

Research Article

The Prevalence of Contagious Bovine Pleuropneumonia in Cameroon: A Case Study Garoua Central Abattoir, Cameroun

Abel Wade¹, Aboubakar Yaya¹, Abdul Dahiru El-Yuguda², Hermann Unger³, Nafarnda¹, Wesley Daniel^{4*}, Enem Simon Ikechukwu⁴ and Godwin Oyeamechi Egwu⁴

¹Bacteriology Service, Animal Pathology Department, National Veterinary Laboratory Cameroon

²Department of Veterinary Microbiology and Parasitology, Faculty of Vet Medicine, University of Maiduguri, Nigeria

³CRP coordinator, International Atomic Energy Agency (IAEA), Austria

⁴Faculty of Veterinary Medicine, University of Abuja, Nigeria

***Corresponding author**

Nafarnda Wesley Daniel, Faculty of Veterinary Medicine, University of Abuja, Abuja, Nigeria, Tel: 2348139778581; Email: nafarnda@yahoo.com

Submitted: 22 May 2015

Accepted: 23 June 2015

Published: 05 August 2015

Copyright

© 2015 Daniel et al.

OPEN ACCESS

Keywords

- Cameroon
- Contagious bovine pleuropneumonia
- Prevalence

Abstract

A one year (February 2009 - January 2010) prevalence study on contagious bovine pleuropneumonia (CBPP) was conducted among cattle slaughter at the Garoua central abattoir in the northern region of Cameroon. Lung and pulmonary lymph node samples were collected from 384 randomly selected cattle carcasses during meat inspection and taken to National Veterinary Laboratory for analysis using culture and PCR techniques. Abattoir visit was done once a week and any 5th carcass was sampled and records of gross lesions, age, gender and breed of each sampled cattle were taken. Based on gross lesion examination, 114 (29.7%) of the 384 carcasses examined were classified positive for CBPP. From breed distribution, the red Mbororo presented a prevalence of 32%, the white Fulani (33%), the Adamawa Gudali (13%) and non-specified breeds had lower rates 0%, most likely resulting from the low number of animals. Male had a slightly higher prevalence of 35% than female with 28%. The age distribution showed the 5 to 10 year-old age group presented a significantly ($p < 0.05$) higher prevalence rate (46%) than the 0 - 5 years (17%) and those of 10 years and above (11%). Only 1.6% of the samples gave a positive isolation, while the PCR found 3.4% positive. This is the first laboratory-based prevalence study of CBPP conducted in Cameroon. The use of PCR in CBPP surveillance especially in test and slaughter process for disease eradication programme is recommended.

INTRODUCTION

Contagious bovine pleuropneumonia (CBPP) is a highly contagious disease of ruminants caused by *Mycoplasma mycoides* subsp. *mycoides* SC (MmmSC; SC = small colony), with a major impact on livestock production and a potential for rapid spread. It is manifested by anorexia, fever and respiratory signs such as dyspnoea, polypnoea, cough and nasal discharges in cattle [1,2]. CBPP is endemic in many African countries, and the Sahara region is under constant threat due to the carrier status of its host. The disease spread alarmingly during the 1990s, infecting several countries previously free from the disease, causing greater

losses in cattle than any other diseases, including rinderpest [3]. Due to high economic losses caused by CBPP in endemic regions, OIE declared CBPP one of the most serious contagious animal diseases and listed it in the group of notifiable animal diseases of high socio-economic impact and is regarded as one of the major transboundary animal diseases (TADs) [4]. Factors contributing to the current resurgence were thought to include collapse in Veterinary Services [5], increased and unrestricted animal movements due to drought, war or civil conflicts [6], and poor vaccine efficacy [7,8]. CBPP represents a major constraint to cattle production in Africa and is regarded as the most serious infectious animal disease affecting cattle on the continent [9,10].

Although cattle production in Cameroon is a major economic activity, there is paucity of data on the prevalence of CBPP in the country. This is the first laboratory-based study conducted with the principal aim of determining CBPP prevalence at the Garoua central abattoir in the northern region of Cameroon.

