Detection of Contagious bovine pleuropneumonia in condemned cattle lungs at Morogoro municipal abattoir in Tanzania

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SUMMARY

Control of re-emerged Contagious bovine pleuropneumonia (CBPP) in Tanzania in 1990s left spots of unvaccinated animals in various areas. Some of these animals were carriers of CBPP and have presumably continued to be sources of infection to other animals. We made an abattoir follow-up of slaughtered animals to understand whether the disease is still present in Tanzania. A total of 13 condemned lungs due to CBPP-like lesions at Morogoro municipal abattoir were collected from November 2011 to April 2012 and examined grossly, histologically and bacteriologically. Typical gross lesions of CBPP including expanded interlobular septa, sequestration, coalescing lungs, and fibrinonecrotic exudation were observed. Histologically, we observed fibrinonecrotic exudates filling and expanding the alveoli, desquamation of alveolar epithelial cells, lymphoplasmacytic infiltration in the interalveolar septa and around bronchi, bronchioles, and blood vessels, and vasculitis with subsequent vascular rupture and hemorrhage. *Mycoplama* cultures in two samples isolated *Mycoplasma* organisms with "fried egg appearance", typical of *Mycoplasma mycoides mycoides* small colony type, the causative agent of CBPP. We conclude that CBPP is still prevalent in Tanzania and continues to pose a potential impending epidemic in the future.

Keywords: CBPP, *Mmm*SC, Abattoir, histopathology, apparently healthy cattle, Tanzania

INTRODUCTION

Contagious bovine pleuropneumonia (CBPP), caused by *Mycoplasma mycoides mycoides* small colony (*Mmm*SC) type, is an acute, subacute or chronic infectious disease of cattle and water buffaloes. It is currently considered one of the main stumbling blocks to the growth of the livestock industry on the African continent. Direct or indirect yearly losses due to CBPP in Africa are estimated to be around 2 billion US dollars (FAO, 2004).

In Tanzania, the first outbreak of CBPP occurred in 1916 and was controlled in 1946. The second occurred in 1955 and was successfully controlled in 1965. The third outbreak occurred in 1990 and has not been controlled; instead it has widely spread in the country partly because of poor diagnosis (Melewas, 1999; Kapaga *et al*., 2005). As a result of this spread, in 2001 the government of Tanzania declared CBPP a national disaster and started implementing a long term program aimed at controlling and eventually eradicating the disease. However, the program did not work well and the disease continued to spread. By the year 2005 the disease had affected all regions of mainland Tanzania except Kilimanjaro and Lindi (Kitalyi, 2005). This outbreak is estimated to have caused more than 350,000 cattle deaths valued at about 25 million US dollars. Indirect losses through reduced production and lost trade opportunities are estimated to be 12.5 million US dollars. Until 2005, conservative estimates put the annual losses from CBPP to be about 2 million US dollars. In the same year the government declared CBPP as the most economically important disease of cattle in the country (Kapaga *et al*., 2005; Kitalyi, 2005).

Similar to many other African countries, effective vaccination is the only viable control strategy at present in Tanzania. However, no single vaccine has proven efficient in a complete protection of ruminants against CBPP. For instance, following the 1990 outbreak, vaccinations were done using a combined Rinderpest and CBPP vaccine (Bisec vaccine) from 1990 to 1993, but could not prove effective. It was replaced by T1-SR vaccine that was used extensively in 1995 to 1996. Again, this vaccine proved ineffective and was replaced with T1-44 in 1996 that is in use to the present. Besides the ineffective vaccines, vaccination programs were difficult to implement because the disease had widely spread in the country (Melewas, 1999; Kitalyi, 2005). The very wide infected area, limited resources, failure of herdsmen to present the whole herd for vaccination or repeat the vaccination as required, and their refusal to continue vaccination following vaccination reactions, were some of the reasons for the failure of CBPP control program (Mlelwa, 1999; Kitalyi, 2005). It was therefore decided to tackle a manageable area at a time. Thus in 2003 the plan started in Southern highlands (Mbeya,

Rukwa, and southern part of Iringa) where CBPP cases were frequent, moving northwards. The program was designed to vaccinate cattle three times in the first year, followed by annual vaccination for another 4 years, after which evaluation was to be done. This approach was time consuming, tiresome, and impracticable.

