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SURVEY OF CONTAGIOUS BOVINE PLEUROPNEUMONIA IN TRADE CATTLE SLAUGHTERED AT ABATTOIRS IN NORTH-CENTRAL NIGERIA

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Abstract

Contagious bovine pleuropneumonia (CBPP) is one of the most important infectious and contagious disease of cattle in sub-Saharan Africa and whose control is urgently needed. A cross-sectional study was conducted to investigate prevalence of CBPP and associated animal factors that predisposed to the disease in trade cattle slaughtered in Niger State, North-central Nigeria between January and May 2014. A total of 525 trade cattle were selected by systematic sampling approach from five purposively selected central abattoirs in the State. Serum samples were analyzed using c-ELISA and lung lesions determined at post-mortem inspections. The OpenEpi 2.3.1 software was used for statistical analyses. Descriptive and analytical statistics were used to present the results. Associations between animal characteristics (predictor variables) and sero-positivity as well as presence of CBPP lung lesions (outcome variables) were tested using Chi-square tests and likelihood backward logistic regressions. A sero-prevalence of 31.8% (95% CI: 27.93, 35.89) in live cattle before slaughtered was observed. Also, 29.5% (95% CI: 25.74, 33.53) of the slaughtered trade cattle had CBPP lung lesions at post-mortem inspections. Cattle in age group 4–5 years were more likely (OR 2.00; 95% CI: 1.17, 3.39) to be significantly exposed to Mycoplasma mycoides subsp. mycoides (Mmm) infection. Cows were more likely to be significantly predisposed to Mmm infections. Furthermore, bunaji breeds were more likely (OR 2.26, 95% CI: 1.42, 3.59) to be significantly predisposed to Mmm infections. This study has shown the need to combine more reliable serological tests with post-mortem examinations to improve active surveillance of CBPP in trade cattle. These dual approaches to investigation of CBPP and identification of intrinsic factors predisposing to infection should be institutionalized as elements of epidemio-surveillance and control strategies of the disease in sub-Saharan Africa.

Keyword: Abattoir, CBPP, c-ELISA, lung-lesions, trade cattle, Nigeria.

ÉTUDE DE LA PLEUROPNEUMONIE CONTAGIEUSE BOVINE CHEZ LES BOVINS COMMERCIALISES ABATTUS DANS LES ABATTOIRS DU CENTRE-NORD DU NIGÉRIA

Résumé

La pleuropneumonie contagieuse bovine (PPCB) est l'une des maladies infectieuses et contagieuses les plus importantes des bovins en Afrique subsaharienne dont le contrôle nécessite une intervention urgente. Une étude transversale a été menée, entre janvier et mai 2014, dans le but d'étudier la prévalence de la PPCB et les facteurs animaux associés prédisposant à la maladie, parmi les bovins commercialisés abattus dans l'État du Niger au centre-nord du Nigeria. Au total, 525 bovins commercialisés ont été choisis par échantillonnage systématique dans cinq abattoirs centraux sélectionnés à dessein dans cet État. Les échantillons de sérum ont été analysés en utilisant l'ELISA de compétition (c-ELISA), et les lésions pulmonaires ont été déterminées en procédant aux inspections post-mortem. Le logiciel OpenEpi 2.3.1 a été utilisé pour les analyses. Des statistiques descriptives et analytiques ont été utilisées pour la présentation des résultats. Les associations entre les caractéristiques (variables de prévision) et la séropositivité des animaux ainsi que la présence de lésions pulmonaires de la PPCB (variables de résultats)

