivers; and Agro-geographical zone C or Northern zone with eight LGAs, large grazing areas, many stock routes, and a porousinternational border with the Republic of Benin. The State experiences two distinct seasons, rainy season that spans between April and October and dry season between November and March. It has a mean annual rainfall of about 1,600 mm, humidity of 104 %, and average lowest and highest temperatures of about 27 °C and 39 °C, respectively (MLFD 2013).

Materials and methods

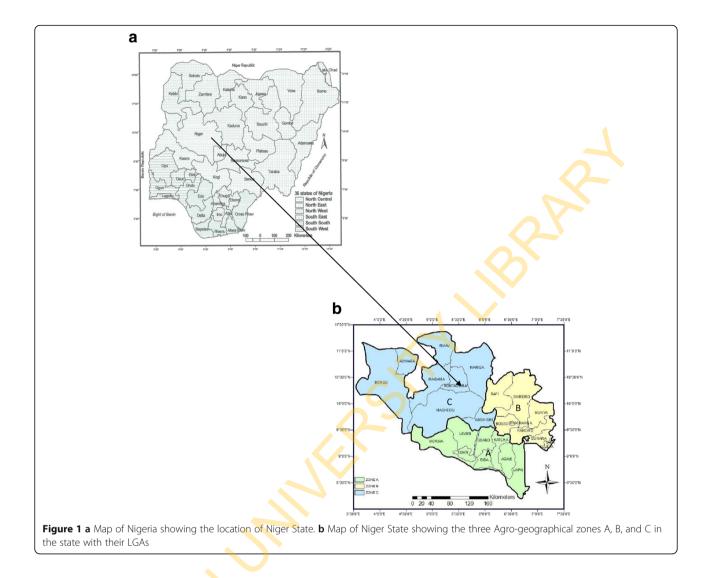
Study design, populations, and definitions

A cross-sectional sero-geospatial survey was carried out between January and August 2013. It involved blood sample collection from cattle in nomadic and sedentary pastoral cattle herds and disease mappings of sero-positive herds. The target populations were the nomadic and sedentary pastoral herds, and their cattle of all ages, sexes, and breeds domiciled in the State during the period of the survey.

For the purpose of this research, a nomadic pastoral cattle herd was defined as a herd in the Fulani ethno-cultural group that keeps mainly cattle, has a large herd size, and has all-year-round movements with large-range grazing and watering and with no permanent homestead. Asedentary pastoral (agro-pastoral) cattle herd was defined as a herder that keeps more cattle and cultivates few crops, a herd which is average of 50 cattle per herd size, is semisettled, has limited movements, and is on low-range grazing. The herd is often given supplementary feeds of crop residues, particularly during the critical dry season period.

Sample size and sampling procedure

For cattle, a simple random sampling method (Thrusfield 2009) was used and sample size was determined using expected CBPP prevalence of 8.7 % (Alhaji 2011) at 95 % confidence level. One hundred and twentyfive nomadic herds and 125 sedentary herds were purposely selected across the State. Sample size for the nomadic cattle was determined at 2 % margin of error, giving a size of 765 cattle; and that for sedentary cattle was determined at 3 % desired absolute precision, giving a size of 375 cattle.



A two-stage sampling procedure was used. In the first stage, herds were selected in the three existing agrogeographical zones (AGZs) using a purposive sampling procedure because sampling frames for them could not be obtained at the time of the survey due to their scattered nature. In the southern and eastern AGZs, 40 nomadic and 40 sedentary herds were selected in each, while 45 herds from each of these management systems were selected in the northern AGZ. In the second stage, seven nomadic cattle and three sedentary cattle were randomly selected and sampled in each of the respective herds, and a total of 765 nomadic and 375 sedentary cattle were sampled by card balloting, respectively.

The study protocol for the survey was approved by the Niger State Ministry of Livestock and Fisheries Development, Minna Research Ethics Committee (reference MLFD/NGS/671).

