

A Study on Contagious Bovine Pleuropneumonia in Khartoum State, Sudan

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ABSTRACT

The study was carried out to investigate the occurrence of contagious bovine pleuropneumonia CBPP in Khartoum state. One-hundred twenty-two pneumonic lung tissue samples were collected from different slaughterhouses (116 samples most of which from local breed cattle) and from the field (six samples from cross breed cattle). Two-hundred and fifty-seven serum samples were collected randomly from cattle in different areas of the state. Tissue samples were cultivated using the standard mycoplasma procedures. *Mmm* was isolated from three pneumonic lungs collected from the field while no isolates were recovered from slaughterhouse samples. Histopathological sections from the positive samples revealed the typical picture of the CBPP which include fibrinonecrotic pneumonia within filtration of inflammatory cells and fibrin and distention of interlobular septae. One hundred and eight out of 257 serum samples were found positive for antibodies against *Mmm* using complement fixation test (CFT). Findings of this study confirmed the presence of CBPP in Khartoum state by the isolation and identification of the causative agent.

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Introduction

Contagious bovine pleuropneumonia (CBPP), which is caused by *Mycoplasma mycoides* subsp. *mycoides* (*Mmm*), is a highly contagious respiratory disease primarily of cattle [1]. The Pan African programme for the Control of Epizootics (PACE) has identified CBPP as the second most important transboundary disease in Africa after rinderpest. The disease represents a real problem to cattle health and production in Africa and estimates of annual losses in some countries account for 3.7 million Euros per country in the African sub-Saharan region [2]. Although the disease was eradicated in many developed countries in the world, Africa is still suffering and the incidence of the disease is continuously increasing in some countries. Reasons for the persistence of CBPP in Africa include uncontrolled cattle movement, cessation of vaccination campaigns and decreased surveillances [3].

The causative agent, which was first isolated by Nocard E. and Roux E. [4], is a member of the "Mycoplasma mycoides cluster". The cluster includes five biochemically and antigenically similar mycoplasma of animal importance [5]. Formerly, *Mmm* was known as *Mmm* (small colony SC- type) and grouped together with *Mmm* (large colony LC- type) in a single subspecies. Later, the two mycoplasma, based on genetic differences, were separated in different subspecies and referred to as *Mycoplasma mycoides* subsp. *mycoides* and *Mycoplasma mycoides* subsp. *Capri* respectively [6].

In the Sudan, the disease was first reported in 1875 in Darfur Province then it spread gradually to the most parts of the country except the Northern Sudan [7]. In Khartoum state, the disease was reported sporadically [8] but the actual status in the State was not clear.

The current research was carried out to study the current status of CBPP in Khartoum state using bacteriological and serological techniques.

Materials and Methods:

Samples were collected from different slaughterhouses in Khartoum State located between latitudes 15° 8` - 16° 39` N and longitudes 31° 36 34° 25` e between January– March. A total of 1786 animals were investigated and 116 pneumonic lung

tissue samples were collected from animals showing lung lesions (table 1). Pneumonic lung tissues, lymph nodes and sera were also collected from six animals showing typical clinical signs and PM lesions of CBPP at Hilat Kuku in Khartoum North. A total of 257 serum samples were collected randomly from dairy farms and cattle herds in Khartoum State.

Collection of Samples:

Tissue specimens (5 cm³) were aseptically cut from the interface between affected and normal tissue and placed in sterile plastic bags, labeled and transported within hours at 4 °C to the Central Veterinary Research Laboratory in Soba. For histopathology, small pieces (about 2 cm³) representing the pneumonic tissue was fixed in 10% formal saline.

Table 1. Sources of lung tissue samples collected from different slaughterhouses in Khartoum State.

Slaughter house	No. of Animals	condemned lungs	Pneumonic lungs
Khartoum	1020	78	58
East Nile	77	7	4
Al Kadro	220	7	5
Al Bogaa	110	15	12
Al Huda	94	14	12
Alsbaloga	35	13	13
Ganawa	230	15	12
Total	1786	149	116

For collection of serum, ten ml of blood samples were collected from the jugular vein in sterile vacutainers. Samples were left at room temperature for one hour then at 4 °C overnight. Sera were aspirated with sterile pipette after centrifugation (3000 rpm for 10 minutes), placed in sterile containers and stored at – 20°C till used.

Bacteriology:

Isolation and identification of mycoplasma was carried out using standard procedures. A liter of basic medium of beef heart infusion (Oxoid) supplemented-with 10 g tryptose, 30% yeast extract (20 ml), 20% inactivated horse serum, 50% glucose (2ml), in addition to penicillin 100,000 IU (0.33 ml) and 10% thallium acetate (0.25 ml) was used in the study [9,10].

