EFFICACY OF LONG-ACTING OXYTETRACYCLINE IN TREATING CONTAGIOUS BOVINE PLEUROPNEUMONIA AND POTENTIAL FOR CARRIER STATUS IN TREATED CATTLE

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DECLARATION

This is my original work and has not been presented for a degree or any other award in a university or any other institution.

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DEDICATION

To my family the Otinas who have instilled in me the need to better myself in all aspects of life. My husband, Simon Dzombo -you have continued to support my quest for further education. My daughter, Marcela Nzalambi -your sacrifice has been great as I pursued my academic endeavors.

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LIST OF ABBREVIATIONS

CBPP	Contagious bovine pleuropneumonia
KALRO	Kenya Agriculture and livestock Research Organization
Mmm Sc	*Mycoplasma mycoides mycoides* Small colony
FAO	Food and Agriculture Organization
c-ELISA	Competitive Enzyme-Linked Immunosorbent Assay
CFT	Complement fixation test
OIE	Office International des Epizooties
ml	Millilitres
Kgs	Kilograms
^{0}C	Degrees Celsius
β	Beta
α	Alpha
OD	Optical Density
µl	Micro litres
PI	Percent inhibition
PM	Post mortem
df	Degrees of freedom
Min	Minutes
CFU	Colony Forming Unit

ABSTRACT

Contagious bovine pleuro-pneumonia (CBPP) is currently one of the most important diseases affecting cattle in Sub-Saharan Africa with great economic implications. It causes restrictions in trade, loss of productivity and loss of life in affected cattle. This respiratory disease has been controlled by strategies including restriction of animal movement, vaccination, test and slaughter. However, these strategies have had challenges in implementation in some places where it occurs due to: cultural practices encouraging aggregation of cattle; husbandry system that involves constant cattle movement; quality of vaccines delivered in vaccination campaigns; lack of funds for setting infrastructure to control movement; and funding compensation in test and slaughter exercises. This has resulted in surges of outbreaks of the disease in areas where it had been controlled. Due to these challenges, other control strategies have been advocated including the use of antibiotics. The objectives of this study were: (1) to assess the efficacy of one of the antibiotics used, long-acting Oxytetracycline (®Alamycine, Norbrook Laboratories Limited), in treating contagious bovine pleuropneumonia in cattle, and (2) to assess whether the treated animals become carriers. .

Thirty cattle were bought from a CBPP-free area and brought to KALRO research station in Muguga. They were naturally-infected by exposing them to known-infected cattle; earlier infected with pathogenic *Mycoplasma mycoides* variety *mycoides*, small colonies (*Mmm SC*). After 42 days of contact, the thirty cattle were randomly divided into two groups of 15 each: Treatment group: treated with Oxytetracycline [a single dose 20% Oxytetracycline each (20 mg/Kg body weight), deep intramuscular] and Control group; given a placebo (saline). . The two groups were followed-up for another 31 days post treatment, during which progression of disease was assessed through clinical-sign observations, serology (complement fixation test) and mycoplasma isolation from naso-pharyngeal swabs. Cattle that showed severe illness were killed humanely by captive bolt stunning and exsanguination, post mortem examination

done, and samples of infected tissues collected. Then the carrier-status experiment followed, where each of the two groups was mixed with 5 CBPP-free (sentinel) cattle for one month; the sentinels were then tested to see if mycoplasma organisms could be isolated from their naso-pharynx.

For the first experiment, significantly ($p< 0.05$) higher rectal temperatures were recorded in the control group particularly between day 7 and 14 post-treatment. The severity of CBPP as determined by clinical observations (general body condition, diarrhoea, appetite, respiratory distress, nasal discharge, cough, and rectal temperatures) was more in the control group; that is: the Oxytetracycline treatment group exhibited milder clinical signs ($p <0.00$) over the same period. No fever (39.5 ^{0}C) was recorded in the Oxytetracycline treatment group, while, in the control group, temperatures were found to be significantly ($p <0.05$) high between days 7 and 14 post treatment.

When sentinel animals were mixed with the treated group (infected and Oxytetracycline treated cattle), none of them yielded mycoplasma organisms from naso-pharynx; thus, there was no evidence of development of carrier status after treatment. On the other hand, when sentinel animals were mixed with the control group (infected and not treated cattle), one of them yielded mycoplasma organisms from pleural fluid; caudal mediastinal, peribronchial lymph nodes.

In conclusion this study has shown that Oxytetracycline treatment of cattle infected with CBPP has some efficacy by reducing the severity of the disease. It has also shown no evidence of carrier-status development after the treatment. However, further studies are recommended to support these findings.

1. INTRODUCTION

1.1 Background information

Contagious bovine pleuro-pneumonia (CBPP) is currently one of the important diseases of cattle following the declaration of eradication of Rinderpest in year 2011 (OIE, 2011). The disease has a high potential for rapid spread. Though the disease was introduced in Africa earlier, in 1853, its importance and control has been overshadowed by various events including the introduction of Rinderpest in 1896-1897, by the South African war, introduction of East coast fever, during the Herero war, and the human influenza pandemic of 1918 (Thiacourt *et al.*, 2003).

The disease causes great economic loss due to: mortality, loss of weight, reduced fertility, reduced milk production, reduced working ability, restriction of trade and indirect cost of controlling the disease (Tambi, 2006). Introduction of the disease to a susceptible herd leads to devastating losses in terms of morbidity and mortality. This was seen when it was re-introduced in Angola in 1969. During this outbreak it led to 75% morbidity and 68% mortality (Thiacourt *et al.,* 2003). The indirect costs include: costs of vaccination, loss of money in reduced trade where restricted movements are applied and money spent in compensation to farmers during test and slaughter campaigns (Tambi, 2006; Thomson, 2005).

The cost of eradicating the disease from Africa is estimated at 300-450 million British Pounds (FAO, 2003). Using participatory epidemiologic approach to understand the dynamics and impact of the disease to the affected communities, a global upsurge of the disease was estimated to cost 2 billion US dollars, an equivalent of 1.7 billion British pounds (FAO, 2003). Within a country, the disease leads to restriction of movements due to confinements as a result of outbreaks. It is also one of the most devastating trans-boundary diseases of cattle in Africa (Chandapiwa, 2011) due to export restrictions imposed on infected countries by the disease-free countries.

Many efforts are thus employed in attempts to control and eradicate the disease. In Italy, Botswana and Portugal, like most parts of Europe, stamping out strategy was applied; it includes designation of infected areas, intensive surveillance, and reduction of susceptible animals in affected areas by slaughter and disposal, decontamination of infected areas, restriction of animal movement and quarantine of affected animals to reduce spread of the disease (Provost, 1987; Ayling *et al*., 2000).