Serum collection and enzyme immunoassay

Ten millilitres of whole blood was taken from the jugular vein of each selected cow, using a sterile 10-ml syringe and $18 \times 1\frac{1}{2}$ in. gauge needle for each animal. These were immediately placed into an ice bath slanted and transported to the laboratory within seven hours. The clot was allowed to form in the syringe in the field before transportation. The sera were later transferred into plastic tubes and centrifuged at 3,000 rpm for 20 min and then decanted into cryovials, which were identified before storage at -20 °C until analysed.

Commercial *M. mycoides* subsp. *mycoides* (*Mmm*) Antibody Test Kit CBPP, ELISA version: P05410/02 (Institut Pourquier-CIRAD/IDEXX Laboratories, Montpellier, France), was used according to the manufacturers' instructions for serological analysis. Only samples with percentage of inhibition (PI) \geq 50 % were considered positive for the presence of *Mmm* antibodies.

The c-ELISA detects antibodies to CBPP even at chronic stage (FAO 2003).

Geospatial data collection and mapping

Geo-coordinates of the selected 125 nomadic and 125 sedentary herds were taken and recorded. The vector data were collected using a hand-held Garmin *eTrex* GPSTM receiver and stored before they were transferred into Excel spreadsheets.

Electronic maps of Niger State were obtained from the Niger State Geographical Information System Office, Minna. All the maps were geo-referenced to real-world coordinate system with respect to known reference points, based on latitude, longitude, and WGS 84 datum. Spatial data were digitized using ArcGIS 9.3 software (Environmental Systems Research Institute (Esri), Inc., Redlands, USA) and coverage edited to remove digitization errors such as overshoot, undershoot, dangles, and labels for plotting herd sites (locations). Database was generated based on CBPP sero-positivity information in Excel spreadsheets and linked with the vector layers.

The coordinate data were pooled from the database files (DBF) in Excel spreadsheets to plot locations of all risk herds on Niger State digital maps geo-referenced in the GIS platform. Images on-screen were digitized through the Windows operating environment of ArcGIS under the following three main themes: line boundaries of the 25 LGAs and the three AGZs joining together (polygons), CBPP risk herd locations (points), overlapped by the number of cattle sero-positive of the disease (proportional circles). Data were attached into the polygons of the LGAs and AGZs using common identifiers in the software. Query tool of ArcGIS was used to identify the proportions of cattle sero-positive of the disease overlaying each risk herd location under each agro-geographical zone.

Data management and analyses

Collected data were summarized and entered into a Microsoft Excel 7 spreadsheet and stored. Open Source Epidemiologic Statistics for Public Health (OpenEpi) version 2.3.1 (Dean et al. 2009) was used for the statistical analysis. Descriptive and analytical statistics were used to describe the obtained data. In the descriptive analysis, frequency and proportion were used. Associations of impacts of agro-geographical zones and sero-geospatial burden of CBPP on pastoral cattle production systems were examined by univariate analysis using chi-square tests (X^2). Further, likelihood stepwise backward multivariate logistic regression models were used to determine final associations on only factors that were significant at p < 0.05 during univariate analysis. Outcomes with p < 0.05 were considered statistically significant in all analyses. The goodness of fit of the model was assessed using the Hosmer-Lemeshow test statistic. Variables remained in the model if they significantly improved the fit (p < 0.05).

Statistical significant relationship of the two pastoral cattle populations was also determined by comparing the confidence intervals of their cattle-level sero-positivity. When confidence intervals of two population proportions are compared and did not overlap, their differences are considered to be statistically significant (Schenker and Gentleman 2001).

The analysis of attribute data using GIS can be broadly categorized into visualization, exploration, and modelling (Pfeiffer and Hugh-Jones 2002). In this study, visualization was used to present proportional circle CBPP maps in herds at risk of the disease. A herd was considered positive if at least one cattle in it was sero-positive on c-ELISA test. Only coordinates of herds with sero-positive cattle were used in the mapping.