Lung tissue samples were pulverized in broth media. The suspension was three tenfold diluted in the broth medium and each dilution was plated on the same solid medium. The pleural fluid was inoculated directly without previous dilution into broth medium. Both cultures were incubated micro-aerophilically (in humid chamber for solid cultures) at 37°C for three to ten days.

After purification, the isolates were identified by cultural (excluding the L-form bacteria, colonial morphology, rate of growth and the characteristic faint turbidity into broth medium), biochemical (Digitonin test) and serological (growth inhibition test using specific anti sera as described by [11] methods. Isolates were aliquoted in 2-ml volumes and stores at – 20°C.

Serology:

Complement fixation test (CFT) was used to examine serum samples for antibodies against Mmm as described by Lindley [12].

Histopathology:

Pneumonic lung tissue samples, fixed into 10% formalin, were prepared for histopathological examination according to Bancroft and Steven [13].

Results:

Isolation of Mycoplasma mycoides subsp. mycoides:

No mycoplasma was isolated from slaughter-house samples. Three mycoplasma isolates were recovered from three out of six cases showing typical clinical signs and P.M. lesions. The isolates were identified as Mmm depending on cultural characteristics (figure 1), digitonin test (figure 2), and growth inhibition test.

Histopathology:

Figures (3), (4) and (5) show different histopathological pictures of hepatized lung tissue samples from which Mmm isolates were recovered. In figure (3), widened interlobular septa, due to presence of fibrinous necrosis and cellular infiltration were observed. Figure (4) shows extensive loss of airspace due to necrosis and infiltration of neutrophils. Figure (5) shows hyperemia of alveolar wall capillaries and infiltration of few fibrin and neutrophils.



Figure (1). Colonial morphology of *Mmm* of recent field isolate grown on heart infusion agar after 3 days of incubation (×40).

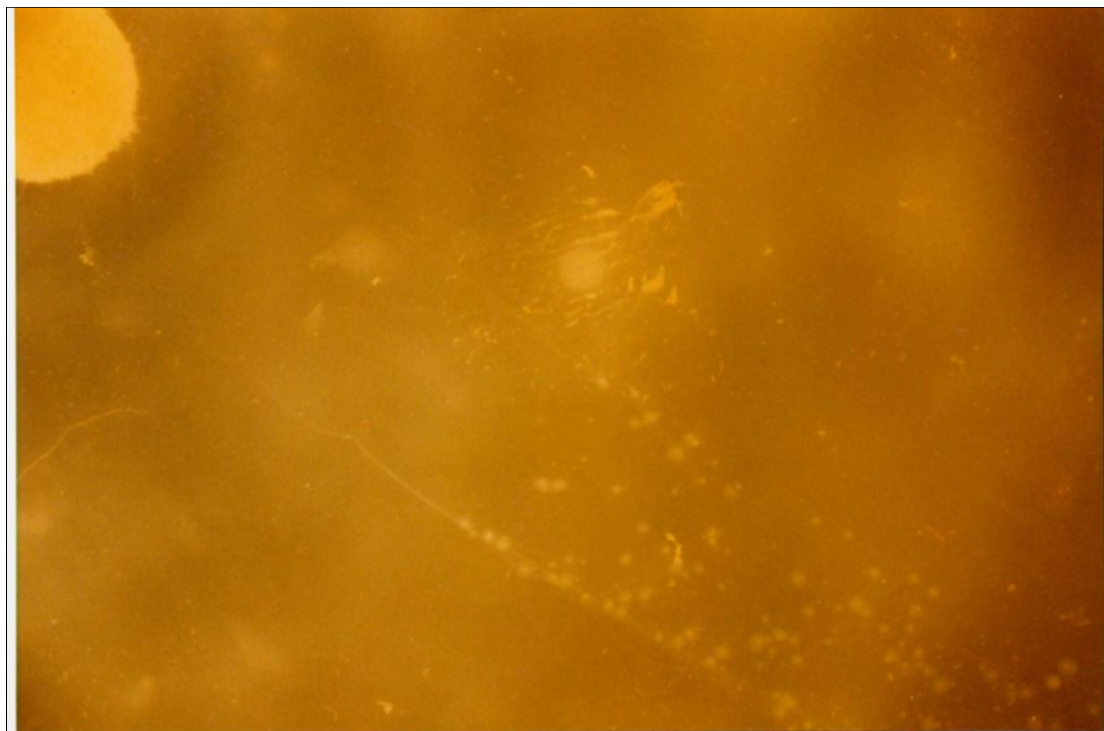


Figure (2). Sensitivity of the isolates to digitonin (x 10).