In Africa the most widely practiced control strategy currently is vaccination. This strategy faces a host of challenges ranging from the quality of vaccines produced, cost of repeated campaigns, handling of the vaccines and inadequate infrastructure (Rweyemamu *et al*., 1995; Waite and March, 2001; Thiacourt *et al*., 2003). Failure of vaccination strategy to control the spread of the disease has led to reconsideration of alternative strategies including use of antibiotics. Although, officially, antibiotics are not recognized for treatment of CBPP, their use has been wide spread especially in pastoral communities. A possible cure may be found from three classes of antibiotics that are effective against mycoplasmas: Tetracyclines, Fluroquinolones and Macrolides (Mitchell *et al.*, 2012). There is presumed possibility of antibiotic treated cattle being carriers, acting as reservoirs of infection for susceptible cattle (Thiaurcourt *et al*., 2004); however, this has not been supported with scientific evidence. Oxytetracycline has been shown to reduce inflammation in vaccine inoculation site. In double dose, it has been shown to reduce clinical signs, formation of sequestrations and lung lesions in cattle infected with CBPP.

This study explores whether animals suffering from contagious bovine pleuropneumonia benefit from treatment with long acting Oxytetracycline (single dose 20% Oxytetracycline) and whether they eventually become carriers and spread the disease.

1.2. Objectives

1.2.1General objective

To assess the efficacy of long-acting Oxytetracycline in treating contagious bovine pleuropneumonia in cattle and potential for carrier status in treated cattle

1.2.2 Specific objectives

- To determine the efficacy of a single-dose long-acting Oxytetracycline in treating contagious bovine pleuropneumonia in experimentally-infected cattle.
- To assess the potential of developing a carrier status in cattle experimentally infected with *Mmm SC* and treated with a single dose of long acting Oxytetracycline.

1.3 Justification of the study

Contagious bovine pleuropneumonia continues to have adverse impact on cattle husbandry in Africa. Animal movement control has been hard to implement, test and slaughter needs compensation, hence both strategies are not acceptable to most of the African pastoralist communities, due to socio - cultural practices, food insecurity and poverty levels. Vaccination faces a host of challenges. Antibiotics may be the alternative Africa is looking for. Tylosin has been used in some experiments however it is more expensive to use than vaccination strategy hence the need for more studies to be done to understand impact of antibiotics on CBPP control. Oxytetracycline is already the most widely used antibiotic in livestock. It has been shown to reduce loss due to CBPP infection. Long acting preparation of Oxytetracycline was considered for this study as it avails an antibiotic that can be administered once, reducing chances of abuse and drug resistance. It is also available at a fairly reasonable price. Understanding of Oxytetracycline impact on CBPP control will serve as a benchmark for other antibiotics that will be investigated for use in CBPP control. The

understanding of the effect of Oxytetracycline on clinical course and carcass weight will provide a control strategy that is acceptable, from social-economics perspective.

2. LITERATURE REVIEW

2.1 Etiology of contagious bovine pleuro-pneumonia

Contagious bovine pleuro-pneumonia (CBPP) is a disease of cattle characterized by cough, nasal discharge, dyspnea, tachypnea and presence of lesions including fibrinous pleurisy and pneumonia (Mbengue *et al*., 2013). It is caused by *Mycoplasma mycoides mycoides Small Colony (Mmm SC).* The organism belongs to the genus *Mycoplasma*, some members of which are important livestock bacterial pathogens worldwide (Fischer *et al.*, 2012). The genus has the smallest free-living bacteria which are usually 0.2-0.3 µm in diameter, have no cell walls, can pass through bacteria-excluding filters and elude lysis. *Mycoplasma mycoides mycoides SC* belongs to '*Mycoplasma mycoides* cluster' which comprises closely related ruminant pathogens including *Mycoplasma capricolum* subsp. *Capripneumoniae* (the agent of contagious caprine pleuropneumonia), *Mycoplasma capricolum* subsp. *Capricolum, Mycoplasma leachii* and *Mycoplasma mycoides* subsp. *Capri* (Xiaoxing *et al*., 1996).

Mycoplasma mycoides subsp. *Mycoides* SC is a pleomorphic bacterium that grows under aerobic and anaerobic conditions; it can be coccoid, filamentous or star shaped. It is sensitive to temperature and can be inactivated by temperatures of 56^0 C and 60^0 C. It can also be inactivated by 0.05% formaldehyde, 0.01% mercuric chloride, and 1% phenol. Serial passage in cattle or culture reduces its virulence and pathogenicity. In environment out of the host, the organism can survive for three days in the tropics and two days in temperate conditions. In frozen pleural fluid it can survive for ten years (Thiacourt *et al.*, 2003).

2.2 Occurrence and distribution of the disease

Contagious bovine pleuropneumonia is currently a disease of Africa (FAO, 2003). It used to occur all over the world, except for South America and Madagascar, at one time or another, but has now been eradicated in Australia, North America and Europe (Xiaoxing *et al*, 1996; FAO, 2003; Thiacourt *et al.,* 2003; Chandapiwa, 2011). Asia may also be free from the disease. It was first described in Europe in 1564. The disease was introduced to South Africa from Netherlands in 1853. Between 2005 and 2014, 27 countries in West, Central and East Africa reported outbreaks of the disease. It is endemic in Angola and Northern part of Namibia (FAO, 2003). In Kenya, it is present in the Karamajong ecosystem (bordering Uganda and Sudan), the Somali ecosystem in the eastern part of the country and in the Maasai ecosystem (Kariuki-Wanyoike *et al*., 2014). In Tanzania, endemic foci were also identified by FAO (FAO, 2003). The disease is in the A list of diseases by OIE as it requires special attention due to its capacity of rapid spread and socio-economic impact to communities where it is endemic (Xiaoxing *et al.,* 1996). Age, sex and breed determine the prevalence of the disease in cattle, with female zebus being most susceptible to the infection (Mbengue *et al.*, 2013).

Spread of the disease is determined by three important factors: intensity of the infection; closeness of contact; and density of susceptible cattle (Thiarcout *et al*., 2004). Sources of infection include aerosol droplets of bronchial secretion and nasal discharge from clinical or subclinical cattle that are actively shading infective microorganisms. Sources of infection can also be by carrier cattle that have recovered from the infection and chronically infected animals (lungers). Other suspected sources of infection are aerosol droplets of infected urine, and fresh and frozen cattle semen (Thiacourt *et al*, 2004). Experimentally, the disease has been induced by naso-tracheal intubation with *Mmm SC* (Scacchia *et al.,* 2011; Isabel *et al.,* 2012). In Africa, congregation of cattle at kraals, watering points and livestock markets has

favoured the spread of the disease. Other conditions that have exacerbated the disease spread are socio-cultural practices like transhumance, dowry payments, cattle rustling and civil strife (Thiacourt *et al.,* 2003; Chandapiwa, 2011).