MATERIALS AND METHODS

Study area and sampling

The Garoua central abattoir was used for this study based on its location in the northern region of the country where most transhumance cattle pass. The abattoir survey was conducted from February 2009 to January 2010. Approximately 40 heads of cattle were slaughtered daily at this abattoir and any 5th inspected carcass constituted a systematic random sample. A total of 384 bovine carcasses were randomly selected for systematic sampling out of 2,458 bovine slaughtered at the abattoir during the study period. Visits to abattoir were conducted weekly in the early morning between 6 am and 7:30 am. A total of 1,195 samples composed of 384 lung fragments (left or right), 384 mediastinal lymph nodes and 384 tracheo-bronchial lymph nodes were collected during meat inspection. Age, sex, breed, animal origin and CBPP gross lesions (hepatisation, marbling, nodulation of lungs, hypertrophy of pulmonary lymph nodes and excessive plural fluid of amber colour [11] were recorded during sample collection. Collected samples were identified and transported on ice to LANAVET (National Veterinary Laboratory) for analysis.

Laboratory analysis of samples

Culture, isolation and identification of Mmm^{Sc} strains: Samples were cultured aerobically in modified heart infusion broth (HIB) (Difco-France, Bordeaux) and anaerobically in modified heart infusion agar (HIA) (Difco- France, Bordeaux) and incubated for 3 to 5 days at 37°C on arrival at the laboratory. The modified HIA and HIB were obtained by adding Neopeptone, bacto-casitone, glucose, horse serum, thellium acetate and penicillin G, respectively. A cut of 1 cm² of tissue sample was used to make an impression smear on the agar (HIA) in the Petri dish, before immersing it in the broth medium. For the pleural fluid samples, a drop was used for culture on HIA and 1 ml - 2 ml of the fluid was also mixed with the broth medium (HIB). Samples were serially diluted in other tubes up to 10⁻³ to reduce contaminants. Growth culture was examined daily. Typical colonies were subjected to growth inhibition test on HIA using anti Pg1 serum. Biochemical tests such as glucose fermentation, hydrolysis of arginin, triphenyltetrazolium (TTZ), phosphatase and digitonin tests were used for identification using OIE standard procedures. Any positive isolate for growth inhibition, glucose, TTZ and digitonin tests, but negative for arginin and phosphatase tests was considered as Mmm^{Sc} strain.

Sample preparations for PCR

A 1.5 ml volume of each cultured sample was spined for 10 min at 13 000 rpm, the supernatants were discarded and the process repeated to concentrate the Mmm^{Sc} isolates as pellet at the bottom of each tube. Pellets were washed in 1.5 ml of 1% phosphate buffer saline, and then re-suspended in 50 µl of sterile de-ionised water in which 100 µl of lysis buffer was added. The mixture was incubated in water bath for 1 hour at 60°C, then at

95°C for 5 minutes. The prepared samples were then stored at +4°C for short storage, otherwise at -20°C for longer storage. Similarly, tissue fragments that could not grow on HIA and HIB were grinded in distilled water and centrifuged at low speed (8000 rpm for 1 min), and the supernatant used as described in culture samples.

Identification of Mmm^{Sc} by PCR

This was performed according to protocol using M^{Sc}1/M^{Sc}2 primer set as described by [12]. The PCR amplification was run in a reaction mixture containing 1 µl of sample template added to 49 µl of reagent mixture composed of 41 µl of molecular water, 5 µl of 10X Qiagen Taq buffer (containing 15mM of MgCl₂), 0.5 µl of dNTP mix (30 mM AT/15 mM GC), 1 µl of each primer set (20 µM) and 0.5 µl of Qiagen Taq (5 U/ µl). The 50 µl final reaction volume were loaded in the thermocycler (Eppendorf 3700) and subjected to an initial denaturation at 94°C for 5 min, followed by 40 cycles of 95°C for 30 sec, 53°C for 30 sec and 72°C for 1 min, then a final extension step of 72°C for 5 min. The products were kept at 4°C for electrophoresis.

Electrophoresis of amplified products

The assay was run on a 3% agarose (Fischer Scientific, Walldorf Germany) gel containing ethidium bromine at 80 volts for 30 min. The products were read under UV illumination adapted to molecular imaging software. A PCR product of 275 base pairs (bp) was expected for all Mmm^{Sc} strains.]

RESULTS AND DISCUSSION

The result of CBPP prevalence among slaughtered cattle at Garoua central abattoir in Cameroon using gross lesion, isolation and PCR are presented in Table 1. Based on gross lesion examination, 114 (29.7%) of the 384 carcasses examined were positive of CBPP. The breed, gender and age distribution of these are presented in Tables 2, 3 and 4. The red Mbororo presented higher prevalence of 22.6% followed by the white Fulani (5.2%) and the Adamawa Gudali (1.3%) (Table 2). Significantly ($p < 0.05$), more female (22.7%) were found to be positive compared to the male (7.0%) (Table 3). The age distribution showed the 5-10 years old having significantly ($p < 0.05$) higher prevalence rate (21.9%) than the 0 - 5 years and the 10 years and above (2.1%). Only 1.6% prevalence rate was observed using isolation method, while the PCR method gave prevalence rate of 3.4% (Table 3). Figure 1 show the results of electrophoresis of CBPP positive samples in PCR.