Results

Sero-geospatial burden distribution of CBPP in nomadic pastoral cattle and herds

Of the 765 cattle tested in pastoral nomadic herds, 16.2 % (124/765; 95 % CI 13.7, 19.0) were sero-positive of CBPP. The overall nomadic herds with CBPP sero-positive cattle in the nomadic production system were 47.2 % (59/125; 95 % CI 38.2, 56.3) (Table 1). The sero-geospatial burden distribution pattern of nomadic herds at risk of CBPP in the Agro-geographical zones is shown in Figure 2. It was apparent from the background proportional circle map imagery that herds at risk of CBPP were more sero-spatially distributed in Agro-geographical zones C and A than in Agro-zone B (Figure 2).

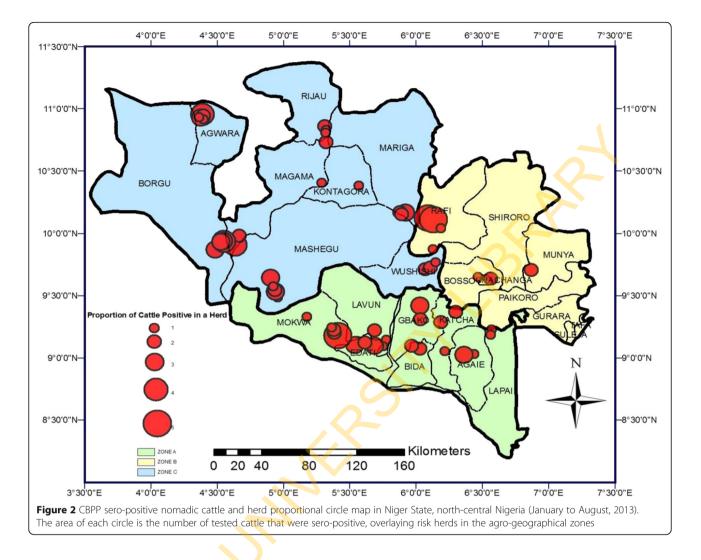
Sero-geospatial burden distribution of CBPP in sedentary pastoral cattle and herds

Of the 375 cattle tested in sedentary pastoral herds, 9.6 % (36/375; 95 % CI 6.9, 12.0) were sero-positive of the

Table 1 Sero-geographical distribution of CBPP in nomadic cattle and herds of Niger State, north-central Nigeria: 2013

Agro-geographical zone	No. of cattle sampled	No. +ve	% +ve (95% Cl)	No. of herds sampled	No. +ve	% +ve (95%Cl)
A (Southern)	245	45	18.4 (13.9, 23.6)	40	23	57.5 (40.9, 73.0)
B (Eastern)	275	17	6.2 (3.8, 9.5)	45	7	15.6 (6.5, 29.5)
C (Northern)	245	62	25.3 (20.2, 31.0)	40	29	72.5 (56.1, 83.4)
Overall	765	124	16.2 (13.7, 19.0)	125	59	47.2 (38.2, 56.3)

Note: No. - number; +ve - positive; -ve - negative; CI - confidence interval, % -percentage



disease. The overall sedentary herds with CBPP seropositive cattle were 27.2 % (34/125; 95 % CI 19.6, 35.9) (Table 2). Sero-geospatial burden distribution pattern of sedentary herds and cattle at risk of CBPP in the Agro-geographical zones is shown in Figure 3. However, it was further apparent from the background proportional circle map imagery that herds at risk of the disease were more sero-spatially distributed in Agro-geographical zones C and A than in Agro-zone B (Figure 3).