2.3 Clinical signs of the disease

The disease has an incubation period of 3 weeks to 6 months and can take various forms. In young calves less than 6 months the disease is mainly associated with lameness due to arthritis; the tarsal and carpal joints are swollen (FAO, 2002; Di Teodoro *et al.,* 2018). In a naive herd the disease is severe and may be characterized by high mortality.

Most of the cases remain sub clinical, especially in areas that have history of use of antibiotic to treat cases or vaccination routines with long intervals (FAO, 2002). However, in the start of an outbreak, 10% of the cases may be of hyper acute form, where the animal dies with no significant clinical signs. The only sign that may be picked is marked fever of over 39.5°C.

In the acute form the animal has marked respiratory distress. The labored breathing is shallow and rapid. The animal has chest pain; it resists percussion of the chest and may grunt from time to time. The nostrils are dilated and the animal may exhibit mouth breathing. At the stage when the animal experiences bouts of shallow, dry and painful cough especially after extraneous activities, it isolates itself, and may have declined appetite, It also stands with neck extended, head lowered, elbows abducted and back slightly arched (FAO, 2002). On auscultation, crepitation, rales and pleuretic friction rubs may be picked. On percussion, dull sounds on the lower thorax may be revealed (FAO, 2002).

In the subclinical form the disease progresses and is more severe than the acute form. The cough is more frequent and pronounced. The animal at this point may show signs of dullness, may isolate itself further and progress to recumbency. The animal also has marked nasal

discharge that ranges in viscosity (watery, mucoid to mucopurrulent) and colour (clear, white to yellow). Blood stained mucoid discharge and diarrhoea may also be observed (FAO, 2002).

Most sub-acute cases transition to chronic form. In the chronic form, clinical signs subside and are more difficult to identify, though the animal may have occasions of intermittent fever. Common signs in this stage is loss of body condition and loss of appetite (FAO, 2002).

2.4 Pathological lesions of the disease

The disease causes lesions that mainly occur in the lungs. Pneumonic areas are unilateral, most often affecting the diaphragmatic lobe (Di Tereodoro *et al.,* 2018). Animals that die from the hyperacute form may only have straw coloured copious pleural and pericardial effusion.

In acute cases, there is yellow turbid fluid in the thoracic cavity, a fibrinous yellow to yellow-grey omlette like lining of the pleura and chest, adhesions of the thoracic organs to the chest and marked marbling of the affected lung; serofibrinous interlobular oedema with red, yellow and grey lobular hepatization (Niwael, 2009; Di Tereodoro *et al.,* 2018). Lympnodes draining the lungs and thoraxic cavity are often enlarged, eodematous, with pinpoint haemorrhages and may sometimes have sequestra or necrotic areas (Di Tereodoro *et al.,* 2018). Sometimes the kidney can be involved with infarcts and haemorrhagic areas.

In the chronic form, fluid, is rarely found in the thoracic cavity; lesions that are more common are fibrous pleural adhesions and necrotic areas that appear as scars or sequestra. The sequestra are pink or white oduorless necrotic areas that vary in size and are surrounded by fibrous capsule that may be as thick as 1 cm (Di Tereodoro *et al.*, 2018). The sequestra tend to shrink with time. In some chronic cases the only lesion seen in the lungs are tags that are associated with complete remission (Muuka *et al*., 2019).

2.5 Diagnosis of the disease

Detection of CBPP can be by observation of clinical signs, pathological findings on post mortem examination, isolation and characterization of causative agent, serology and molecular techniques (Bashriudin *et al.,* 2005; Amanfu *et al*., 2006; Lutta, 2010).

Observation of clinical signs is not a reliable method of diagnosis and often the disease is confused with other respiratory diseases (Masiga *et al.,* 1996; Nicholas, 2000). Demonstrating pathological lesions is a more reliable method of diagnosis as the lesions, sequesters, are almost pathognomonic. Post mortem findings can be effective for passive surveillance in abattoirs (OIE, 2021). For diagnosis in disease-free zones and in control of outbreaks, in live animals, serological tests are more reliable than clinical signs (TerLaak, 1992; Rurangirwa, 1995). However, serological tests pose a challenge of cross reaction with other organisms in the closely-related 'mycoplasma cluster' (Xiaoxing *et al.,* 1996).

Serological tests are divided into two large classes: the primary binding tests and the secondary binding tests (Rurangirwa, 1995). The primary binding tests allow for reaction of antigen and antibody; the resulting complexes being measured with a marking system. A primary binding test has an advantage as it uses all components of the pathogenic organism to detect the host antibodies and all activities, the isotypes, of host antibodies to the pathogenic organism are directly detectable. Immunofluorescence and Competitive Enzyme-Linked Immuno-Sorbent Assay (c-ELISA) tests, both direct and indirect, are two tests for CBPP diagnosis that belong to this class of serological tests. The secondary binding tests involve two stages: the first being the antibody antigen reaction; and the second stage depends on whether the antigen antibody complex is soluble or particulate. If soluble, the antibody antigen complex will form a precipitate - examples of such tests are the agar gel immunodiffusion test and counter current immunodiffusion test. If particulate, the antibody-antigen complexes will agglutinate - examples of such tests are agglutination tests (slide

agglutination test and latex agglutination). In some incidences there is activation and fixation of complement system by the antibody-antigen complexes and complement fixation test (CFT) can be used.

The Office International des Epizooties (OIE) recommends competitive ELISA (c-ELISA) and CFT tests for detection of CBPP.

2.6 Prevention and control

Various strategies have been employed to control or eradicate CBPP including disease surveillance, controlling animal movement, vaccination and test and slaughter (Xiaoxing *et al.*, 1996; Newton and Morris, 2000; Thiacourt *et al.*, 2003; Chandapiwa, 2011; Mbengue *et al.*, 2013). These strategies pose various challenges in Africa where the disease is currently confined. In sub-Saharan Africa the test and slaughter strategy may pose challenges including inability of government to compensate farmers, socio-economic factors, and cultural practices and straining food security in the already starving populations (Thomson , 2005). Restriction of cattle movement may not be achievable as was in the 1960s; it requires heavy investment including fencing which is very expensive (FAO, 2003). Its implementation may also be hampered by socio-cultural practices, animal husbandry and communal land uses (Thiacourt *et al.*, 2003). Vaccination is emphasized in Africa, although it has not been effective in the control of the disease due to various reasons ranging from the quality of vaccines produced, handling of the vaccines (Thiacourt *et al.*, 2003; Rweyemamu *et al.*, 1995), cost of repeated campaigns, and inadequate infrastructure.

