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Full Length Research Paper

Experimental studies on the effect of long-acting oxytetracycline treatment in the development of sequestra in contagious bovine pleuropneumonia-infected cattle

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The effect of long-acting oxytetracycline treatment in the development of sequestra in contagious bovine pleuropneumonia (CBPP)-infected animals was determined. Thirty five clinically healthy zebu cattle, negative for CBPP antibodies (as tested by a competitive ELISA), were infected with *Mycoplasma mycoides* subsp. *mycoides* small colony variant - field isolate. All the animals were monitored for clinical signs of illness and regularly sampled for serological and bacteriological analysis. Nine animals were treated with long-acting tetracycline upon the first evidence of clinical signs of illness. The 26 remaining animals were left untreated and served as control animals. Post-mortem examinations were performed to assess the presence or absence of CBPP gross lesions. The experimentation lasted for 10 months during which, 13 animals (all untreated) died with acute lung lesions. When the 22 surviving animals (13 untreated and 9 treated) were slaughtered at the end of experimentation, chronic lesions were observed in all of them. Among the 13 untreated animals, 10 had visible lung sequestra and 3 had cicatrical lesions indicative of resolved lung lesions. Conversely, among the 9 treated animals, only 1 had a small lung sequestrum and the remaining 8 had pulmonary adhesions. The results presented demonstrate that, under the experimental conditions used, treatment with oxytetracycline did not result in significant sequestra formation in CBPP-infected animals. Full field validation is required in order to confirm these findings.

Key words: Contagious bovine pleuropneumonia, antibiotherapy, oxytetracycline, Lung sequestra, experimental transmission, Mali.

INTRODUCTION

Contagious bovine pleuropneumonia (CBPP) is an important infectious disease of cattle caused by *Mycoplasma mycoides* subsp. *mycoides* SC (*MmmSC*) that is characterized by a severe fibrinous exudative pleuropneumonia. It used to be included on the former 'Office of International Epizooties' the disease was included in the list A of diseases because of its high transboundary and infectious potential (http://www.oie.int). Now that rinderpest is largely under control in Africa, CBPP remains one of the most important animal diseases in tropical Africa causing great economic loss due to animal mortality, weight loss, reduced working ability, reduced fertility and indirect costs due to control programmes (Tambi, 2006).

In Africa attempts to control CBPP by stamping out, a

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combination of vaccination, animal movement management and quarantine of affected animals have been met with limited success because of the difficulties and financial costs of implementing such control measures (Sylla et al., 1995). The use of antibiotics to treat CBPP disease in the field is common, although official policy is contrary to this practice, due to the possibility of creating carrier animals which are thought to be sources of fresh infection in a herd, development of antimicrobial resistance and anti-biotic residues in meat and milk products (FAO, 1967). Currently, there is little or no experimental evidence to support the hypothesis of carrier status created by the use of antibiotics for CBPP disease treatment. Studies are therefore, urgently needed in this area to further elucidate the role of antibiotics in the generation or not of carriers of CBPP disease (FAO, 2002).

This study was therefore, conducted with the principal objective of assessing the role of long-acting oxytetracycline treatment in the development of sequestra in CBPP-infected animals. This antibiotic is the most widely used antibacterial agent in the field throughout many African countries.

METHODLOGY

Experimental animals

Thirty five clinically healthy zebu cattle, 3 - 6 years old and negative for MmmSC antibodies as tested by competitive enzyme linked-immunosorbent assay (c-ELISA), were purchased from several herds and conveyed to facilities at the Central Veterinary Laboratory (CVL), Bamako, Mali. Upon arrival, the animals were ear-tagged for identification and underwent a two-month mandatory quarantine period during which they were bled twice to establish their serological status with regards to CBPP disease. The animals were also treated against internal and external parasites and vaccinated against pasteurellosis and blackleg infections.

Experimental infection

After the quarantine phase, 21 of the 35 animals were artificially infected with *MmmSC* field isolate (N^{o} 342/07) by intra-tracheal intubation. The strain used was isolated from an active CBPP field outbreak in Mali and stored at the CVL mycoplasma isolate bank at -80°C. Mycoplasma organisms were cultured in Gourlay medium at 37°C and harvested in log phase growth by centrifugation at 10 000 × g for 20 min and washed once in PBS buffer. The number of organisms was determined by microtitration in liquid media and concentration was adjusted to 5×10^{8} CFU (colony forming units) in PBS buffer. While awaiting the results of the microtitration, the organisms were kept at -80°C.