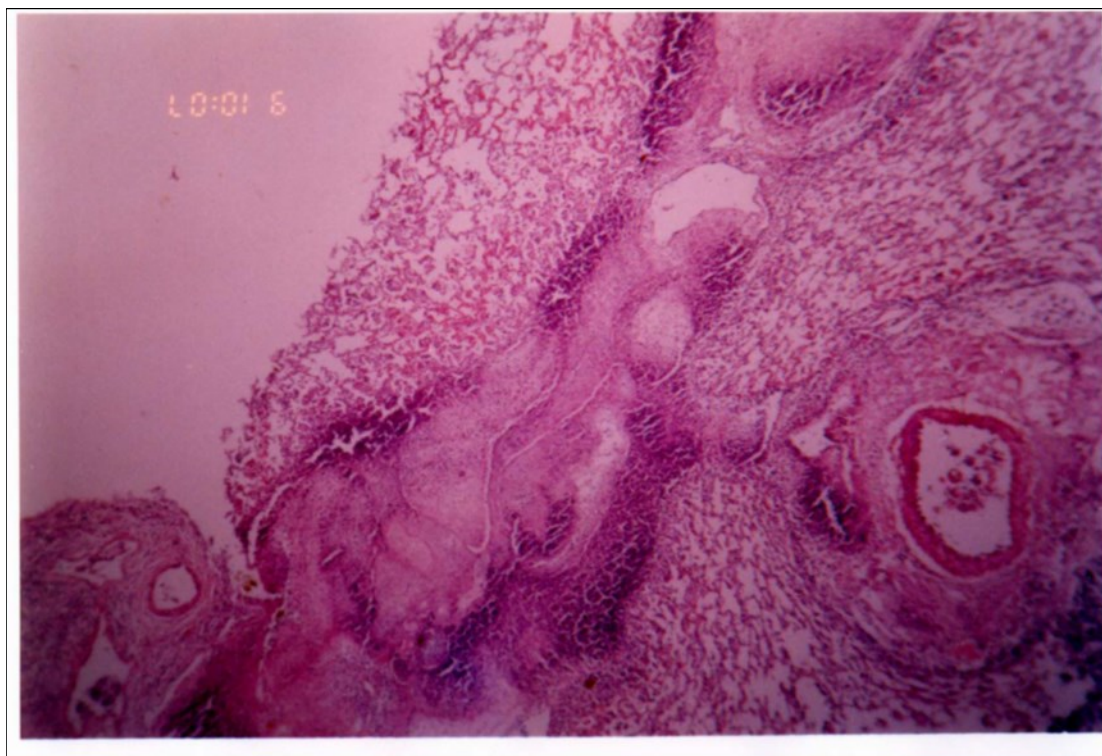


Figure (3): Widened interlobular septa in pneumonic lung tissue (H&E ×200).