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ont été évaluées en utilisant des tests de Chi-carré et les régressions logistiques de vraisemblance. Avant l'abattage, une séroprévalence de 31,8% (IC 95% : 27,93 ; 35,89) avait été observée chez les bovins. De plus, les inspections post-mortem ont révélé que 29,5% (IC 95% : 25,74 ; 33,53) des bovins abattus avaient des lésions pulmonaires dues à la PPCB. Les bovins âgés de 4 à 5 ans étaient plus susceptibles (OR 2.00; IC 95%: 1,17 ; 3,39) d'être significativement exposés à Mycoplasma mycoides subsp. Mycoïdes (Mmm). De plus, les vaches étaient plus susceptibles d'être significativement prédisposées aux infections à Mmm. En outre, les races bunaji étaient plus susceptibles (OR 2,26, IC 95%: 1,42, 3,59) d'être significativement prédisposées aux infections Mmm. Cette étude a fait ressortir la nécessité de combiner des tests sérologiques plus fiables avec des examens post-mortem, afin d'améliorer la surveillance active de la PPCB chez les bovins commercialisés. Cette double approche d'enquêtes sur la PPCB et l'identification de facteurs intrinsèques prédisposant à l'infection devraient être institutionnalisées en tant qu'éléments de stratégies de surveillance épidémiologique et de contrôle de la maladie en Afrique subsaharienne.

Mots-clés : abattoir, PPCB, c-ELISA, lésions pulmonaires, bovins commercialisés, Nigeria

Introduction

Contagious bovine pleuropneumonia (CBPP) is an infectious and contagious respiratory disease of cattle with high economic impacts in sub-Saharan Africa (Tambi *et al.,* 2006). It is caused by Mycoplasma mycoides subsp. mycoides (Mmm), previously further specified as small colony (SC) type (Manso-Silván *et al.,* 2009), and characterized by sero-fibrinous interlobular edema 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 (Schnee *et al.,* 2011; Tardy *et al.,* 2011). Affected cattle developed dyspnea due to damage to the lungs, and a proportion of them died from respiratory insufficiency (Radostits *et al.,* 2007).The disease is transmitted by direct contact between infected and susceptible cattle (Vilei and Frey, 2010). While CBPP was eradicated from USA in the mid 20th century, it was not eradicated from Europe until the end of the 20th century and still persists in many African countries, especially in the West, Central, East, and parts of Southern Africa (Nicholas *et al.,* 2008), though has not been reported in North Africa (Amanfu, 2009).

CBPP is a major constraint to cattle production in sub-Saharan Africa and is regarded as the most serious infectious animal disease affecting cattle in the continent (Amanfu, 2008; Marobela-Raborokgwe, 2011). The economic impact of this disease is due to huge losses in cattle (Foluso, 2004; Tambi *et al.,* 2006; OIE, 2010). The decline in its outbreaks and burden reports in Nigeria and other affected African countries was due to absence of science-based evidence for disease prevalence, which does not augur well for the implementation of internationally coordinated control programmes as it underpins control and preventive actions (Amanfu, 2009).

Abattoir surveillance has been used in many countries as an important strategy for detection of disease cases and provides essential information that can be utilized for research and disease control purposes (Nunes–Pestica *et al.,* 1990; Nicholas *et al.,* 1996). The use of meat inspection to detect CBPP cases in slaughter facilities is particularly useful in Africa where laboratory capacity for routine disease diagnosis is limited (Phiri, 2006; Cadmus and Adesokan, 2009). Moreover, diagnosis and evaluation of diseases like cysticercosis, *fasciolosis* and CBPP can be made much more reliable at abattoir than other conventional diagnostic methods (Swai *et al.,* 2013). For accurate diagnosis of CBPP, gross pathological changes in the affected organs and tissues (Nunes–Pestica *et al.,* 1990) backed with sensitive and specific serological reactions (Egwu *et al.,* 1996) are required.

Although cattle production is a major economic activity in Nigeria, there is paucity of epidemiological based information on burden and associated predisposing biological factors for CBPP in trade cattle slaughtered at abattoirs in the country, while prevalence of the disease varies from one geographical area

to another. Many cattle traders are unaware of the existence of CBPP in apparently healthy animals at slaughter. Generation of such data would be a convenient and inexpensive source of information that would assist in the development of surveillance and control strategies for CBPP in endemic African countries. This study, therefore, was aimed at investigating abattoir based prevalence of contagious bovine pleuropneumonia in slaughter trade cattle using post-mortem and serological examinations in Niger State, North Central Nigeria. Our Null hypothesis was, that potential animal factors cannot influence occurrence of CBPP in cattle slaughtered at abattoirs. The examined cattle were under zero vaccination status because the last CBPP vaccination campaign in the state was carried out in November 2011. Moreover, 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 3-5 years (FAO, 2002; FAO, 2004).