For the intra-tracheal intubation method, a lubricated 11 mm outer diameter polyethylene nasal-oesophageal tubing (bronchoscope) was inserted into the nostril of the animal and pushed gently along the nasal septum until seated firmly in a bronchus. Usually, a cough reflex indicated the successful delivery to the bronchus. Fifty ml of mycoplasma organisms (5×10^{8}) were delivered into the lung followed by 50 ml of sterile 1.5% of agar solution to rinse the bronchoscope (Dedieu et al., 2005). Intubated animals were kept apart, checked daily for body temperature and other symptoms suggestive of CBPP disease (coughing and dyspnea), to observe disease progression.

Upon the manifestation of the first clinical signs of illness (10 – 15 days post intubation), the 21 animals were added to the remaining 14 animals in order to reproduce the disease by contact exposure. Animals were tagged with numbers from **I**1 to **I**21 for the intubated animals (**I**) and from **C**1 to **C**14 for the contact-exposed (**C**) animals. To increase the pressure of infection, the intubated animals and the contact-exposed animals were kept in permanent and close contact in an isolated and secured pen. They spent the daytime in the courtyard of the pen with free access to feed and water, and in confinement within the pen at nighttime (Niang et al., 2004).

Antibiotic treatment

In order to assess the effect of long acting oxytetracycline treatment in the development of sequestra in CBPP-infected animals, 9 animals comprising of 3 intubated animals (**I**2, **I**3 and **I**5) and 6 contact- exposed animals (**C**1, **C**2, **C**4, **C**7, **C**11, and **C**14) were treated, upon the first evidence of clinical signs of illness (respectively, 10 - 15 days post intubation and 24 - 73 days post contact) with long acting oxytetracycline (20%) following the manufacturer's instructions (dose rate of 1 ml/10 kg of body weight). The treatment started with varying periods after infection, but still immediately following manifestation of the clinical signs. The 26 remaining animals comprising 18 intubated animals and 8 contact-exposed animals were left untreated and served as control animals.

Clinical and post-mortem examinations

Animals were observed daily for signs of clinical disease including cough, nasal discharge, lethargy, respiratory distress, anorexia, weight loss and eye discharges. In addition, rectal temperatures and respiratory rates were recorded within two-day intervals over a period of 49 weeks post-infection.

Post-mortem examinations were performed on animals that died or were culled *in extremis* during the experimentation or those that were slaughtered at the end of the experimentation that lasted approximately 10 months. Lungs and adjoining regional lymph nodes were thoroughly examined for gross pathological lesions suggestive of CBPP (specifically the presence of lung sequestra, the determination of their size and consistency).

Sample collection

From all the animals, serum samples were collected sequentially at one week intervals during the first month post-infection and then 2 - 4 week intervals during the remaining period of experimentation. Blood for serum samples were allowed to clot at room temperature and centrifuged to harvest the serum samples that were aliquoted and stored at -20°C until tested for specific antibodies (Ab) to MmmSC by cELISA and the complement fixation test (CFT).

Tracheobronchial lavage fluids were collected only once from the intubated animals immediately following the onset of the disease and then from all remaining intubated and contact-exposed animals at slaughtering. Samples of lung tissues, pleural fluid, lung sequestra, lymph nodes and kidneys were also collected from animals which died during the experiment or were slaughtered at the end of the experimentation.

Samples of tracheobronchial lavage fluid, lung tissues, pleural fluid, lung sequestra, kidney tissues and lymph nodes were processed immediately for mycoplasma isolation.

Laboratory analysis

For isolation of *Mmm*SC, ten-fold serial dilutions of each sample were made in Gourlay and Brain Heart Infusion (BHI) broth media.

Table 1. Scale of animal scoring system.

Indicators of infection	Score
1. Serology (c-ELISA PI)	
PI<40	0
PI = 40-50	1
PI >50	2
2. Pyrexia	
< 38.5°C	0
38.5°C - 39.5°C	1
> 39.5°C	2
3. Lethargy	
absent	0
mild	1
severe	2
4. Cough frequency	
Absent	0
Moderate	1
Severe	2
5. Pulsation	
< 63	0
=63–70	1
> 70	2
6. Respiratory frequency	
< 22	0
=22–25	1
> 25	2
7. Animal died from the disease	2
8. Autopsy results	
Active lesions (hepatisation and pleural fluid)	2
Size of chronic lesions (sequestrum)	
Absent	0
< 5 cm	1
5 – 20 cm	2
> 20cm	3
Resolving lesions (fibrotic scars and adhesions)	1

PI (Percentage of Inhibition).

Confirmation of *Mmm*SC was made by growth inhibition test on solid medium after two to three sub- passages in liquid medium. To test specific antibodies to *Mmm*SC in serum samples, CFT and cELISA were both carried out according to the protocols provided with the kits (CIRAD-EMVT, Montpellier, France). The CFT was carried out on two fold serial dilutions of heat inactivated (56°C for 30 min) serum samples. The cELISA was carried out using a single 1:10 dilution of raw serum samples.