DISCUSSION

In this investigation, more Red Mbororo breed (70.3%) was

Table 1: Prevalence of CBPP among cattle slaughtered at Garoua central abattoir based on breeds.

Breeds	No. examined	No. positive (%)	Rates
Red Mbororo	270	87 (32)	87/270
White Fulani	67	22 (33)	22/67
Adamawa Gudali	39	5 (13%)	5/39
Other breeds*	8	0 (0%)	0
Total	384	114 (29.7%)	

* Other breeds include *Muturu*, *Ndama* and unclassified breeds

Table 2: Gender distribution of CBPP among cattle slaughtered at Garoua central abattoir.

Sex	No. tested	No. positive (%)	Rates
Male	77	27 (35.0%)	27/77
Female	307	87 (29 %)	87/307
Total	384	114 (29.7%)	

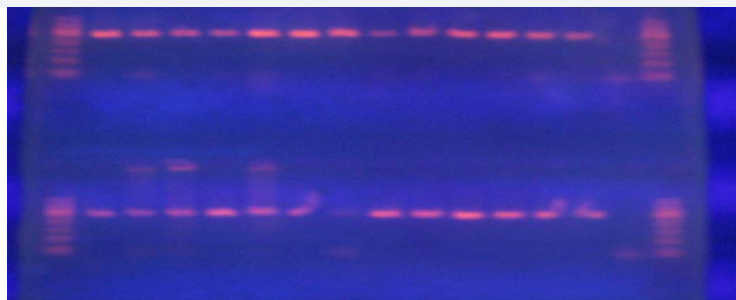
Table 3: Age distribution of CBPP gross lesions at Garoua central abattoir.

Age (years)	Age (years)	No.(%) positive	Rates
2-≤5	128	22 (17%)	22/128
5-≤10	182	84 (46%)	84/182
>10	74	8 (11 %)	8/74
Total	384	114 (29.7%)	

Table 4: CBPP prevalence based on different diagnostic techniques.

No. tested	No.(%) positive with gross lesions	Isolation	Polymerase chain reaction
384	114 (29.7%)	6 (1.6%)	13 (3.4%)
384	11 (2.8%)	6 (1.6%)	13 (3.4%)
114	11 (9.6%)	6 (5.2%)	13 (11.4%)

encountered among the breeds of cattle slaughtered at the Garoua central abattoir, followed by the Adamawa Gudali breed (10.1%). This may be because most popular breed of cattle in the northern region is the Fulani Red Mbororo breed. The few Adamawa Gudali came from the Adamaoua region where the breed is known to be the most popular. In addition, more red Mbororo cattle (22.6%) showed CBPP gross lesions compared to the White Fulani (5.7%) and the Adamawa Gudali (1.3%). No case was found in other breeds probably because of the few number recorded during this study. The gender distribution of the cattle with CBPP lesions showed more females than the males. When considering age, the study showed that majority of cattle slaughtered are mature adults with an average age of five years and above. In this perspective, animal owners do gain more by selling adult animals for slaughter compared to the young ones which cannot get good market because of low meat quality and quantity. Because CBPP is a chronic disease, more cattle do harbour the causative agent with time due to multiple exposure and other factors. This may justifies why CBPP lesions were found mostly in cattle aged 5 years and above. The overall gross lesion-based prevalence was 29.7%, in the laboratory, out of 384 carcasses, 6 (1.6%) samples were positive using isolation while PCR detected 13 (3.4%) positives. Results obtained in this study confirm the endemic nature of CBPP in the northern region of Cameroon as described



Figures

M	1	2	3	4	5	6	7	8	9	10	11	12	13	14	M
---	---	---	---	---	---	---	---	---	---	----	----	----	----	----	---

Figure Legend 1: Amplified positive polymerase chain reaction samples on an agarose gel.