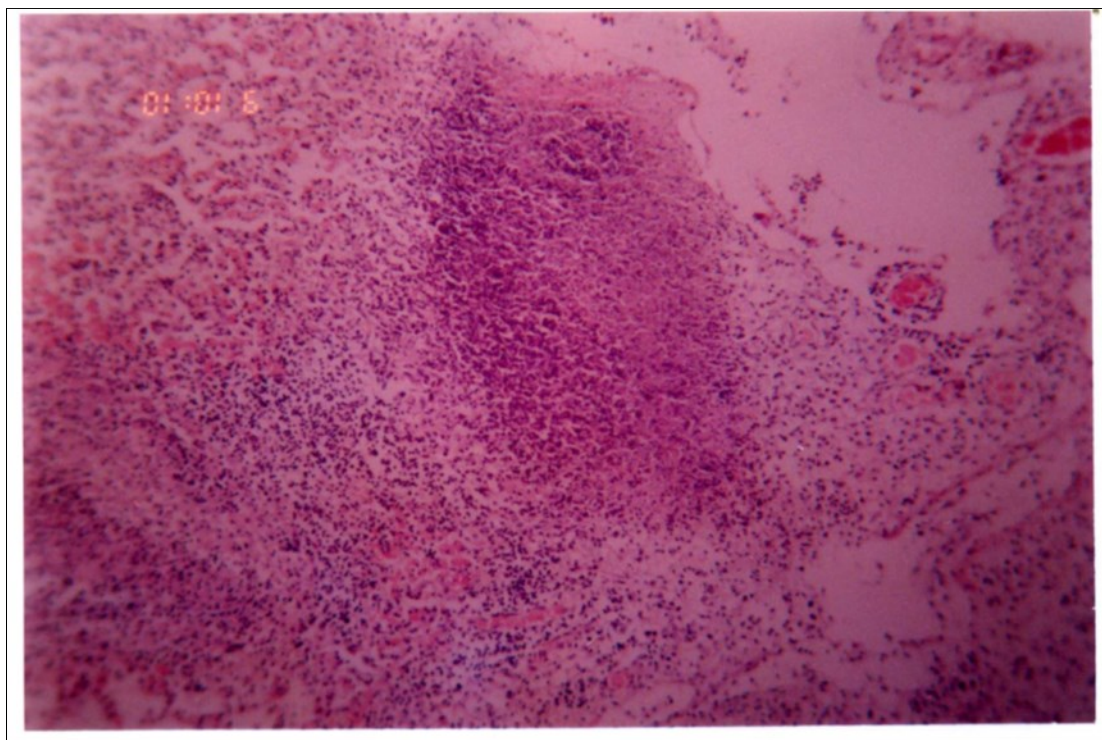


Figure (4): Extensive loss of air space due to necrosis and infiltration of neutrophils (H&E ×100).

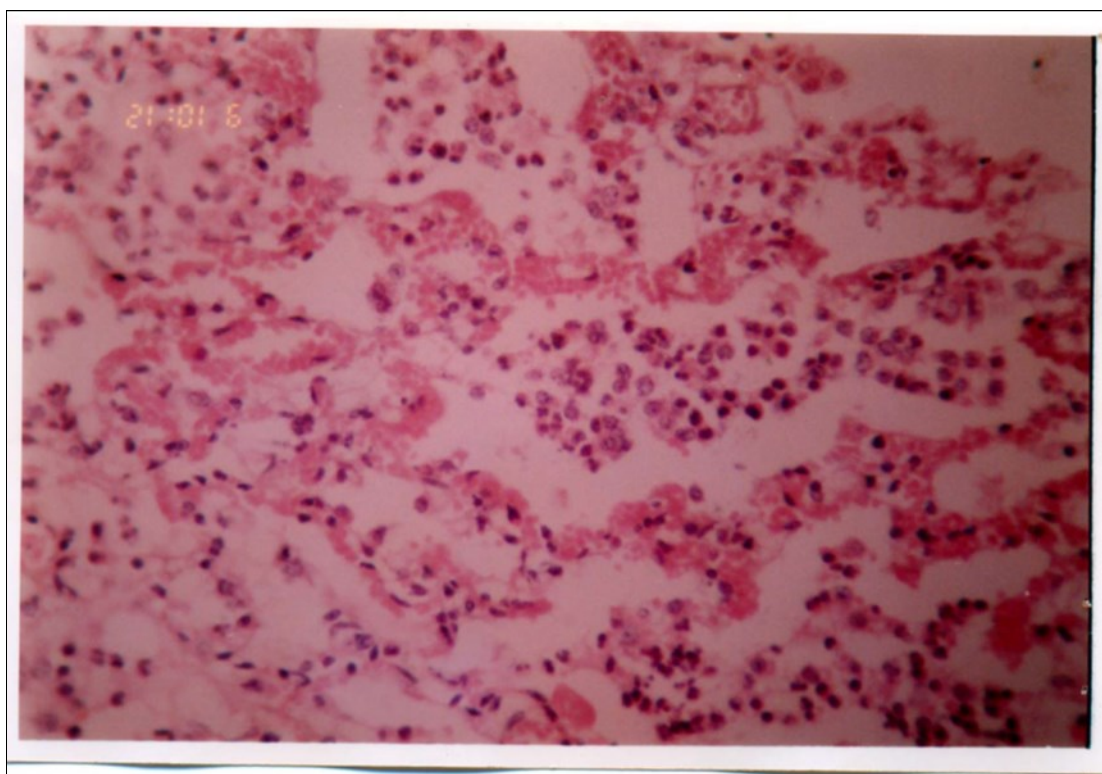


Figure (5): Hyperemia of alveolar wall capillaries, presence of few fibrin and neutrophils infiltration (H&E ×400).

Serological tests:

Complement fixation test (CFT):

Two hundred and fifty-seven serum samples were tested for antibodies against Mmm with the CFT. Antibodies were detected in 108 (42.2%) of animals (Table 2).

Table 2. Results of CFT for serum samples collected from different dairy farms in Khartoum State.