Materials and methods

Study area

The survey was conducted at five central abattoirs in Niger State, North-central Nigeria. These slaughterhouses are located in Minna (coordinate N09.64655°; E006.54436°), Suleja (N09.13716°; E007.20159°), Bida (N09.08570°; E006.02027°), Kontagora (N1o.41057°; E005.46677°) and New-Bussa (N09.86805°; E004.48343°). Niger State is located in the Southern Guinea Savannah ecological zone of Nigeria, between latitude 8o 20' N and 11o 30' N, and longitude 3° 30' E and 7° 20' E. It is the largest State in Nigeria in terms of land mass, covering an area of 86,000 km^2, representing about 9.3% of the total land area of the country. The State has large grazing areas, many stock routes and an international border with the Republic of Benin, which is porous. It experiences two distinct seasons, rainy season that spans between April and October, and dry season between November and March. The mean annual rainfall is 1600 mm with duration of about 180 days. It has humidity of 104% and average lowest and highest temperatures of about 27°C and 39°C, respectfully. The State has an estimated cattle population of about 2.4 million cattle in 2012 (MLFD, 2013).

Study design and population

A cross-sectional survey was conducted on cattle slaughtered at five slaughterhouses between January and May 2014. It involved blood sample collection from cattle prior to slaughter and post-mortem inspections of the slaughtered cattle from which blood samples were taken. The study population was apparently healthy cattle of ages two years and above, both sexes (bulls and cows), and available breeds (bunaji and bokologi) brought to the abattoirs by livestock traders and livestock keepers for slaughter during the period of the survey.

Selection study abattoirs and trade cattle

A total of five central abattoirs were purposefully selected for the study from the five major towns in the State. Accordingly, 525 cattle were selected from the target populations using systematic sampling procedure with, every 5th cattle examined for slaughter selected. We assumed a comparable number of cattle slaughtered in each of the five abattoirs and took an equal number of 105 cattle in each slaughterhouse. Studied animals, presented asymptomatic, were bled before slaughtered and blood samples taken for sera. Post-mortem inspections involved visual observations, palpations and incisions of the suspected lungs (Phiri 2006).

Post mortem and serum samples collection

Gross pathological lesions on the suspected lungs were recorded after inspection. Serum samples detailed for serological analysis was also collected before slaughtered. Age, sex, breed and CBPP gross lesions of sequestration, hepatisation, marbling, nodulation of lungs, hypertrophy of pulmonary lymph nodes and excessive plural fluid of amber colour were

recorded at post-mortem inspections (OIE, 2001).

For serum, 10 ml of whole blood was taken from jugular vein of each selected cattle, using a sterile 10-ml syringe and 18 × 1½" gauge needle per animal. This was immediately placed into an ice bath slanted and transported to the laboratory within seven hours. The clot was allowed to form in syringe at the lairage before transportation. All daily collected sera were later transferred into plastic tubes and centrifuged at 3000 rpm for 20 min and then decanted into cryovials, which were identified before storage at -20 °C until analyzed.

Serological analysis

A competitive enzyme linked immunosorbent assay (c-ELISA), developed by the OIE Collaborating Centre for the diagnosis and control of animal diseases in tropical countries (Le Goff and Thiaucourt, 1998) was used. It detected antibodies in infected herds even if they persist for a longer period of time (FAO, 2003; Niang *et al.,* 2006). The test performance on CBPP has sensitivity of 99.9 % and greater specificity of more than 63.8 % (OIE, 2014). The c-ELISA was conducted using commercial *M. mycoides subsp. mycoides* (*Mmm*) Antibody Test Kit (CIRAD/IDEXX Institut Pourquier Laboratories, Montpellier, France) according to manufacturers' instructions. Optical densities (ODs) were measured at 450 nm using the Photometer Spectra Fluor (Tecan, Crailsheim, Germany). Only positive serum samples with percentage inhibition (INH%) cutoff value of 50 % and above were considered as positive.