Animal scoring system

In an attempt to integrate the various criteria of infection (clinical signs, post-mortem findings, mycoplasma isolation and serological responses) recorded during the experimental period, for a better evaluation of animal status, a scoring system scale for CBPP according to Hudson and Turner (1963) was adopted as indicated in Table 1.

Table 2. Summary of clinical, pathological, microbiological and serological data on animals within the untreated group.

Animal N°	Clinical disease		Gross lung lesions		Laboratory results	
	Main clinical signs recorded	Disease outcome	Type of lesions	Size of sequestra	MmmSC Isolation	c-ELISA
I1	Cough, weakness, lethargy, diarrhea	Death	A, H	NA	+ (B, L)	+
I4	Cough, nasal discharge, dyspnea, prostration, difficulty of movement	Death	A, H, PF	NA	+ (B, L, PF)	+
I6	Cough, dyspnea, lethargy	Recovery	A, S (multiple)	Large (6/10 cm) (6/15 cm)	+ (B)	+
I7	Cough, dyspnea, lethargy	Recovery	A, S	Large (14/18 cm)	+ (B, S)	+
I8	Cough, nasal discharge, diarrhea, prostration, difficulty of movement	Death	H	NA	+ (B, L)	-
I9	Cough, dyspnea, lethargy, weakness	Recovery	A, S	Large (7/15 cm)	+ (B)	+
I10	Cough, dyspnea, nasal discharge, lethargy	Recovery	A, S	Large (8/15 cm)	+ (B)	+
I11	Cough, dyspnea, nasal discharge, lethargy	Recovery	A, S	Large (4/15 cm)	+ (B)	+
I12	Cough, lethargy	Death	A, H	NA	+ (B)	+
I13	Cough, lethargy	Recovery	A, H, CL	NA	+ (B)	+
I14	Cough, nasal discharge, dyspnea, weakness	Recovery	A, S (liquid)	Large (5/15 cm)	+ (B)	+
I15	Cough, diarrhea, weakness, lethargy	Death	H	NA	+ (B, L)	-
I16	Cough, dyspnea, weakness	Death	A, H, PF	NA	+ (B, L, PF)	-
I17	Cough, nasal discharge, lethargy	Recovery	A, H, CL	NA	+ (B)	+
I18	Cough, nasal discharge, dyspnea, weakness	Death	A, H	NA	+ (B, L)	-
I19	Cough, lethargy	Recovery	A, S	Large (6/13 cm)	+ (B)	+
I20	Cough, lethargy	Recovery	A, S	Small (3/5 cm)	+ (B, S)	+
I21	Cough, diarrhea, lethargy, difficulty of movement	Death	A, H	NA	+ (B, L)	+
C3	Cough	Recovery	A	NA	-	+
C5	Cough, lethargy	Death	H, PF	NA	+ (L, PF)	+
C6	Cough, lethargy	Death	A, H, PF	NA	+ (L, PF)	-
C8	Cough, lethargy	Death	H, PF	NA	+ (L)	-
C9	Cough, lethargy	Death	A, H, PF	NA	+ (L, PF)	+
C10	Cough, lethargy	Death	Absence	NA	-	+
C12	Cough, lethargy	Recovery	A, S	Large (8/9 cm)	+ (S)	+
C13	Cough, lethargy	Recovery	A, S	Large (11/21 cm)	-	+

A (Lung adherence); H (Lung hepatization); PF (Pleural fluid); S (Sequestrum); CL (Cicatrical lesions); B (Bronchoalveolar lavage fluid); L (Lung); NA (Not applicable); + (Positive); - (Negative).

Statistical analysis

Because of the small sample size, the Fishers Exact test was used to compare the various parameters of infection in animals within the treated and untreated groups. The Wilcoxon–Mann–Whitney test, which uses non-parametric analysis of two populations having the same location, was used to test differences of total clinical scores between the 2 groups.

RESULTS

Results of the overall clinical outcome (including course of clinical signs and mortality), the pathology, the frequency and size of sequestra, the serology and the isolation of mycoplasmas within both untreated and treated groups were summarized in Tables 2 and 3,

Table 3. Summary of clinical, pathological, microbiological and serological data on animals within the treated group with long-acting oxytetracycline.

Animal N°	Clinical disease		Gross lung lesions		Laboratory results	
	Main clinical signs recorded	Disease outcome	Type of lesions	Size of sequestra	*MmmSC* Isolation	c-ELISA
I2	Cough, nasal discharge, lethargy	Recovery	A	NA	+ (B)	+
I3	Cough, lethargy	Recovery	A	NA	+ (B)	+
I5	Cough, nasal discharge, lethargy	Recovery	A, CL	NA	+ (B)	+
C1	Cough	Recovery	A	NA	-	+
C2	Cough	Recovery	A	NA	-	+
C4	Cough	Recovery	A	NA	-	+
C7	Cough	Recovery	A, S	Small (0.1/0.2 cm)	-	+
C11	Cough, lethargy	Recovery	A	NA	-	+
C14	Cough, lethargy	Recovery	A	NA	-	+

A (Lung adherence); S (Sequestrum); CL (Cicatrical lesions); B (Bronchoalveolar lavage fluid); NA (Not applicable); + (Positive); - (Negative).