The failure of CBPP control program has resulted in spots of unvaccinated animals and more virulent field strains that have been potential sources of outbreaks now and again. Unfortunately, these outbreaks are loosely handled leading to a more wide spread of chronic asymptomatic infections. Many farmers are however, unaware of the infection in apparently healthy animals that are sporadically detected at abattoirs. In addition, most livestock stakeholders are ignorant of the presence of the disease and the potential impending epidemic in case of stress. It is under this background that the current study was designed to investigate for possible presence of CBPP in apparently healthy slaughtered cattle at Morogoro municipal abattoir in Tanzania.

MATERIALS AND METHODS

Study area

The study was conducted at Morogoro municipal abattoir. This abattoir receives animals from various areas as far as a diameter of 200 km around Morogoro town. However owing to cattle trade and trekking, some of the animals came beyond this diameter. The study involved visiting the abattoir to collect condemned lungs that had suspected CBPP lesions i.e. consolidated, fibrinous, edematous and with enlarged interlobular septa, after receiving a phone call from meat inspectors stationed at the abattoir. The study was carried out between November 2011 and April 2012.

Gross examination of samples

Collected lungs were transported to the Veterinary Pathology laboratory at the Faculty of Veterinary Medicine, Sokoine University of Agriculture, Morogoro. Thorough gross examination was done and findings recorded. Thereafter, some pieces of lungs were taken and fixed in 10% neutral buffered formalin (NBF) for histopathology. The rest of the lung lobe was frozen for microbiological examination.

Histopathology

Tissue fixation (10% NBF) was done for at least 24 hours. This was followed by tissue processing and paraffin embedding in a routine manner. After obtaining tissue blocks, sectioning was done by rotary microtome to obtain 4 microns tissue sections. The thin tissue sections on glass slides were deparaffinized, stained with hematoxylin and eosin (H & E), mounted, and observed under light microscope (Olympus BX 41, Japan) and photographs taken by digital camera (Olympus DP21, Japan).

Culture of *Mmm*SC

With the aid of a sterilized pair of forceps and a scalpel blade, 5 pieces of lung tissue from each collected lung were carefully trimmed and each piece smeared on one angle of four culture plates containing *Mycoplasma* Experience media (Reigate, UK). The smear was then streaked on the other area of the plates by means of a sterile wire loop. Subsequently, the plates were incubated at 37°C in a humid atmosphere containing 95% air-5% CO_2 and inspected under stereo microscope daily for 7 days for *Mmm*SC growth.

RESULTS

Gross observations

All the 13 collected lungs were unilaterally affected with most severe lesions on diaphragmatic lobes. The affected lungs were enlarged, non-collapsing, and covered with fibrinous exudates. On cut surface, a clear brownish to yellowish edema fluid oozed, the interlobular septa were prominently enlarged, and the parenchyma was marbled with yellowish and reddish-brown patterns (Figure 1). In some areas, sequestration was evident with coagulative necrosis surrounded by prominent fibrous connective tissue.

Histopathology

Slide review for histopathological appearance of routinely processed and stained (H & E) sections revealed various changes in the airways, lung parenchyma, and blood vessels. The bronchi and bronchioles were surrounded by dense aggregates of lymphocytes and plasma cells (Figure 2). Their lumina were filled with fibrin, edema and karyorrhectic and degenerate lymphocytes, plasma cells and macrophages (Figure 2, 3). The bronchial epithelium, submucosa, smooth muscle layer and the cartilage were also infiltrated with lymphocytes and plasma cells (Figure 4).

We observed massive congestion and hemorrhages of capillaries in the interalveolar septa particularly in places where the lungs were moderately affected and the tissue architecture appeared more normal (Figure 3). In severely affected areas, the interalveolar septa were diffusely expanded and filled with massive fibrin, edema, and cellular debris. The later was due to karyorrhexis and necrosis, and heavy infiltration with viable and degenerate lymphocytes, plasma cells, macrophages and few polymorphonuclear leukocytes (Figure 5).