Data management and analyses

Collected data were summarized and entered into Microsoft Excel 7 spreadsheet (Microsoft Corporation, Redmond, WA, USA) and stored. The Open Source Epidemiologic Statistics for Public Health (OpenEpi) version 2.3.1 software (Dean *et al.,* 2009) was used for the statistical analysis. Descriptive and analytical statistics were used to describe the obtained data. Descriptive statistics was used to determine the prevalence of the disease. The associations between individual cattle characteristics (independent variable) with negative or positive serological outcomes and absence or presence of CBPP lesions in lungs (dependent variables) were first investigated using univariable analysis by Chi-square (X^2) tests. All factors found to be biologically plausible and significant were finally subjected to multivariate logistic regressions using likelihood stepwise backward logistic regression models to control for confounding and test for effect modification. Furthermore, $p<0.05$ was considered statistically significant at all analyses.

Ethical clearance

The study protocol was reviewed and approved by the Ethical Clearance Committee of the Niger State Ministry of Livestock and Fisheries Development, Minna, Nigeria. Permissions were obtained from the Area Animal Health Offices in the State to carry out the study at the respective abattoirs.

Results

CBPP sero-prevalence and associated animal factors in trade cattle at ante-mortem examinations

Sero-prevalence of CBPP in trade cattle examined at ante-mortem was 31.8% (167/525; 95% CI: 27.93, 35.89). The sero-prevalence of CBPP in live animals at the five slaughterhouses is presented in Table 1.

All animal factors of age, sex and breed were significantly ($p<0,05$) associated with CBPP sero-positivity during univariate analysis. On subsequent logistic regressions, only cattle in age group 4–5 years were more likely (OR 2.00; 95% CI: 1.17, 3.39) to be significantly exposed to *Mmm* infection. Also, cows were more likely (OR 2.00; 95% CI: 1.34, 2.93) to be significantly predisposed to *Mmm* infection than bulls. However, bunaji breeds were two times more likely (OR 1.72, 95% CI: 1.12, 2.65) to be significantly predisposed to *Mmm* infection than bokoloji breeds (Table 2).

Table 1: Sero-prevalence of CBPP in trade cattle sampled at ante-mortem examination in abattoirs of Niger State, North-central Nigeria: 2014

Abattoir	Number of live cattle sampled	Number of negative	Number of positive	Proportion (%)	95% Confidence interval
Suleja	105	86	19	18.10	11.60, 26.33
Minna	105	73	32	30.48	22.25, 39.77
Bida	105	68	37	35.24	26.56, 44.73
Kontagora	105	61	44	41.91	32.75, 51.50
New-Bussa	105	70	35	33.33	24.82, 42.75
Total	**525**	**358**	**167**	**31.81**	**27.93, 35.89**

Table 2: Animal factors associated with CBPP sero-prevalence in trade cattle examined at ant-mortem in abattoirs of Niger State, North-central Nigeria: 2014

Factors	Number negative Row (%)	Number positive Row (%)	Odds ratio	95% Confidence interval	P-value
Age					
2-3 years	73 (76.0)	23 (24.0)	1.00		
4-5 years	161 (61.5)	101 (38.5)	2.00	1.17, 3.39	0.009
≥6 years	124 (74.3)	43 (25.7)	1.01	0.61, 1.97	0.754
Sex					
Bulls	164 (76.6)	50 (23.4)	1.00		
Cows	194 (62.4)	117 (37.6)	2.00	1.34, 2.93	<0.001
Breed					
Bokoloji	115 (76.2)	36 (23.8)	1.00		
Bunaji	243 (65.0)	131 (35.0)	1.72	1.12, 2.65	0.012

Statistically significant at p<0.05

Table 3: Proportions of slaughtered trade cattle with CBPP lesions in their lungs at abattoirs in Niger State, North-central Nigeria: 2014