2.7 Economic impact of the disease

Livestock production accounts for 40% of agricultural GDP, 90% employment and 95% of total family incomes in the ASAL areas in Kenya. Arid and semi-arid land (ASAL) that make about 80% of Kenyan land mass is where 75% of cattle in the country are kept. Most of the

populations in ASAL areas rely on pastoralism, which has been shown to be a sustainable use of the scarce resources (Kenya Ministry of Agriculture, 2008; Perry and Grace, 2009). Livestock produced under pastoralism has annual livestock/meat off-take of 0.155 billion British pounds and 0.319 billion British pounds (Nyariki and Amwata, 2019).

Though livestock is not a predominant factor of the poor, improved productivity of livestock was shown to have an accelerated effect on economic growth as it has high returns on land and labour, which are predominant factors of the poor. It hence has a significant role in poverty reduction and narrowing of the income gap (SNV, 2008; Engida *et al*., 2015). Endemic and transboundary diseases have a devastating effect on the poor communities in the developing countries. They hinder the pathway of alleviation of poverty through livestock by reducing productivity as treatment increases the cost of production (Perry *et al*., 2002).

Kenya has challenges controlling movement of animals within and across its borders (Kairu - Wanyoike, 2015). This is due to its porous borders facilitating informal movements, social-economic activities of communities that practice pastoralism and instability in countries neighbouring it. Kenya also relies heavily on import of livestock to satisfy its domestic demand while sustaining its top export market; United Arab Emirates, Saudi Arabia, Bahrain, South Sudan and Kuwait (Marube, 2022; Noor, 2011). Though this has resulted in establishment of the largest livestock market in East and Central Africa, Garissa livestock market, it also increases the exposure of the country to transboundary diseases including CBPP.

CBPP is one of the most serious transboundary diseases of cattle that is endemic in many countries in Africa including Kenya and its neighbours. In Kenya, the disease is a threat to all actors in the beef value chain (Chandapiwa, 2011; Kairu -Wanyoike, 2015). It has been

difficult to emphasize the importance of the disease in most African countries due to inadequate active surveillance and animal disease outbreak reporting, hence insufficient data (Kairu -Wanyoike, 2015; Alhaji *et al.,* 2020). This can be attributed to political interference on data publication, insufficient financial support, inadequate access and use of diagnostic tests (FAO, 2003; Perry and Grace, 2009). In Kenya the disease was estimated to have an annual economic impact of about 6.232 million British pounds (Onono *et al.,* 2014). The disease leads to loss of market opportunity as it leads to trade restriction; it causes unmet demand where culling is applied (FAO, 2003; Thomson *et al*., 2005; Tambi, 2006; Chandapiwa, 2011).

The disease has adverse effect on human welfare; it has immediate effect of reduced access to nutrition where milk production drops or animal loses condition. This also occurs when the 'test and slaughter' system is applied (Thomson, 2006). Introduction of the disease to a susceptible herd leads to devastating losses in terms of morbidity and mortality. This was seen when it was re-introduced in Angola in 1969. It led to 75% morbidity and 68% mortality (Thiacourt *et al.,* 2003). Imposing quarantines interferes with access to resources such as water, pasture, access to livestock markets and the human socialization associated with aggregation at these points. The alteration of animal movement may result in degradation of pasture lands that may affect the farmers long after the control has been lifted. For pastoral community, livestock is also a currency hence a sign of wealth; thus the disease poses a threat to social structure by increasing the vulnerable (Perry *et al*., 2002). Strategies that have worked in most developed countries where free status of the disease has been attained may not be implementable in Kenya, as in many of other African countries, due to socio-economic activities, husbandry system, financial viability and sustainability; hence alternatives of dealing with the disease must be sought (FAO, 2003, FAO 2015).

2.8 Treatment

Failure of vaccination to control spread of CBPP has led to consideration of antimicrobial use in control of the disease in endemic areas (FAO, 2003; Nicholas *et al.*, 2007); antibiotics are already widely used for treatment of CBPP and other respiratory conditions among pastoral communities (FAO, 2003). They have also been widely employed to treat post CBPP vaccination reaction in inoculation sites. However, the use of antibiotics has not been encouraged since they may not kill the *Mycoplasma*, hence give rise to carriers which end-up shedding the organisms and contaminate the environment (Thiacourt *et al.*, 2003). Some studies, however, have recorded contrary results – the treated animals did not transmit the disease to in- contact susceptible cattle (Windsor and Masinga, 1977; Huebschle *et al*., 2006; Niang *et al.*, 2010). The main antibiotics used in treatment of CBPP and other respiratory diseases in pastoral communities in Kenya are Tylosin, tetracyclines and sulfadimidine (Muindi, 2014).

Tylosin was shown to have beneficial effects on clinical course of CBPP and alleviation of reaction to vaccination. However, to achieve this, the drug had to be administered twice daily for five days (Windsor and Masiga, 1976). The cost of using Tylosin in treatment of CBPP was found to be more than the cost of vaccination. The period of treatment under which this was done is long for animal treatment especially in pastoral livelihoods. Treatment with long duration of repeat application poses a challenge in regime adherence and possibility of developing antimicrobial resistance has been observed in treatment of typhoid fever and tuberculosis in humans (Chung *et al.,* 1987; Kimani *et al.,* 2021).

Studies have also shown that use of long acting Oxytetracycline has benefits of alleviating clinical signs, reducing mortality of cattle infected with CBPP, reducing lung lesions and preventing sequestration (Niang *et al.,* 2010). However, the question of its efficacy as used in

the field, being hampered by dosage, frequency of administration and route of injection, needs to be investigated. The impact of the antibiotic use on spread of the disease, including: effect on surveillance; infection in a herd and duration of clinical disease in herds, also needs to be investigated. This study was carried out to fill some of the gaps.

3. MATERIALS AND METHODS

3.1 Study site

The study was done at the Kenya Agriculture and Livestock Research Organization (KALRO) Muguga, at the Bacteriology department. Muguga is 28 km west of Nairobi, in Kiambu County. The location has an altitude of 1675 m, latitude 1^{0} 13' S, and longitude 36^{0} 38' E. The mean annual rainfall is 1200 mm and the mean annual evaporation is 1716 mm. The institute (KALRO) has the capacity to quarantine infected cattle; it also has adequate land for seclusion of sentinel herd and technical capacity for diagnosis of contagious bovine pleuropneumonia (CBPP), both microbiologically and through post mortem examination.