Source of samples	No. of samples	Positive sample	% of +ve
East Nile	104	35	33.60%
Northern Khartoum	30	3	10%
Hilat Kuku	13	13	100%
Elmahlab 2	11	11	100%
Kafori	12	8	66.60%
Elmahlab 3	20	10	50%
Omdurman	38	18	47.30%
Southern Khartoum	17	5	29.40%
Different sample	10	10	100%
Total	257	108	42.20%

Discussion:

The persistence of CBPP in most sub-Saharan countries in Africa including the Sudan necessitates continuous screening of cattle herds to detect positive animals and subsequently eliminate them to get rid of sources of infection. In this study, the situation of CBPP in Khartoum state was studied. The investigation include abattoir monitoring and screening of random serum samples of cattle for antibodies against Mmm using the recommended complement fixation test. Abattoir monitoring is regarded as a cheap and highly effective method of surveillance for CBPP because it can detect the silent carriers (chronically and subclinically infected cattle) [14]. Samples collected from different slaughter houses in Khartoum State revealed no isolation of Mmm. These results confirmed the previous study of Ameera M. [9] and other veterinary reports which as certain that no cases of CBPP were detected in slaughterhouses in Khartoum state. These results were expected due to the fact that no typical lesions (pleuropneumonia) of CBPP were found in all examined animals. In addition, only

healthy animals that are fat and free from clinical signs can pass the antemortem examinations. It was observed that most slaughtered animals were local breeds which may be more resistant to infection as it was reported that there are variations in disease susceptibility in cattle [15,16] added that cattle susceptibility to the disease depends on many factors such as the type of animal husbandry, individual resistance and other factors.

Three isolates of Mmm were recovered from three out of six crossbreed cattle that showed typical clinical signs and postmortem findings of CBPP. The source of these animals was dairy farms in Hilat Kuku, Khartoum north. Hilat Kuku is a large market and represents a center, for raising of dairy and beef cattle, in which productive animals are selected from the whole country. Accordingly, cattle from different regions and breeds are brought together giving a good chance for the transmission of CBPP from silent carriers to susceptible cross breeds (local with Holstein-Friesian) as seen in this study. The silent carrier animals are usually obtained by the use of antibiotics (mainly tylosin) to cure animals with respiratory symptoms or when routine vaccination has been practiced with long intervals between campaigns [15]. Treatment of animals affected by CBPP leads to the disappearance of clinical signs but they develop sequestra in their lungs from which life mycoplasma may be secreted intermittently to infect susceptible animals. These isolation results confirmed previous reports of the Administration of Animal Health and Epizootic control, Ministry of Animal Resources [8] which stated that sporadic cases of CBPP were observed in certain areas of the state including Khartoum north. However, diagnosis of the disease in that reports was based only on clinical signs and postmortem findings and no isolation of the causative agent was previously achieved in the state.

Although no advanced techniques like PCR were used to identify the isolates, procedures used in this study were quite enough to confirm the incidence. Affected animals with CBPP were diagnosed based on the clinical signs, the PM findings, the typical histopathological picture in addition to the isolation of the causative agent and its subsequent identification using cultural and biochemical procedures. Identification of the isolates was confirmed by the growth inhibition test as recommended in OIE manual [10]. The histopathological sections of diseased lungs, from

which Mmm (SC) was isolated, showed typical histopathological picture of CBPP. The CBPP lesion comprises abronchiolar necrosis and oedema which progress rapidly to an exudative serofibrinous bronchiolitis with extension to the alveoli and uptake of alveolar fluid into tissue spaces [17], lymphatic vessels and ultimately septal lymphatics [18]. With stasis, lymphatic vessels become thrombosed and ultimately fibrosed [19]. The histological section of the lung in acute stage of the disease showed odema in the lymphatics of the interlobular septa and interstitial tissue and massive infiltration of fibrin, macrophage and neutrophils into the alveolar lumen [20]. Also there was presence of lymphocytes and alveolar macrophages around the lymphatic vessels and septa margin [21]. These findings supported the isolation results and gave additional evidence for the diagnosis of the disease.

In this study, 108 out of 257 randomly collected cattle serum samples were found positive for antibodies against Mmm using complement fixation test (CFT). CFT is there commended test by the OIE for determining disease free status of cattle herds [10]. The test is reported to detect nearly all sick animals with acute lesions and few animals in the early stage of the disease or animals with chronic lesions. The percentage of positive animals in this study was clearly high (42.2%) and this may indicate that CBPP is spreading in Khartoum state and that may be due to uncontrolled animal movement, cessation of vaccination and decreased surveillances as stated by [3].

It was concluded from the present study that CBPP is present in Khartoum state and serological screening indicated that 42.2% of the investigated animals were positive. This situation necessitates a prompt action to control the disease in the state and prevents its spread.

Conflict of Interest :

The authors have declared that no competing interests exist.

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