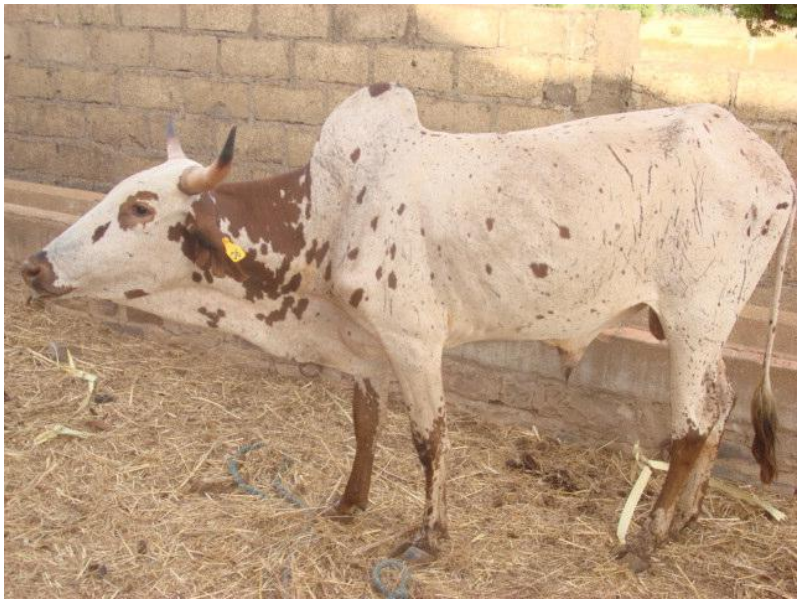

Figure 1. Acute clinical form of CBPP observed in an untreated animal (**I** 4) one month after intubation. Note the neck extension of the animal indicating a respiratory distress. Saliva is leaking from the mouth.

respectively.

Clinical and post-mortem findings on animals within the untreated group

All of the 26 untreated animals comprising 18 in the intubated group and 8 in the contact-exposed group showed clinical signs of CBPP characterized by cough, prostration with difficulty of movement, dyspnea, nasal discharges and weakness (Table 2 and Figure 1). While the onset of the disease was rapid in the former group which varied from 10 - 15 days post intubation, it was delayed in the latter and varied from 24 - 73 days post contact. The recorded rectal temperatures were high and averaged around 40°C in both groups.

Thirteen animals of which 8 in the intubated group (**I**1, I4, I8, I12, I15, I16, I18 and I21) and 5 in the contact-exposed group (**C**5, **C**6, **C**8, **C**9 and **C** 10) died during the course of the experimentation. At necropsy, acute pulmonary lesions characteristic of CBPP (lung hepatization and pleural fluid) were observed (Table 2, Figures 2, 3 and 4).

The 13 remaining animals, 10 in the intubated group (I6, I7, I9, I10, I11, I13, I14, I17, I19 and I20) and 3 in the contact-exposed group (C3, C12 and C13) showed

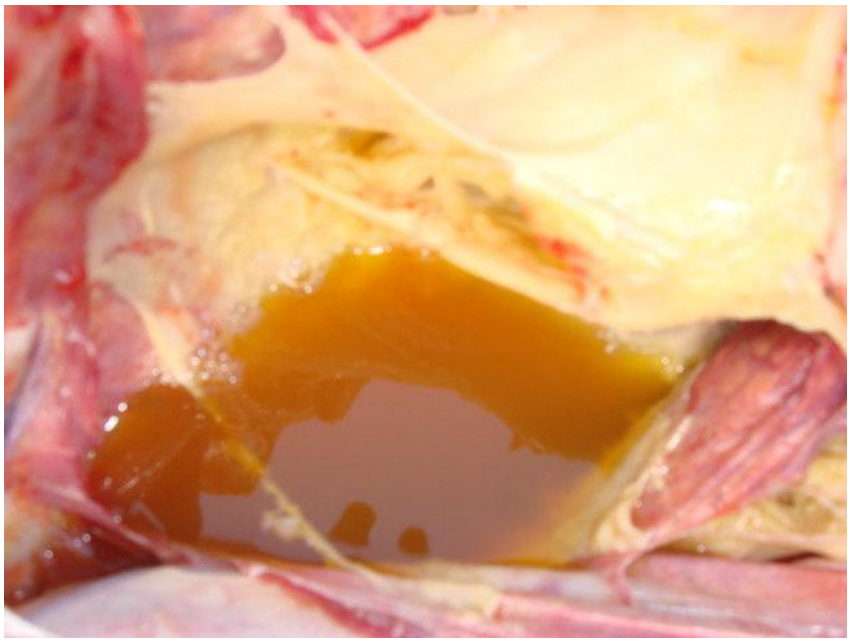

Figure 2. Acute gross lung lesions of CBPP in an untreated animal (**C**6). The thorax has been opened and an abundant quantity of clear yellow fluid (pleural liquid) is visible in the cavity.