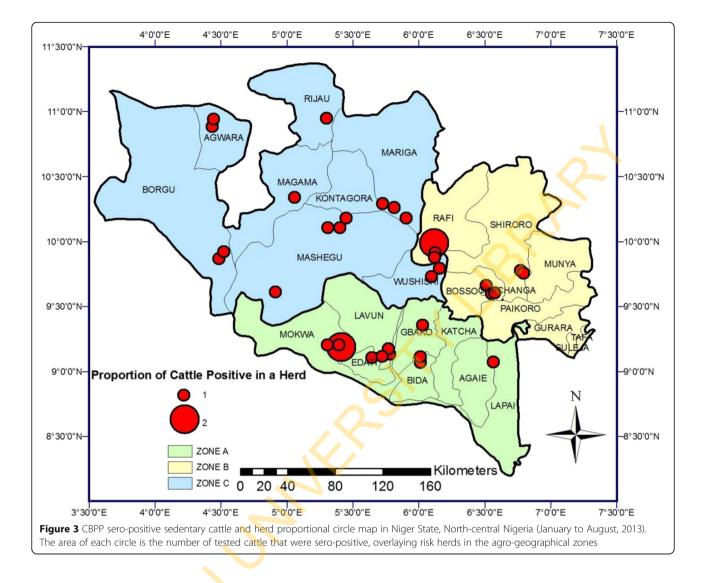
Association of agro-geographical zone impacts and sero-geospatial burden of CBPP in the nomadic pastoral production system

At the univariate analysis in the nomadic production system, there were significant impacts of agro-geographical zones on CBPP sero-geospatial burden ($X^2 = 36.13$; p < 0.001). At the herd level, there was also significant association between impacts of agro-geographical zones and CBPP sero-geospatial burden ($X^2 = 30.06$; p < 0.001). At the multivariate regressions, Agro-geographical zone A

Table 2 Sero-geographical distribution of CBPP in sedentary cattle and herds of Niger State, north-central Nigeria: 2013

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Agro- geographical zone	No. of cattle sampled	No. +ve	% +ve (95% Cl)	No. of herds sampled	No. +ve	% +ve (95%Cl)
A (Southern)	120	12	10.0 (5.5, 16.4)	40	11	27.5 (14.6, 43.9)
B (Eastern)	135	9	6.7 (3.3, 11.9)	45	8	17.8 (8.0, 32.1)
C (Northern)	120	15	12.5 (7.5, 19.4)	40	15	37.5 (22.7, 54.2)
Overall	375	36	9.6 (6.9, 12.9)	125	34	27.2 (19.6, 35.9)

Note: No. - number; +ve - positive; -ve - negative; Cl - confidence interval, % - percentage



was more likely (OR 3.42; 95 % CI 1.90, 6.15) to have significant impacts on cattle-level sero-geospatial burden than Agro-zone B. Also, Agro-geographical zone C was more likely (OR 5.14; 95 % CI 2.91, 9.08) to have significant impacts on cattle-level sero-geospatial burden than Agro-zone B (Table 3).

Association of agro-geographical impacts and sero-geospatial burden of CBPP in the sedentary pastoral production system

At the univariate analysis in this production system, there was no significant association between agro-geographical zone impacts and CBPP sero-geospatial burden at

Table 3 Multivariate logistic regressions of associations of agro-geographical zones impacts with CBPP sero-geospatial burdens in nomadic cattle population of Niger State, north-central Nigeria: 2013

		-	-					
Variable	No. of cattle -ve	No. of cattle +ve	Odds ratio (95% CI)	P-value	No. of herds -ve	No. of herds +ve	Odds ratio (95% Cl)	P-value
Zone B (Eastern)	258	17	Ref.		38	7	Ref.	
Zone A (Southern)	200	45	3.42 (1.90, 6.15)	0.001*	17	23	7.35 (2.65, 20.39)	0.001*
Zone C (Northern)	183	62	5.14 (2.91, 9.08)	<0.001*	11	29	14.31 (4.94, 41.46)	<0.001*

Note: No. – number; +ve – positive; -ve – negative, Cl – confidence interval, *statistically significant at p < 0.05

both the cattle level ($X^2 = 2.52$; p = 0.29) and herd level ($X^2 = 2.04$; p = 0.36).