3.2 Study animals

3.2.1 Type and source of the study cattle

The study cattle were of zebu (*Bos indicus*) breed, male castrate cattle of ages between 24 and 36 months based on dentation, weighing between 100- 240 kgs. They were sourced from Kakamega in Western Kenya, a CBPP free zone. Individual animals were physically examined prior to enrollment. Only cattle that were healthy and sero- negative for CBPP were recruited into the study. The cattle were held in designated paddocks and pens within the institute.

3.2.2 Preparation of the study cattle before experimentation

The cattle were ear-tagged for identification, treated for helminths and ticks control. They were also vaccinated against lumpy skin disease, foot and mouth disease and anthrax before inclusion into the study. They were then placed in quarantine for a month. Institute animal

care and use committee approval (Kalro/VSRI/ACUC010/07/02016), as per animal welfare regulations, was also obtained (Appendix 1).

3.3 Study design

This was a laboratory experimental study where experimentally-infected cattle were randomly allocated to 2 groups: treatment (Oxytetracycline) group and control group. The cattle in both groups were followed for a period of 31 days post-treatment and outcome determined in both groups; development of CBPP and development of carrier status.

3.4 Sample size determination

Sample size was calculated using the formula in Dohoo *et al*. (2003):

$n = [Z\alpha\ (2\ \bar{p}\ \bar{q})^{½} + Z_{\beta}\ (p_1q_1 + p_2q_2)^{1/2}]^2 / (p_1 - p_2)^2$ $n = [1.96\ (2xo.44x0.56)^{½} + 0.84\ (0.68x0.32+ 0.2xo.8)^{1/2}]^2 / (0.68 - 0.2)^2$

Where n= the required sample size for each group, P_1 = *a priori* estimate of CBPP prevalence at 68%; average of expected prevalence in high risk to very high risk group 53%-80%, (Yansambou *et al.*, 2018) for treatment to be biologically and economically viable it has to reduce prevalence to 20% (P_2).

$q_1 = 1 - p_1$.

$\bar{p} = (p_1 + p_2)/2$, $\bar{q} = 1 - \bar{p}$

Z_{α} $Z_{0.05}$ = 1.96 the value of Z_{α} required for (2-tailed test) confidence = 95%

Z_{β} $Z_{0.80}$ = - 0.84 the value of Z required for (1-tailed test) power = 80%

Each treatment having 15 cattle

3.5 Challenge description and treatment allocation

This study consisted two groups of 15 cattle each, both experimentally infected, through contact transmission by 60 cattle that had been previously infected by endotracheal intubation, with pathogenic *Mycoplasma mycoides* variety *mycoides*, small colonies (*Mmm SC*). The contact transmission was by housing through the night in the same house and paddocked together during the day. The description of endotracheal intubation of the 60 cattle and source of infective material are described in detail in Appendices 2 and 3.

On day 42 post mixing/exposure, when 20% of the in-contact (experimental) cattle had shown fever (high temperature) of above 39.5^0 C for three consecutive days (an indication of infection), the animals were randomly allocated into two groups (designated 1 and 2) of 15 animals each, raised in different pens and treated as described; : Group 1 was treated with long-acting Oxytetracycline (®Alamicycine 20%, Norbrook laboratories LTD; Batch, 6176_600C; manufacture date, April 2016; expiry date, April 2019) at dose of 20 mg/kg body weight , while Group 2 was injected with a placebo (saline; at 6 mg/kg body weight); it served as the control group. The animals were then monitored for development of clinical signs (temperature changes, laboured breathing, other); done daily every morning, and antibody titres, using complement fixation test (CFT); bleeding, for serum was done every two weeks. Naso-pharyngeal swabs (for mycoplasma isolation) were also collected every two weeks. Animals which showed severe signs of sickness (high temperature; other clinical signs) were not allowed to continue with the experiment; they were humanely killed by captive bolt stunning and exsanguination and respective post-mortem examination done. Those that survived were humanely killed at the end of the experiment and post-mortem examination done on them. All pathological lesions seen were recorded; lung lesions were scored as given in Appendix 4.

At post-mortem examination, lung tissue with pathology, lymph nodes, pleural and broncho-alveolar lavage were collected and used to culture for the *Mycoplasma (Mmm)* organisms.The veterinarians making observations and the animal attendants were blinded to the allocation and status of the cattle to reduce observation bias.

Thirty one days after the treatment-period, each of the two experimental groups of cattle (each consisting 15 animals) was mixed with 5 clean cattle (sentinel herd) and followed-up for 2 months, to assess respective transmission of infection to the sentinel herd (carrier status.). Evidence of cross-transmission was based on post-mortem examination of the 5 sentinels and isolation of mycoplasma organisms from respective various tissues including; pleural fluid, broncho alveolar lavage fluid, peribronchial lymph node, retro pharyngeal, caudal and cranial mediastinal lymph nodes. Naso-pharyngeal swabs (for mycoplasma isolation) were also collected every two weeks.

3.6 Clinical assessments

General health observations on all the study cattle were made daily and recorded by animal attendants on data capture forms (Appendix 5). The attendants also recorded the rectal temperatures of the cattle using data capture forms each morning (Appendix 6). Clinical observations were made on the cattle using data capture forms (Appendix 7) according to the scoring system shown in (Appendix 8).

3.7 Sample collection, handling and processing

3.7.1 Naso-pharyngeal swab samples

Cotton-tipped swabs, with individual plastic sheaths were used to collect naso-pharyngeal swab-samples. After sampling the swabs were replaced into their sheets and then transported in cool boxes with ice packs to the bacteriology laboratory in KALRO, Muguga and

processed immediately. The nasal swabs were collected every two weeks on same day as bleeding. Collected samples were recorded on data capture sheets (Appendix 9).

3.7.2 Post mortem samples

At necropsy, the following samples were collected for isolation and identification of mycoplasma: pleural fluid; peribroncheal (left and right) lymph nodes; caudal-mediasternal lung lymph nodes; retropharyngeal lymph nodes (for the sentinel herd); lung tissue (at least 5 grams including a sample from a representative lesion, where present) and: broncho-alveolar lavage fluid (where no lesions were seen in the lungs). The tissue samples were collected individually and placed in marked sterile polythene bags, placed in cool boxes with ice packs and transported to the bacteriology laboratory in KALRO Muguga for processing. All fluid tissue was collected in sterile Bijou bottles or Falcon tubes and were processed immediately at the KALRO laboratory. Collected samples were recorded on data capture sheets (Appendix 10).