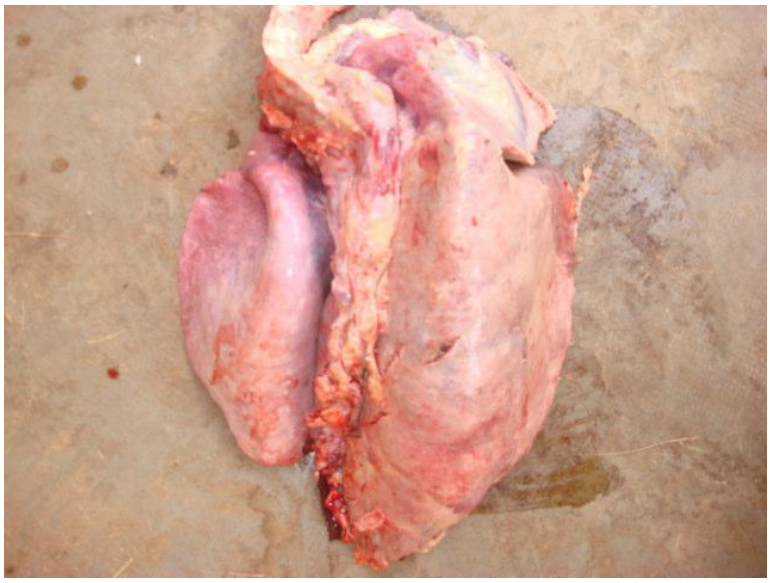

Figure 3. Acute gross lung lesions of CBPP in an untreated animal (**I**4). Note the volume and the consolidation of the left lung.

clinical signs of the disease for a long period of time (approximately 4 - 5 months) and recovered thereafter. When they were slaughtered at the end of experimentation, chronic lesions were observed in all of them. In the intubated group, 8 had visible lung sequestra (I6, I7, I9, I10, I11, I14, I19 and I20) and 2 had pleural adhesions and cicatrical lesions, indicative of resolved lung lesions (I13 and I17). Among the contact-exposed group, 2 animals (C12 and C13) had visible lung sequestra and the remaining one (C3) had pleural adhesions and cicatrical lesions, indicative of resolved lung lesions. In both groups, the sequestra were large and varied in size from about 5 - 21 cm in diameter (Table 2, Figures 5 and 6). The presence of multiple sequestra was observed only in one animal (I6). In some cases, the lymph nodes were inflamed.