Relationship between nomadic and sedentary pastoral cattle populations

The relationship between the nomadic and sedentary pastoral cattle populations, in terms of husbandry management systems, was assessed. The overall cattle-level sero-positivity confidence intervals of the two populations were compared and found not to overlap (Tables 1 and 2) and therefore significantly different populations. This indicated that the two populations were independent of one another, due largely to variations in husbandry systems, although the predisposing factors that influenced CBPP occurrence under each system in the agro-zones were likely to be the same.

Discussion

This study has shown that GIS techniques can be used to provide descriptive quantitative proportional circle maps of CBPP burden, which are useful for promotion of surveillance of the disease in pastoralists' settlements of Nigeria. One of the most powerful benefits of GIS has been its ability to integrate different spatial databases into a single environment for possibilities of improving surveillance and control programmes for infectious diseases and zoonoses (Longley et al. 2005; Rinaldi et al. 2006; Haghdoost et al. 2007).

In this survey, a total of 93 cattle herds were involved in the proportional mappings of CBPP in the two pastoral production systems. The mappings were done based on 160 sero-positive cattle data. The c-ELISA used was developed by the OIE Collaborating Centre for the Diagnosis and Control of Animal Diseases in Tropical Countries and validated in several African and European countries. It has true specificity of at least 99.9 % and sensitivity of 63.8 % to 70 % (Le Goff and Thiaucourt 1998; OIE 2008). The test detects exposures to only natural infections without cross-reactions with other induced antibodies by similar antigens (OIE 2014). The overall cattle-level CBPP seropositive burdens in nomadic and sedentary pastoral herds were 16.2 % and 9.6 %, respectively. The proportion of geo-referenced nomadic herds at risk of the disease was 47.2 %, and that of the sedentary herds was 27.2 %. This study shows that CBPP is endemic in pastoral communities of north-central Nigeria.

The proportional circle imageries served as visualized descriptive augmentations to the estimated sero-positivity in the herds. The survey has shown variable spatial distribution burdens of the disease in the agrogeographical zones. This suggests that risk factors for the disease are spatially distributed and varied from one agrogeographical zone to another. On visualization, densities of CBPP sero-geospatial burdens were high across AGZs C and A in both pastoral production systems compared to AGZ B. These distribution patterns may have occurred due to high cattle population densities and many transboundary stock routes that traverse through the zones (MLFD 2013), contagiousness of CBPP, and many point exposures at watering and grazing available in the zones (Alhaji 2011). The high sero-geospatial burden distribution patterns obtained in the two production systems, which are invariably extensive in nature, are in agreement with the previous reports that the extensive nature of the husbandry systems exposed stocks to different geographical factors, which exacerbate CBPP occurrence (Nawathe 1992; Nwanta and Umoh 1992; Jiuqing et al. 2011). The higher CBPP sero-geospatial burden in the nomadic system than in the sedentary system is in consonance with previous findings that burden of CBPP varies according to the cattle management system and tends to be higher in more extensive cattle husbandry systems (Windsor 2000).

The observed high sero-geospatial burdens of the disease visualized in AGZs C and A in the two production systems cannot be unconnected with the fact that the two zones have many factors that predisposed to CBPP occurrence, with high concentrations of pastoral cattle herds, many stock routes for transits of cattle on seasonal movements from northern Nigeria to the southern parts, and high close contacts of cattle at grazing and watering points as a result of available pastures especially during dry seasons. Furthermore, AGZ C has a common international border with the Republic of Benin that is porous, which promotes interactions of infected and susceptible cattle. Ecological factors that have to do with availability of water and grazing pastures have been reported to lead to increased movement of cattle over long distances to the areas in search of pastures and water during dry seasons, with resultant exacerbation of the disease due to stress (Amanfu 2009). Similarly, Teshale et al. (2015) has reported a significant association of CBPP sero-positive impacts with agro-ecology in the Southern Zone of Tigray Regions, Northern Ethiopia.