The alveoli were filled and expanded by fibrin, edema and variable number of lymphocytes, plasma cells, and macrophages (Figure 3, 5). Depending on the severity the alveolar epithelium

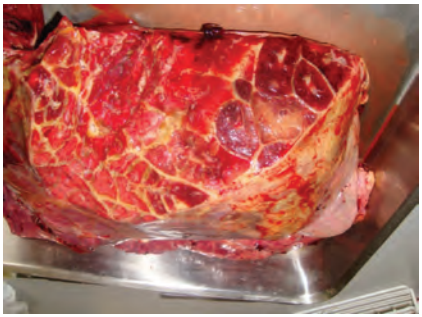

Figure 1. Cut surface of condemned lung at abattoir. Reddish-brownish-yellowish marbling of lung parenchyma and prominent interlobular septa.

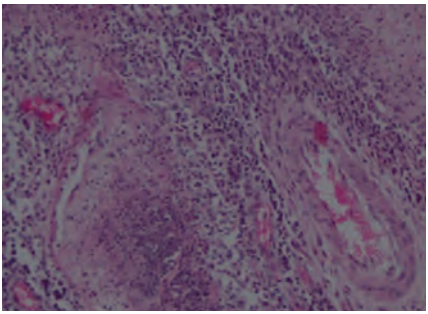

Figure 2. Section of cattle lung showing a bronchiole (left) and a peribronchiolar artery (right). The structures are heavily surrounded by mononuclear leukocytes, the bronchiole is filled with exudates composed mainly of fibrin and necrotic (some viable) mononuclear leukocytes and is inflamed (bronchiolitis), ruptured and releasing the exudates to the parenchyma. The endothelium of the blood vessel is damaged and vasculitis is at initial stages. H&E, x200

was intact or desquamated. In more severe cases, alveoli were no longer recognized due to degenerated, desquamated, and necrotic epithelium as well as fibrinonecrotic exudates and lymphoplasmacytic infiltration.

Blood vessels in affected areas were congested, hemorrhagic and inflamed (vasculitis) (Figure 2, 6, 7) and some contained fibrin (Figure 3). The inflammation was evidenced by disruption of tunica media and adventitia and presence of inflammatory cells and karyorrhectic debris. In some cases the endothelium was hypertrophied. A dense lymphoplasmacytic aggregation was observed around the blood vessels (Figure 2, 6, 7).

Isolation of *Mycoplasma* results

Daily observation under stereo microscope of cultures for *Mmm*SC revealed that only 2 out of the 13 collected lung samples grew Mycoplasma organisms. The observed Mycoplasma colonies were small, brownish-red, and had characteristic fried egg appearance with an elevated central spot, typical of *Mmm*SC colonies (Figure 8).

DISCUSSION

Control of CBPP in developing countries is still a challenge. This is exemplified by the current study which vividly demonstrates presence of the disease at Morogoro abattoir in Tanzania despite the control efforts instituted by the Government. This abattoir receives cattle from several places within the country through cattle trade and trekking. The detection of

CBPP in this abattoir signifies presence of the disease in several places in the country since most of the cattle slaughtered at this abattoir are bought from local cattle markets brought by dealers from different places within Morogoro region and other nearby regions like Dodoma, Pwani, Iringa, Tanga and Singida. Hence the spread of CBPP at the moment could be wider than expected. There is therefore, a need to make a close ollow up on the epidemiology of the disease in the country to assess its prevalence.

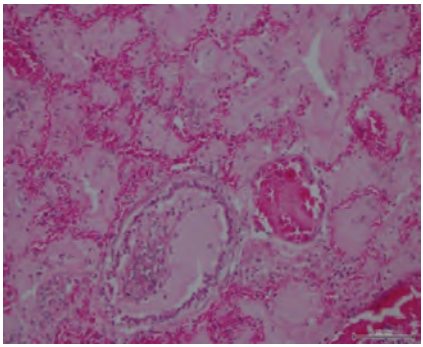

Figure 3. Lung parenchyma. The bronchioles, alveoli and blood vessels are filled with fibrin and edema. In addition, karyorrhectic and degenerate lymphocytes, plasma cells and macrophages are components of the bronchiolar and alveolar exudates. The interalveolar septa are hyperaemic, haemorrhagic, expanding, and infiltrated with lymphocytes and plasma cells. H&E, x200

Control of CBPP in Europe and America has been successful through diagnosis, restricted cattle movement and test, and slaughter and compensation policies.