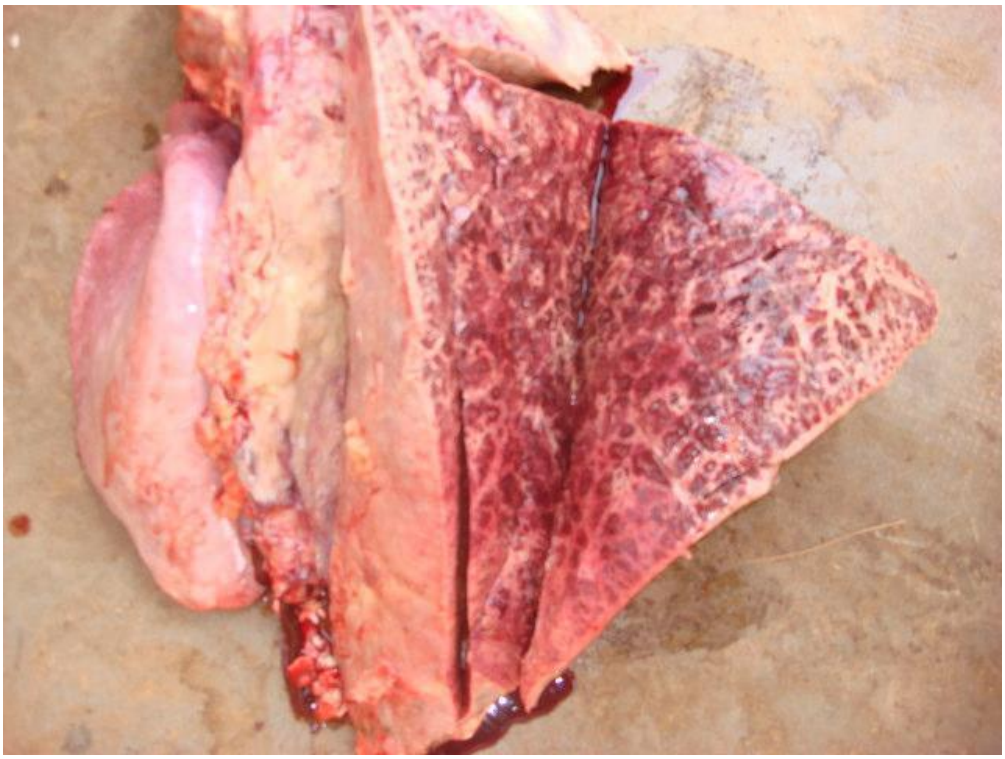

Figure 4. Acute gross lung lesions of CBPP in an untreated animal (**I**4). On cross-section, the surface of the lung exuded large amount of serous fluid and showed a pronounced interlobular thickening with a marbled appearance.

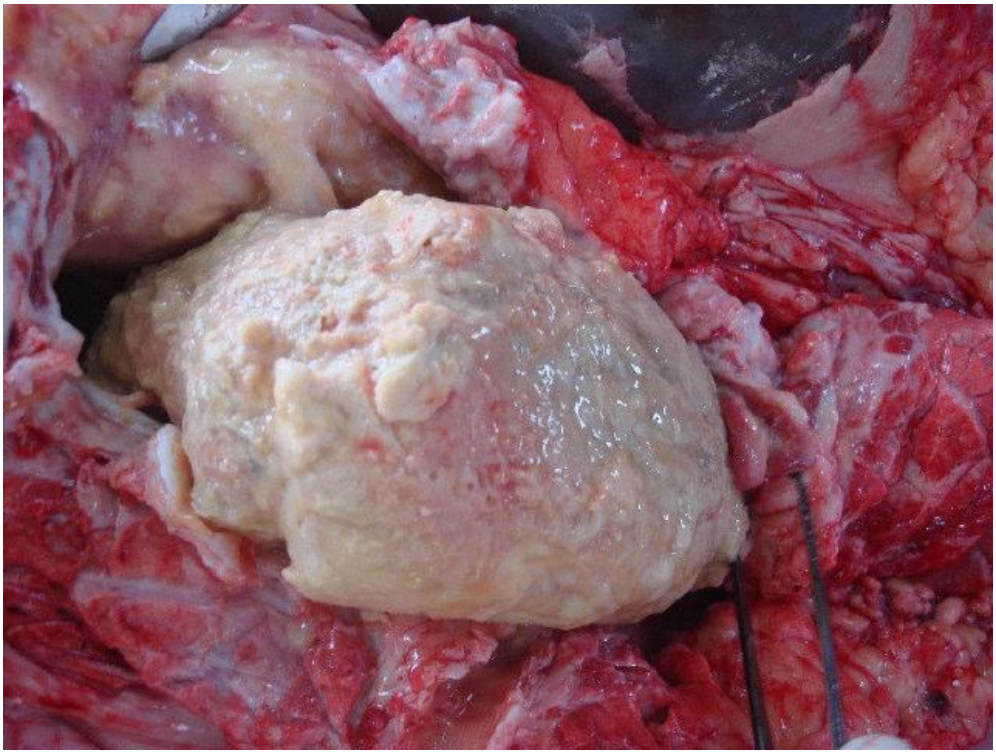

Figure 5. Chronic gross lung lesions with a visible sequestrum observed in an untreated animal (**I**10) . On incision of sequestrum, the necrotic contents shelled out easily leaving a thick capsular wall.

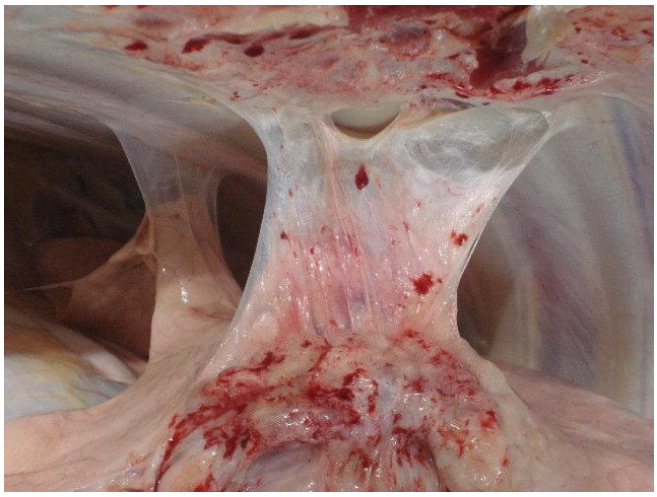

Figure 6. Chronic gross lung lesion with adherence observed in an untreated animal (**C**12).