3.7.3 Blood collection and serum harvesting

Blood was collected from the jugular veins of the cattle into vacutainer tubes (BD ®vacutainers; Becton, Dickson and Company USA) without anticoagulant; the vacutainers were respectively labeled with date of collection, the animal identification and the sample type. The vacutainers with blood were put on holding racks in a vertical orientation and transported to the bacteriology laboratory in Muguga in a cool box. At the laboratory, the vacutainers were kept at room temperature for four hours and then refrigerated at $4^{o}C$ overnight. The following day the vacutainers were kept at room temperature for an hour, then centrifuged at 1500 revolutions per minute for 15 minutes. The separated serum was decanted and 2 ml aliquoted into cryovials (Nunc®; Sigma-Aldrian, Germany) that were labeled with the date of collection, the animal identification number, and the sample type, and stored at -

35^0C until testing. Blood samples collected per bleeding recorded as per data capture sheets (Appendix 9).

3.7.4 Procedure for Complement fixation test

The test was done following the method of OIE 2021. Cryovials with serum samples were first arranged on a cryovial holder rack and a plate layout drawn. Each serum sample was then diluted ten-fold on a microtiter plate as follows: 90 µl of buffer was put on to wells to which test serum was to be put; columns 1 to 10. Ten microliters (10 µl) of undiluted test serum was added to the respective wells and mixed thoroughly. Twenty-five microliters (µl) of each of the diluted serum was then picked with a micropipette and put onto another microtiter plate, the working plate, maintaining the plate lay out (Appendix 10). Twenty-five microliters of antigen and then 25 µl of complement were added to the diluted serum mixing thoroughly each time. The process was repeated for all samples in the layout.

Columns 11 and 12 on the working plate, were used for control settings as follows: column 11 (11A-11H) for positive serum control; column 12 for antigen control (12A); complement control (12B-12E); hemolytic control (12F) and negative serum control (12G and 12H).

For antigen control, 25 µl of phosphate buffered saline was added, instead of the test serum; then twenty-five microliters of antigen followed by 25 µl of complement and mixed. For the hemolytic control well, 75 µl of buffer was added, to replace antigen, serum and complement - this was to test whether the sensitized rbcs would hemolyse without presence of complement.

For the known positive serum control on the 11A well, 25 µl of the undiluted known positive was added. On well 11B 11H positive serum control was diluted ten-fold as for the test serum; then 25 µl of antigen and 25 µl of complement were added to each dilution well, as for the test serum.

On the complement control wells, then 25 µl (working dilution) of complement was serial diluted and added from 12B to 12E on the working plate.

When setting was completed, the plate was sealed and incubated at 37^0 C for 30 minutes, with gentle shaking after which 25 µl of the hemolytic system (rbcs plus hemolysin) was added to all the wells on the plate then incubated at 37^0 C for another 30 minutes, with shaking. The plate was then kept at 4^0 C overnight to allow for settling in the wells and plates read. The control wells were read first. Positive reaction was indicated by no hemolysis, while negative reaction was indicated by there being hemolysis. The positive serum (2 to 4 score in the first reading) were serial diluted (10^{-1} to 10^{-4}) and CFT redone in the same procedure as before; titre was taken as the highest dilution showing no hemolysis in the positive serum that were serial diluted.

3.7.5 Culture and identification of mycoplasma

3.7.5.1 Processing of organ/ tissue inoculum

The organ/tissue was cut, while placed near a Bunsen burner flame, using a scalpel blade mounted on a blade holder. The scalpel blade was first flamed and used to scorch the surface of the organ to be excised; it was flamed again and used to debride the scorched area. After this, a pea sized piece of the organ/tissue was cut out and was inoculated into Gourlay (broth) medium by immersing the solid tissue in the media, to culture for *Mmm*SC following the modified Newing's method (Gourlay, 1964). The inoculated medium was then incubated at 37^0 C and 5% humidity overnight.

3.7.5.2 Inoculating media with nasal swab

The swabs were placed into 3 mls of Gourlay (broth) medium in bijou bottles and then agitated/swirled briskly for three seconds; the bottles having been opened near a Bunsen burner flame to avoid introduction of contamination. The inoculated medium was then

incubated at 37^0 C and 5% humidity overnight. The suspensions were then used to culture *Mmm*SC following modified Newing's method (Gourlay, 1964).

3.7.5.3 Inoculating media with pleural and broncho-alveolar lavage fluid samples

To inoculate media with pleural fluid or broncho alveolar lavage fluid, 1000 µl of the fluid was aspirated using a pipette, introduced to Gourlay-broth medium in the Bijou bottles and agitated by several aspirations and releasing into the Bijou bottles; the bottles having been opened near an open Bunsen flame to avoid introduction of contamination. The culturing was done following modified Newing's method (Gourlay, 1964); incubated at 37^0 C and 5% humidity overnight.

3.7.5.4 Culturing the mycoplasma using modified Newing's method (Gourlay, 1964)

This stage started with initial inoculation into Gourlay broth done and the culture incubated at 37^o C overnight. Before the inoculation exercise, the Gourlay medium was initially prepared and stored at 4^o C for 3 days; to observe for contamination. It was then taken out, 2.7ml aliquoted and incubated at 37^o C for 30 minutes to make it ready for inoculation with the sample.

After 24 hours, the cultures were removed from the incubator and observation for growth made; this was done by observing colour change, turbidity and filamentous growth. To observe for the filamentous growth, the bottles were gently lifted and observed against the light; looking for a tree-like, filamentous growth which collapsed with even the slightest swirling. A colour change from red to yellow was taken as indication of growth in the media. The broth culture was observed daily and observations recorded. Inoculated media with marked turbidity on the first day of reading/incubation were discarded as they were assumed to be contaminated; inoculation was then redone on a fresh medium, from the original sample.

On the third day, sub-culturing was done by serial titration (1:10) of the cultures in 3 bottles with media and process repeated as above. The reading was done for up to 21 days after which all samples giving negative reactions were discarded. Whenever any bottle had complete colour change (third day onwards), confirmation of culture organism was done by plating on Gourlay agar plates:

First, clean agar plates were put in the incubator for thirty minutes to reduce condensation water in them. The plates were then brought out, divided into four quarters and each marked on the bottom side position, respective to the culture in the Bijou bottles.