Electrophoresis	of positive	PCR products	on a 3% agarose gel
M:	molecular	marker	(50bp, Qiagen)
1:	Positive	control	sample (Rita strain)
2:	Sample		210
3:	Sample		273
4:	Sample		258
5:	Sample		339
6:	Sample		341
7:	Sample		342
8:	Sample		47
9:	Sample		374
10:	Sample		373
11:	Sample		329
12:	Sample		186
13:	Sample		377
14:	Negative control sample (H ₂ O)		

by [13]. The prevalence rates observed in this study are higher compare to those earlier observed in Nigeria [14,15] during a post-mortem examinations based study of CBPP in slaughtered cattle. Similarly, the ratio obtained in this study is higher than those earlier reported in Chad [16]. However, the prevalence rate observed in this study was lower than those from other in African countries [17,18]. Also, out of 500 lung tissues and sera samples of cattle from CBPP endemic areas in Nigeria, 27.4% and 32% were seropositive for CFT and cELISA, respectively [19]. In a similar study conducted among 56 herds using 793 serum samples from two zones (Jijiga and Shinille) of the Ethiopian Somali region, the overall CBPP herd sero-prevalence was 30.4% [20], whilst the sero-prevalence based on agro-ecology was found to be highly significant in lowlands with 39.0% compared to areas of higher altitude with 6.7%. Higher overall serological values based on cross-sectional surveys have been reported from endemically infected areas of southern Sudan [22,23]. Prevalence based on gross examination is higher than those obtained using laboratory diagnostic CBPP techniques. This shows that many other diseases are responsible of gross pathological findings on the lung tissues. Some samples with lesions suggestive of CBPP were tested negative while others that did not present any gross lesion tested positive for CBPP indicating that Mmm^{Sc} can be shaded even by a host diagnosed to be negative of CBPP lesions using imaging systems such as echography or radiology. Culture and isolation techniques require the presence of viable pathogens that can grow on a medium whereas molecular technique relays on the presence of the genetic material (DNA) of Mmm^{Sc} in the sample. Another reason for isolation failure is the abusive use of oxytetracyclines and other Mmm^{Sc} sensitive antimicrobial compounds on animals. This confirmed that the application of isolation technique to cell-wall-less organisms such as Mmm^{Sc} is really difficult and time consuming and requires skilled manpower and vigilance. Similarly, in a study conducted on Italian cattle, Mmm^{Sc} was isolated from only 4 of the 20 cattle in group A and 6 of the 17 cattle in group B using PCR [24]. This proves that PCR is more sensitive than isolation technique; therefore, the use of PCR in CBPP surveillance especially in test and slaughter process for disease eradication programme is recommended. This is the first laboratory-based prevalence study of CBPP conducted in Cameroon.

LIMITATIONS

The limitation of study is the limited number of samples and study area coverage. Nevertheless, our findings are the first laboratory-based prevalence study of CBPP conducted in Cameroon.

ACKNOWLEDGMENTS

The authors wish to acknowledge the collaboration of some MINEPIA staff in charge of meat inspection at the Garoua central abattoir including the regional delegate for the North. All the LANAVET staffs are hereby acknowledge specially the General Director and his collaborators for administrative assistance.