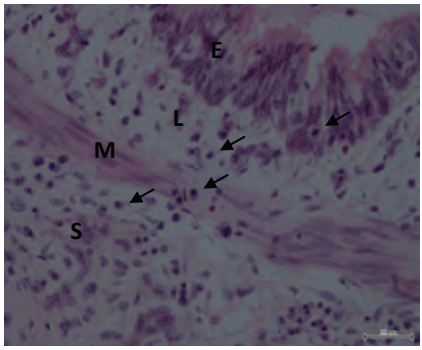

Figure 4. Histological section of cattle lung showing part of a bronchus with lymphoplasmacytic infiltration (arrows) in the epithelium, lamina propria, smooth muscles, and submucosa. E, epithelium; L, lamina propria; M, smooth muscle; S, submucosa. H&E, x400

In endemic areas of Africa the control is challenged by lack of capacity to diagnose carrier asymptomatic animals and impractical policies on restricted cattle movement and test as well as slaughter and compensation. As a result CBPP continues to pose potential impending epidemics like the ones that occurred in late 1990s (Bölske *et al*., 1995; Melewas 1999; Kapaga *et al*., 2005). The control measures instituted to these epidemics included limited cattle movement and vaccination. Unfortunately, due to inefficient vaccine, wide infected area, limited resources, failure of herdsmen to present the whole herd for vaccination or repeat the vaccination as required, and their refusal to continue vaccination following vaccination reactions (Melewas 1999; Abdo *et al*., 2000; Thiaucourt *et al*., 2000; Mbulu *et al*., 2004; Kitalyi 2005), many animals

were not vaccinated. This created spots of apparently healthy infected carrier animals that kept on spreading the disease as evidenced in this very study. The situation is exacerbated by large numbers of pastoralists in Tanzania whose animals are continuously at the risk of contracting CBPP due to constant movement in search of water

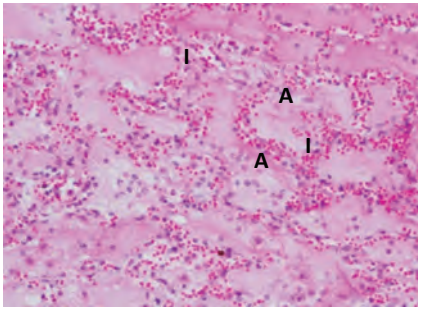

Figure 5. Histological section of alveoli (A) and interalveolar septa (I). The interalveolar septa are hemorrhagic, diffusely expanded and filled with fibrin, oedema, and cellular debris. The later was due to karyorrhexis and necrosis, and heavy infiltration with viable and degenerate lymphocytes, plasma cells, macrophages and few polymorphonuclear leukocytes. H&E, x400

and pasture (Schnier *et al*., 2009).

The incapability to diagnose CBPP carrier asymptomatic animals is a major factor to the continual spread of the disease. Currently, diagnosis of such animals is possible only at the abattoir during meat inspection since the pathological lesions of CBPP are distinctive (Ferronha *et al*., 1990; Di Francesco *et al*., 1998). The unilaterally

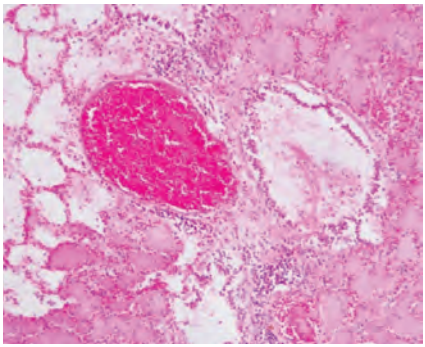

Figure 6. Lung section showing congested blood vessel, perivascular and peribronchiolar lymphoplasmacytic aggregation, desquamating bronchiolar epithelium, and fibrinous and oedematous alveoli. H&E, x200