Abattoir	Number of slaughtered cattle sampled	Number of cattle without of CBPP lesions	Number of cattle with CBPP lesions	Proportion (%)	95% Confidence interval
Suleja	105	78	18	17.14	10.82, 25.26
Minna	105	87	27	25.71	18.05, 34.71
Bida	105	70	35	33.33	24.82, 42.75
Kontagora	105	64	41	39.05	30.07, 48.62
New-Bussa	105	71	34	32.38	23.96, 41.76
Total	**525**	**370**	**155**	**29.52**	**25.74, 33.53**

Table 4: Animal factors associated with CBPP lesions in lungs of trade cattle slaughtered at abattoirs in Niger State, North-central Nigeria: 2014

Factors	Number negative Row (%)	Number positive Row (%)	Odds ratio	95% Confidence interval	P-value
Age					
2-3 years	75 (78.1)	21 (21.9)	Ref.		
4-5 years	166 (63.4)	96 (36.6)	2.10	1.20, 3.56	0.007
≥6 years	129 (77.2)	38 (22.8)	1.05	0.58, 1.93	0.877
Sex					
Bulls	175 (81.8)	39 (18.2)	Ref.		
Cows	195 (62.7)	116 (37.3)	2.67	1.76, 4.05	<0.001
Breed					
Bokoloji	123 (81.5)	28 (18.5)	Ref.		
Bunaji	247 (66.0)	127 (34.0)	2.26	1.42, 3.59	<0.001

Statistically significant at p<0.05

Proportions of CBPP lung lesions at post mortem inspection in trade cattle and associated factors

Of the cattle subjected to post-mortem inspections at the slaughterhouses, 29.5% (155/525; CI: 25.74, 33.53) of them had CBPP lesions in their lungs. The proportions of CBPP lesions in the lungs of slaughtered cattle at the respective abattoirs are presented in Table 3.

At the post-mortem inspections, all animal factors of age, sex and breed were significantly (p<0,05) associated with CBPP lesions in the lungs of slaughtered cattle at univariate analysis. At final logistic regressions, only animals in age group 4–5 years were two times more likely (OR 2.10; 95% CI: 1.20, 3.56) to be significantly exposed to Mmm infection. However, cows were more likely (OR 2.67; 95% CI: 1.76, 4.05) to be significantly predisposed to *Mmm* infection than bulls. Also, bunaji breeds were more likely (OR 2.26, 95% CI: 1.42, 3.59) to be significantly predisposed to *Mmm* infection than bokoloji breeds (Table 4).

In the infected lungs, there was pleurisy, loss of lung parenchyma architecture, marbling, consolidation and thickening of interlobular septa, and focal necrotic areas with massive sero-sanquinous fluid. However, in the uninfected lungs, we observed that normal architecture was maintained with no obvious pneumonic lesions. A piece of lung tissue floated when suspended in water and both pleura were clear and distinct.

Discussion

Clinical diagnosis of CBPP is unreliable as initial signs may be slight or non-existent and may be indistinguishable from any severe pneumonia. Therefore, CBPP should be investigated by pathological, microbiological, molecular or serological diagnostic methods. As the pathological lesions of CBPP are distinctive and pathognomonic, abattoir surveillance for CBPP involving lung examination at post-mortem is a practical method for disease monitoring (OIE, 2014). The inherent obstacles against the successful control of CBPP particularly in the sub-Saharan Africa could be linked to inadequate abattoir surveillance and reliable diagnostic tests (Egwu *et al.,* 1996).

The present study had shown that CBPP is enzootic in Nigeria, with abattoir-based sero-prevalence of 31.8% in Niger State using c-ELISA. The observed sero-prevalence was similar to the 32.0% sero-prevalence reported from a CBPP surveillance, using similar test, in Nigeria (Aliyu *et al.,* 2003), but higher than 7.8% sero-prevalence reported from a similar

investigation in some abattoirs around Adama province in Ethiopia (Atnafie *et al.,* 2015). The sero-prevalence observed indicates that there was a considerable levels of cattle challenged with *Mmm* infections without manifesting clinical signs. The detection of positive sera in asymptomatic cattle in this study further confirms the salient nature of CBPP, with likely implication that some of the positive animals were in chronic stage and were carriers, as previously reported (Provost *et al.,* 1987; Alhaji and Babalobi, 2016a).