REFERENCES

1. Blood DC, Henderson JA, Radostits OM. Veterinary medicine. 8th edition. W B Saunders Co. Tulsa, OK, USA. 1994.

2. World Organisation for Animal Health; Office International des Épizooties (OIE) Terrestrial Manual. Contagious Bovine pleuropneumonia; 2014.
3. World Organisation for Animal Health (Office International des Épizooties: OIE). Diagnostic tests for contagious bovine pleuropneumonia (CBPP). Report of the scientific Committee on Animal Health and Animal Welfare. OIE, Paris; 2001; 2-8.
4. Food and Agriculture Organization of the United Nations (FAO). Recognizing contagious bovine pleuropneumonia. FAO Animal Manual Health Manual, FAO, Rome; 2002; 13: 3-17.
5. Provost A, Contagious bovine pleuropneumonia (CBPP) prevention and control strategies in eastern and southern Africa. Report of the Joint FAO EMPRES and OAU IBAR Regional Workshop, Arusha, Tanzania, 4-6 July 1995. Rome, Italy: Food and Agricultural Organization; 1996; p. 109
6. Windsor RS, Wood A. Contagious bovine pleuropneumonia. The costs of control in Central/southern Africa. Ann N Y Acad Sci. 1998; 849: 299-306.
7. Rweyemamu MM, Litamoi J, Palya V, Sylla D. Contagious bovine pleuropneumonia vaccines: the need for improvements. Rev Sci Tech. 1995; 14: 593-601.
8. Thiaucourt F, Yaya A, Wesonga H, Huebschle OJ, Tulasne JJ, Provost A. Contagious bovine pleuropneumonia. A reassessment of the efficacy of vaccines used in Africa. Ann N Y Acad Sci. 2000; 916: 71-80.
9. Amanfu W. Contagious bovine pleuropneumonia (lung sickness) in Africa. Onderstepoort J Vet Res. 2009; 76: 13-17.
10. Marobela-Raborokgwe C1. Contagious bovine pleuropneumonia in Botswana: experience with control, eradication, prevention and surveillance. Vet Ital. 2011; 47: 397-405.
11. World Organisation for Animal Health (Office International des Épizooties: OIE). Diagnostic tests for contagious bovine pleuropneumonia (CBPP). Report of the scientific Committee on Animal Health and Animal Welfare. OIE, Paris. 2001; 2-8.
12. Dedieu L, Mady V, Lefèvre PC. Development of a selective polymerase chain reaction assay for the detection of Mycoplasma mycoides subspecies mycoides SC (contagious bovine pleuropneumonia agent). Vet Microbiology. 1994; 42: 327-339.
13. Yaya A, Manso-Silvan L, Blanchard A, Thiaucourt F. Genotyping of Mycoplasma mycoides subsp. mycoides small colony by multilocus sequence analysis allows molecular epidemiology of contagious bovine pleuropneumonia. Vet Res. 2008; 39: 14.
14. Aliyu MM, Obi TU, Egwu GO. Prevalence of contagious bovine pleuropneumonia (CBPP) in northern Nigeria. Prev Vet Med. 2000; 47: 263-269.
15. Nawathe DR. Resurgence of contagious bovine pleuropneumonia in Nigeria. Rev Sci Tech. 1992; 11: 799-804.
16. Maho A. Etude historique sur la péripneumonie contagieuse bovine au Tchad. Consultancy report produced for the African Union Inter-African Bureau for Animal Resources-Pan African Programme for the Control of Epizootics. AU/IBAR-PACE, Nairobi. 2001.
17. Kane M. Étude historique sur la péripneumonie contagieuse bovine au Burkina Faso, Côte d'Ivoire, Guinée, Mali, Mauritanie, Niger et Sénégal. Consultancy report produced for the African Union Interafrican Bureau for Animal Resources-Pan African Programme for the Control of Epizootics. AU/IBAR-PACE, Nairobi. 2002.
18. Wanyoike SW. Assessment and mapping of contagious bovine pleuropneumonia in Kenya: past and present. MSc dissertation. Freie

- Universität, Berlin, Germany and Addis Ababa University, Addis Ababa. 1999.
19. Fikru R. Herd prevalence of CBPP, bovine tuberculosis and Dictyocaulosis in Budju woreda, West Wellega. DVM thesis. Addis Ababa University, Faculty of Veterinary Medicine, Debre Zeit. 2001.
20. Aliyu MM, Obi TU, Oladosu LA, Egwu GO, Ameh J A. The use of competitive enzyme linked immunosorbent assay in combination with abattoir survey for CBPP surveillance in Nigeria. *Trop. Vet Medicine*. 2003; 21: 35-41.
21. Mekonnen G. Serological, Clinical and Participatory Epidemiological Survey of Contagious Bovine Pleuropneumonia in Somali Region, Ethiopia. Master of Science in Tropical Veterinary Epidemiology Thesis submitted to the Faculty of Veterinary Medicine, Addis Ababa University. Ethiopia; 2004.
22. McDermott JJ, Deng KA, Janatileka TN, Jack MA. A cross-sectional cattle disease study in Kongor Rural Council, southern Sudan. I. Prevalence estimates and age, sex and breed associations for brucellosis and contagious bovine pleuropneumonia. *Prev. Vet Medicine*. 1987; 5: 111-123.
23. Zessin KH, Baumann M, Schwabe CW, Thornburn M. Analyses of baseline surveillance data on contagious bovine pleuropneumonia in the southern Sudan. *Prev. Vet Med*. 1985; 4: 371-389.
24. Nicholas RAJ, Santini FG, Clark KM, Palmer MA, de Santis A, Bashiruddin JB. A comparison of serological tests and gross lung pathology for detecting contagious bovine pleuropneumonia in two groups of Italian cattle. *Vet Rec*. 1996; 139: 89-93.

Cite this article

Wade A, Yaya A, El-Yuguda AD, Unger H, Nafarnda, et al. (2015) The Prevalence of Contagious Bovine Pleuropneumonia in Cameroon: A Case Study Garoua Central Abattoir, Cameroun. *J Vet Med Res* 2(4): 1029.