affected lungs, marmorisation, hepatisation, necrosis, sequestration, and pleurisy and most of the histological findings observed in this study were consistent with the distinctive CBPP lesions previously observed under natural or experimental infection (Ferronha *et al*., 1990; Di Francesco *et al*., 1998; Sacchini *et al*., 2011). The World Organisation for Animal Health, OIE, approves abattoir surveillance of the disease by examining lungs with CBPP-like lesions as a practical method for disease monitoring (OIE 2008). Consistently, the detection of typical pathological lesions in the lungs of slaughtered cattle and subsequent microbiological analysis of sampled organs has been used previously as part of active surveillance of CPBB in other countries (Stärk *et al*., 1995; Bashiruddin *et al*., 1999; Aliyu *et al*., 2000; Cetinkaya *et al*., 2003). Our study therefore serves an awakening

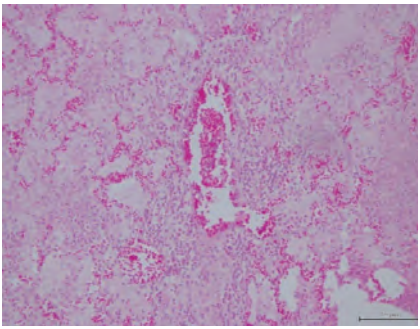

Figure 7. Lung section showing hemorrhagic and inflamed (vasculitis) blood vessel with perivascular aggregation of lymphoplasmacytic leukocytes. H&E, x200

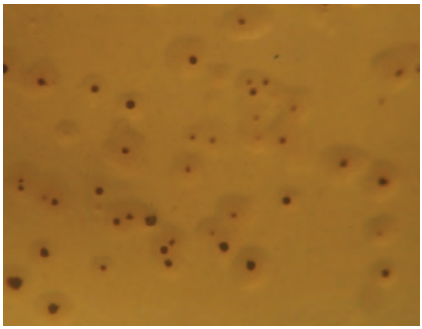

Figure 8. Mycoplasma culture. Lung tissues were trimmed, smeared and streaked on culture plates containing Mycoplasma Experience media before incubation at 37□C in a humid atmosphere of 95% air-5% CO2. They were then observed under stereo microscope at x40. Note the typical "fried eggs" colonies of MmmSC.

call to carry out a thorough surveillance of the disease in the country to assess its magnitude and plans for control measures.

Although all the 13 lung samples were grossly and histologically pathognomonic of CBPP, only 2 (15.4%) cultures grew *Mycoplasma* organisms. This alludes that despite the tedious and delicate technique of *Mmm*SC culture and the longer time it takes (up to 7 days observation in our study) the method leaves out many positive samples. Other researchers have also noted more or less similar results. For instance, in an examination of 11 CBPP affected lungs from Portuguese cattle only 4 (36.4%) isolated *Mmm*SC organisms on culture (Ayling *et al*., 1998). In addition, Cetinkaya *et al*., (2003) cultured 62 abattoir lung samples from Turkish cattle with lesions suggestive of CBPP but only 3 (4.8%) samples grew *Mycoplasma* species. Some of the reasons for this difference may stem on the difficulties in isolating *Mmm*SC partly due to non-viable microbes (Adegboye *et al*., 1995; Cetinkaya *et al*., 2003) and presence of sequester and marbling (Provost *et al*., 1987).

Compared to other diagnostic methods like ELISA and CFT and the lack of diagnostics that can detect carrier animals, abattoir screening of animals remains to be the best option in monitoring CBPP in developing countries where slaughter and compensation is impossible. Abattoir examination of lungs for CBPP diagnosis is cheaper and can easily be handled by meat inspectors. However, to confirm the disease, isolation of *Mmm*SC or application of other techniques such as PCR can be employed.

The finding that CBPP is prevalent in cattle slaughtered at Morogoro abattoir

should alert the livestock stakeholders of the presence of the disease in the country. We presume that these cattle come from Morogoro and other places in the country because of the history provided on cattle market and trekking. This information is pertinent in follow-up studies to explore the prevalence of the disease and propose effective control measures.

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