Clinical and post-mortem findings on animals within treated group

The 9 animals comprising 3 intubated animals (I2, I3 and I5) and 6 contact-exposed animals (C1, C2, C4, C7, C11 and C14) that were treated with long-acting oxytetracycline (20%) upon the first evident clinical signs of illness, recovered. The clinical signs of disease completely disappeared within 4 - 5 weeks after treatment (Table 3).

When these animals were slaughtered at the end of experimentation, the lesions observed, consisted mainly of pleural adhesions. In contrast to the untreated animals, no evident sequestra were detected, except in one animal (**C**7) which showed a very small sequestrum (0.1/0.2 cm) (Table 3).

Integrated scores of clinical, pathological, microbiological and serological data on animals within both untreated and treated groups

The results of the integrated scores of each animal based on the various criteria of infection including course of clinical signs and mortality, the pathology, the frequency and size of sequestra, the mycoplasma recovery and the serological responses for both groups recorded during the whole experimental period are summarized in Table 4.

Considering all criteria with the exception of temperature, pulse rate and respiratory frequency in which there was no significant difference between animals within the 2 groups, the others clinical criteria of infection were significantly lower in the animals within the treated group than in those within the untreated group ($p > 0.01$), and this was reflected in differences in the mortality rate ($p > 0.008$) observed, the mycoplasma recovery rate ($p > 0.004$) and the development of sequestra ($p > 0.002$) . Indeed, of the 26 animals within the untreated group, 13 animals died during the course of the experimentation and among those that survived 76.92% (10/13) developed sequestra. In contrast to the animals within the untreated group, all the animals within the treated group survived. Only one animal (1/9; 11.11%) presented a small sequestrum.

Results of the two groups were also compared by examination of the total clinical scores. While the total clinical score of all the animals within the untreated group was 354, the animals within the treated group had a total score of 89 which was significantly lower ($P > 0.0003$). This score was still significantly lower ($p > 0.002$) when compared to that of the surviving animals within the untreated group which was 169.

Serological responses

As indicated in Table 2, using the cELISA, the majority of the animals (29 /35), irrespective of their status, seroconverted with high titers. Likewise, using CFT, seroconversion was observed in many animals (27/35) irrespective of their status.

Mycoplasma isolation

MmmSC was isolated from the samples of tracheobronchial lavage fluids of all the intubated animals, irrespective of their status, collected during the early stage of the infection (Tables 2 and 3). In contrast, - attempts to isolate mycoplasma from the samples of tracheobronchial lavage fluids collected at slaughtering were unsuccessful. Mycoplasma was isolated from the lung tissues of 7 animals that died from the disease (I1, I4, I8, I15, I16, I18 and I21) and from the sequestral contents of the lung of only 2 animals (I7 and I20). As indicated in Table 2, none of these 9 animals were treated with the long-acting oxytetracycline.

Attempts to isolate mycoplasma from tracheobronchial lavage fluids collected from all contact-exposed animals at slaughtering were unsuccessful. Mycoplasma was isolated from lung tissues collected from the animals (**C**5, **C**6, **C**8 and **C**9) that died during the course of the infection and from the sequestral contents of the lung of only one animal (**C**12). None of these animals was treated with the long-acting oxytetracycline as indicated in the Table 2.

DISCUSSION

Contagious bovine pleuropneumonia (CBPP) is considered a priority disease by the FAO-Emergency Prevention System for Transboundary Animal Diseases (FAO-EMPRES) and other organizations. Controlling the

Table 4. Summary of integrated scores of clinical, pathological, microbiological and serological data on animals within both untreated and treated groups.

Untreated animals				Treated animals	
Died plus recovered		Recovered only			
Animal N°	Clinical score	Animal N°	Clinical score	Animal N°	Clinical score
I1	17	I6	15	I2	14
I4	18	I7	15	I3	9
I6	15	I9	15	I5	10
I7	15	I10	15	C1	9
I8	16	I11	15	C2	9
I9	15	I13	12	C4	8
I10	15	I14	13	C7	10
I11	15	I17	12	C11	12
I12	16	I19	12	C14	8
I13	12	I20	13		
I14	13	C3	10		
I15	11	C12	11		
I16	11	C13	11		
I17	12				
I18	13				
I19	12				
I20	13				
I21	16				
C3	9				
C5	16				
C6	14				
C8	14				
C9	15				
C10	9				
C12	11				
C13	11				
Total score	354	Total score	169	Total Score	89

disease in African countries, however, poses a number of challenges; among which are the difficulties in the application of rigorous sanitary measures due to socio-cultural and economic reasons. Another factor is the limited protection conferred by the current CBPP vaccine against the disease. Alternative means of controlling the disease including antibiotic treatment have always been discouraged without any scientific support. In fact, it has been a common belief that treatment of infected animals with antibiotics compromised the control of the disease by generating a large number of carrier animals (FAO, 1967). For a long time, this understanding has prevented the use of antibiotic treatment in the control of the disease. Reviewing the literature, however, it seems that this perception has not been objectively assessed and there is a lack of empirical evidence to substantiate it.