Using CBPP lesions in lungs, this survey observed post-mortem CBPP prevalence of 29.5% at the abattoirs. This was higher than previously reported 18.8% in a similar CBPP surveillance in Nigeria (Aliyu *et al.,* 2003), 18.4% at the Maiduguri municipal abattoir in Nigeria (Egwu *et al.,* 2012) and 8.6% in slaughter facilities in 10 regions of Tanzania (Noah *et al.,* 2015). However, our observed gross lesions prevalence was similar to the 29.7% reported at Garoua central abattoir in Cameroun (Wade *et al.,* 2015). Varying CBPP lesions manifested in the lungs of infected cattle may be due to differences in breed susceptibility as reported (Egwu *et al.,* 2012).

Movements of cattle across the State as well as the extensive nature of the cattle production system in Nigeria may have contributed to the current observed sero-prevalence and CBPP lesions in lungs. In addition, the position of the State as transit routes for nomadic pastoralists on seasonal migrations between northern and southern parts of the country may have also contributed to the high prevalence. These observations are in agreement with the reports that prevalence of CBPP tend to be higher in cattle herds on extensive management system (Nawathe, 1992; Nwanta and Umoh, 1992).

In this survey, sex, age and breed predispositions were significant ($p<0.05$) to the development of CBPP antibodies and lesions. However, cattle aged 4–5 years were more prone to CBPP lesions and sero-conversions, perhaps because antibodies tend to persist for longer periods during the chronic stage of infection in this age group. This observation is similar to previous reports that older cattle tend to be associated with chronic stage *Mmm* infection (Windsor and Masiga, 1977). Also, because CBPP is more a chronic disease, many cattle tend harbour the causative agent with time due to multiple exposures and other factors, without clinical manifestations. This may justifies why CBPP lesions were significantly found mostly in cattle within 4-5 years age group. The gender distribution of cattle with CBPP lesions significantly ($p<0.05$) shows more cows with the infection than the bulls. Our findings at abattoirs in this study confirmed the enzootic nature of CBPP in Nigeria, as previously reported (Alhaji and Babalobi, 2016a; Alhaji and Babalobi, 2016b).

The control of CBPP is not given the needed attention in trade cattle probably because of the insidious nature of the disease. Previous reports have indicated that majority of cases in apparently cattle remain sub-clinical and affected animals become carriers due to irregular vaccination and sequestration of lesions in the lungs (OIE, 2008; Ezanno and Lesnoff, 2009; Alhaji and Babalobi, 2016b). A very effective way for the control of CBPP is by test and slaughter policy (Tambi *et al.,* 2006), which is also not practicable at livestock markets in developing countries due to logistic reasons. However, this policy can be covered up by the use of post-mortem examinations in combination with serological diagnostic tests, as previously reported for the surveillance of CBPP in developing countries (Nicholas *et al.,* 1996; Danbirni *et al.,* 2010; Egwu *et al.,* 2012). The serological evidence provided in this study, in addition to post-mortem lesions, can provide the necessary platform needed for better monitoring and detection of this disease in Nigeria and other African countries.

Being a cross-sectional study, the survey does not show causal relationship but does demonstrated associations of animal characteristics with occurrence of CBPP at both ante-mortem and post-mortem levels in the State, which is the major limitation of this study.

Conclusions

This study has shown the need to combine more reliable serological tests with post-mortem examinations to improve active surveillance of CBPP in trade cattle. The high CBPP burden was partly due to absence of effective approach to surveillance of the disease in Nigeria. These dual approaches to investigations of CBPP and identification of intrinsic factors predisposing to infection should be institutionalized as elements of epidemio-surveillance and control strategies for the disease in sub-Saharan Africa. Aggressive veterinary extension services to educate cattle traders on the dangers of trading CBPP infected cattle should be pursued. It is also recommended that "trace back" of positive animals detected at abattoirs should be made to their original herds to obtain details of their vaccination history and previous outbreaks, so as to assist policy makers in instituting adequate control and immuno-prophylactic measures.

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