Close to an open flame, the bottles were opened aseptically, then 10 µl were aspirated using a pipette and dropped onto designated quarter on the agar plate. Using a sterile wire loop, the culture was spread on the respective quarter. The process was repeated for all the dilutions of the culture. The plate was also marked with date of plating, sample number and type. The plate was covered with paraffin film to reduce the oxygen tension in the plate and incubated at 37^0C.

The plates were incubated for 3 days taken out and placed on a card board to reduce loss of heat by contact and prevent heat shock. The plates were read against sunlight and observed for colonies. Where colonies were present a mark was made and observed under a microscope to observe the “fried –egg” morphology; rounded with raised middle part. The plates were observed for 14 days and all samples giving negative reactions were discarded.

3.7.5.5 Necropsy

Necropsy examination was done to the study cattle that were killed using KALRO standard operating procedures for CBPP and extent of pathology was respectively scored. The cattle were killed using a captive bolt stunner and exsanguinated. The observations were recorded

on data capture forms (Appendix 10 and 12). The lung tissue lesions were scored using the Modified Hudson and Turner method (Hudson and Turner, 1963**)**; details of which are given in Appendix 3.

3.8 Data handling and analysis

In this study, data were recorded using various capture forms. The raw data were hence recorded on various excel sheets showing parameter of interest, animal identification, treatment group and day of observation. The data collected were captured in three groups: group 1 giving ante mortem observation, rectal temperatures, general health observations and clinical observations; group 2 giving post mortem data (pathological lesions observed and pathological scoring) and group 3 giving results on serological tests and *Mmm SC* isolation.

The ante mortem data, on excel, was divided into 2 periods; pre-treatment (41 days) and post-treatment (31days). These data were exported and analysed using GenStat (10.3DE). The daily mean temperatures of the groups were generated and profiles of the rectal temperatures drawn. The rectal temperature means of the groups were compared using a two- tailed, t- test at 95% confidence level. Clinical signs scores for CBPP and pathology scores of the groups were compared using two- tailed, t- test at 95% confidence level.

4.0 RESULTS

4.1 Effect of long acting Oxytetracycline on the course of contagious bovine pleuropneumonia

4.1.1 Rectal temperature profiles of the two groups of cattle naturally infected with mycoplasma organisms

Thirty heads of cattle that were naturally infected with *Mycoplasma* (*Mmm SC*) organisms were randomly allocated to two groups. The pretreatment mean rectal temperature of the cattle assigned to the control group (15) was 37.43^0 C, while that of those assigned to the treatment group (15) was 37.37^0 C. The difference was not statistically significant ($p< 0.05$, $p=0.113$). Details of pretreatment temperatures of the two groups are shown in Fig 4.1 and Appendix 12.

Post treatment fever (>39.5 ^{0}C) was only recorded in the control group. In the Oxytetracycline treated group, no animal had fever. In seven out of 31 days, particularly between days 7^{th} and 14^{th} post treatment, there was a statistical difference in the rectal mean temperatures of the two groups, with the control group having higher temperatures in 6 of the (7,8,9,10,12 and 14) days (Fig 4.2, Appendix 13).

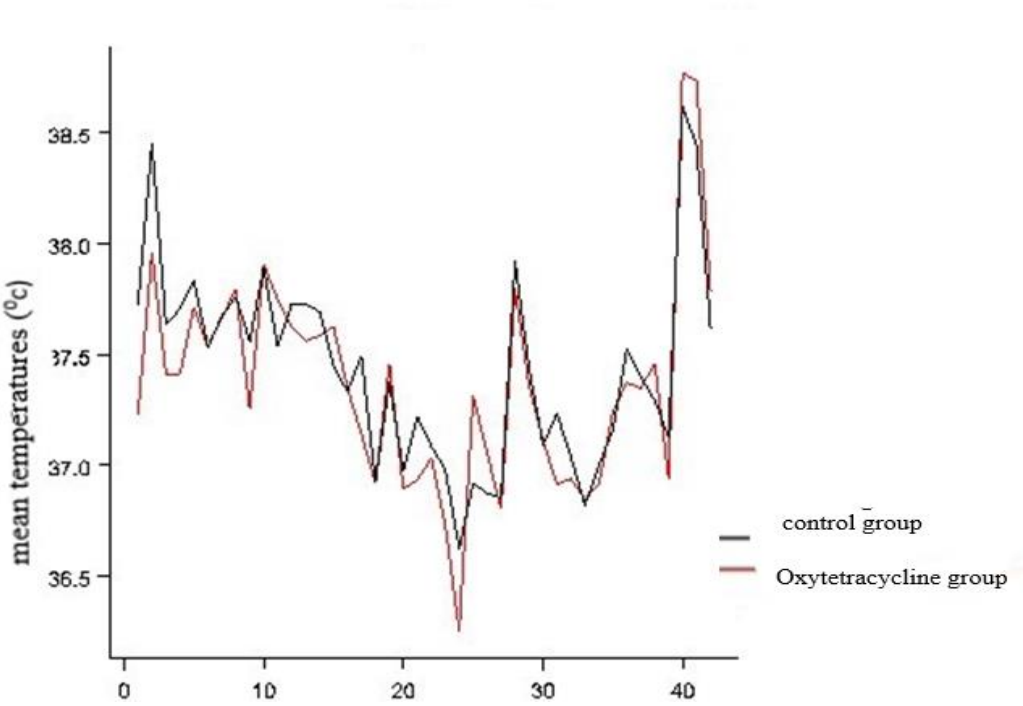

Figure 4.1: Mean temperatures of cattle assigned to control and Oxytetracycline treatment group in the pretreatment period