There was an observed significant difference between the two production systems, indicating that each was independent of another. Therefore, the agro-geographical factors that predisposed to the visualized high sero-geospatial CBPP burden in the nomadic production system were due to its more extensive nature of husbandry practices compared to the sedentary system. The ability of GIS to express spatial dataset variability in different groups has facilitated analysis of diseases in risk maps (Hay et al. 2009).

Veterinary epidemiologists are adopting these new techniques to study a variety of animal and zoonotic diseases (Kshirsagar et al. 2013). These quite uncommonly utilized techniques are not known to have been previously applied in the area of CBPP epidemiology in Nigeria. Therefore, results presented in this survey are of

both practical and scientific value. Sero-geospatial burden distribution knowledge obtained in this study would valuably help to further understand geospatial epidemiological influence of CBPP in cattle populations across Nigeria and some other affected African countries. All these can become important insights for surveillance and control programmes. Visualized maps showing locations of herds at risk of CBPP can help in better drawing of surveillance zones as well as available facilities to implement decided control measures, as previously described (Durr and Gatrell 2004; Jebara 2007).

Although the use of GIS in animal health studies is not entirely new in south-west Nigeria (Babalobi 2007; Adeyemo and Babalobi 2008), its application in livestock diseases in other regions of the country is still rudimentarily reported. To our knowledge, there has not been any spatial dataset generated to present visualized burden of CBPP in Nigeria. Generation of such data would be a better source of visual information that would assist in the development of surveillance and control strategies for the disease in the country. It is very important to explore the potential risk areas on a map rather than in tabular form to assist policy-makers, livestock officers, farmers, and other stakeholders, as reported previously (Rytkönen 2004; Longley et al. 2005).

One limitation of this study is the use data obtained from a cross-sectional study with participating pastoral cattle herds purposively selected due to their mobile nature. For spatial analysis, spatial systematic sampling or stratified random samplings based on knowledge of spatial structure are sometimes more appropriate. Also, we could not fully adjust for clustering in the designed random sampling for the studied cattle. However, the used of central tendency measures would be valuable enough to tolerate the likely imperfections in the confidence intervals.

Conclusions

This study presented a visual map to describe serogeospatial burdens of contagious bovine pleuropneumonia in pastoral cattle herds of Niger State. Our findings suggest that sero-spatial maps of the disease should be institutionalized as an element of active surveillance and control strategies. The visualized proportional circle maps in the Agro-geographical zones using Global Positioning Systems and Geographical Information Systems have shown areas at risk of the disease, showing the devices' usefulness in assessment of contagious bovine pleuropneumonia burdens in pastoral cattle herds. Application of disease risk maps becomes imperative to help in target surveillance and control activities. Routine production of these maps for contagious bovine pleuropneumonia and other infectious animal diseases in Nigeria has the potential to provide a more realistic active surveillance using visual pictures of different disease situations. Used critically and in conjunction with other tools such as sero-diagnosis, the maps would aid policy-makers with practical imageries for livestock disease control decisions in pastoral cattle herds.

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Authors' contributions

NBA conceived of the study, collected and analysed the serological data, and drafted the manuscript. OOB participated in its design and helped draft the manuscript. SS analysed the geo-referenced data. All authors read and approved the final manuscript.

Authors' information

NBA was a doctoral fellow on preventive veterinary medicine and participatory epidemiology at the University of Ibadan (Nigeria). The manuscript is part of his doctoral thesis on 'Epizootiology of contagious bovine pleuropneumonia in the pastoral communities of Niger State, Nigeria'. OOB is a Consultant Epizootiologist and doctoral supervisor of NBA. SS is the head of the Bemote Sensing Unit, Department of Geography, Federal University of Technology, Minna (Nigeria).

Competing interests

The authors declare that they have no competing interests.

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