To address this gap in knowledge on the use of antibiotics for the control of CBPP, animal experimentations were conducted with the objective of assessing the effect of long-acting oxytetracycline treatment in the development of sequestra in CBPP infected animals and the possible role of treated cattle in the spread of the disease.

Overall, the results reported here, indicated that long-acting oxytetracycline treatment did not have a significant effect on the formation of sequestra in CBPP-infected animals, since 76.92% (10/13) of infected and untreated animals developed sequestra when compared to the infected and treated animals in which only one animal (1/9; 11.11%) presented a small sequestrum. This difference was statistically significant between the two groups ($p > 0.002$). To the best of our knowledge, there have been no published studies on the role of long-acting oxytetracycline treatment in the development of sequestra in CBPP-infected animals. Therefore, the present study provides the first experimental attempt to elucidate this issue.

The suggestion that antibiotic therapy could create carrier animals (cattle developing sequestra) which can shed infectious organisms and generate new infections in

susceptible herds is frequently repeated in the literature (Turner, 1954; Provost et al., 1987; Masiga et al., 1996). However, it appears that this perception is not based on scientific experimentations and might be derived from extrapolations made from past treatments with novarsenobenzol which is an arsenic based product and not an antibiotic. The beneficial therapeutic action of this drug in a variety of general infections has been demonstrated (Mornet, 1954). Orue and Memery (1961) showed the development of encapsulated lesions with high amounts of *MmmSC* organisms in cattle treated with novarsenobenzol. They did not demonstrate that these animals could transmit the disease to susceptible cattle. However, the authors suggested that these animals could have disastrous consequences in the epidemiology of the disease by excreting mycoplasmas and therefore, this therapy as well as any other chemical treatments including antibiotics, could favor, not only the persistence of the disease, but also its dissemination. It is precisely based on such untested assumptions that veterinary epidemiologists have called for the banning of antibiotics in the treatment of CBPP. However, there are some scientific data that prove the contrary. Windsor and Masiga (1977) were unable to transmit infection to susceptible cattle from naturally recovered cases harboring sequestra despite prolonged contact and stress. They also failed to produce active infection in recovered cattle harboring sequestra by contact with acute cases of the disease.

The results reported also demonstrated that long-acting oxytetracycline treatment had a positive effect on the clinical course of the disease since all the 9 animals that were treated recovered and clinical signs of disease completely disappeared 4 - 5 weeks after treatment. Conversely, the untreated animals did not recover and 13 animals died with acute lung lesions during the course of the experimentation. These results confirmed our previous experimentations (Niang et al., 2007) in which a high rate of recovery was obtained (10 animals out of 12) following the treatment of naturally CBPP infected cattle in an acute stage of the disease with long-acting oxytetracycline. Treated animals also failed to transmit the disease to susceptible cattle. These are also in agreement with the observations made by Windsor and Masiga (1977) and Yaya et al. (2003). Similarly, Camara (1971), Turner (1960), Hudson and Etheridge (1965) and the FAO Expert Panel (FAO, 1967) described the efficacy of tetracyclines, tylosin, erythromycin and many other antibacterial agents in treating CBPP. Huebschle et al. (2006) studying the effect of danofloxacin in the treatment of naturally infected cattle with CBPP and the role of these treated animals in the spread of the disease, have shown that although clinical and bacteriological cures could not be obtained, treatment significantly reduced the transmission of the disease to healthy in-contact animals. Nicholas et al. (2006) found a large reduction in clinical cases and death following treatment of CBPP-infected animals with Advocin over a six-month period in the Caprivi region of Namibia in which mortality and morbidity were occurring despite vaccination. The authors also indicated that animals at post-mortem examination were devoid of active lesions. Instead, chronic lesions consisting of pleuritis and extensive fibrosis or, infer-quently, small encapsulated sequestra indicators which probably pose little risk to healthy contact cattle were detected. Interestingly, *Mmm*SC could not be detected in any of these chronic lesions. These observations are in accordance with the study's results in that all the CBPP lesions seen in animals treated with the oxytetracycline, consisted of adhesions and a small sequestrum in only one animal. All these chronic lesions were culturally negative for *MmmSC*.

In conclusion, the results of this study demonstrated that treatment with oxytetracycline did not result in significant sequestra formation in CBPP-infected animals. Full field validation is required in order to confirm these findings.

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