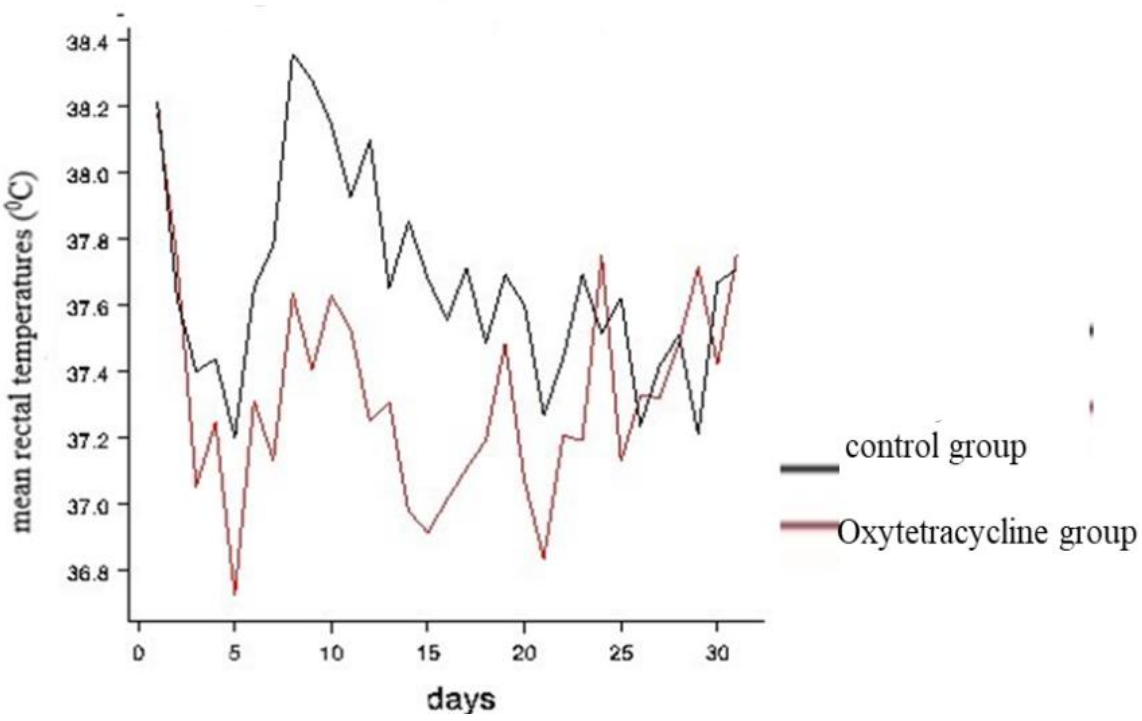

Figure 4.2: Mean temperatures of cattle assigned control and Oxytetracycline treatment group in the post-treatment period

4.1.2 Clinical signs in cattle in the Oxytetracycline and control groups

When infection was established in the cattle by mixing with experimentally infected cattle, 11 of the 15 cattle (73.3%) and 10 of 15 (66.67%) cattle assigned to the Oxytetracycline and placebo control groups, respectively, had shown clinical signs of CBPP infection before the treatment. The most prominent clinical signs in the two groups were depression, respiratory distress and cough (Table 4.1). Other clinical signs observed were nasal discharge, loss of

appetite, diarrhea and loss of body condition. Clinical signs were scored on a scale of 0-3; where 0 is no significant observation for the parameter, 3 the most severe (Plates 4.1 and 4.2). The scoring system is described in details in Appendix 8. As expected, since treatment had not started, the clinical signs scores for the 2 groups were similar except for diarrhea and general body condition ($p < 0.05$). The clinical-sign monitoring was done for 42 days before start of treatment (pre-treatment). Table 4.2 gives a comparison of the mean scores of clinical signs of CBPP in the two groups of cattle pretreatment.

Table 4.1: The most prominent clinical signs of CBPP manifested by naturally-infected cattle

Treatment group	Cattle sick by day of treatment	Cattle with Depression	Cattle with Respiratory distress	Cattle with Cough
Treatment	11	3	4	7
Control	10	5	1	6
Total	22	8	5	13

Plate 4.1: A cow showing emaciation and rough hair coat subsequent to infection with contagious bovine pleuropneumonia, 105 days post infection. This was given a body score of 1 from a scale of 0-3, where 3 is the severest.

Plate 4.2: A cow showing emaciation and rough hair coat subsequent to infection with contagious bovine pleuropneumonia, 131 days post infection. This was given a body score of 2 from a scale of 0-3, where 3 is the severest.

Table 4.2: A comparison of mean scores of clinical signs of CBPP in experimental cattle pretreatment

	Control group Mean score	Treatment group Mean score	t statistic	P value
Appetite	0.0129	0.00114	0.24	0.81
Cough	0.06774	0.09609	1.31	0.191
Depression	0.04032	0.03094	0.87	0.385
Diarrhea	0.001613	0.001629	-0.01	0.0051
Nasal discharge	0.6694	0.4363	-1.21	0.228
Respiratory distress	0.0371	0.04568	-0.59	0.557
General body condition	0.5258	0.7308	2.31	0.021

The two groups of cattle were then monitored for a period of 31 days post-treatment and CBPP clinical scores recorded. All the 7 clinical signs mean scores considered in the experiment were significantly ($p < 0.05$) lower in the Oxytetracycline treated group of cattle than the control group (Table 4.3). Oxytetracycline treatment appeared to modify clinical signs of CBPP.

Table 4.3: A comparison of post treatment clinical signs mean scores of CBPP in experimental cattle

Clinical sign	Mean for Control group	Mean for Treatment group	t-statistic	p- value
Appetite	0.2368	0.0342	6.62	<0.001
Cough	0.5994	0.0433	11.64	<0.001
Depression	0.3206	0.0706	7.78	<0.001
Diarrhea	0.05263	0.00228	3.42	<0.001
Nasal discharge	0.6268	0.5125	2.49	0.013
Respiratory distress	0.6292	0.1139	12.24	<0.001
General body condition	1.294	0.884	7.83	<0.001

4.1.3 Post mortem lesions manifested in the two groups of experimental cattle and mycoplasma isolation

Two groups of cattle were infected by contact transmission and treated with either Oxytetracycline or a placebo. Seventy one percent (10/14) of the cattle in the control group had lesions in the lungs. These included lung sequestration (5), lung hepatization (4) and lung adhesions to the ribs (1). Sizes of the sequesters ranged from 1.5 cm to more than 20 cm. On the other hand, only 4/15 (27%) of the Oxytetracycline treatment group developed lesions in the lungs, including lung hepatization, lung sequestration and fibrous tags formation. Some of the observed lesions that were consistent with CBPP are shown in Plates 4.4 to 4.6. Mean scores of the observed lesions in the control and Oxytetracycline treatment groups were 5.5

and 3.6, respectively. Although the mean score of the lesions were slightly higher in the control group, the difference was not statistically significant (t= -130, p =0.204); this could have been due to low statistical power of the small sample sizes.

Eight of the 14 cattle (57%) from the control group yielded *Mmm SC* organisms on culture. These were isolated from the lungs, caudal mediastinal lymph nodes, left para-bronchial lymph node and pleural fluid. Only one cow in the Oxytetracycline treatment group (1/15; 6.7%) yielded *Mmm SC* on culture of the lungs. This demonstrates that Oxyteteracycline treatment protected the sentinels from CBPP infection. This appears to suggest that Oxytetracycline treatment restricts the spread of *Mmm SC* in the lungs. Plate 4.3 shows isolates of *Mmm SC;* round colonies with elevated centres, fried- egg morphology.

Details of the killings and mycoplasma isolation are given in Appendices 14 and 15.

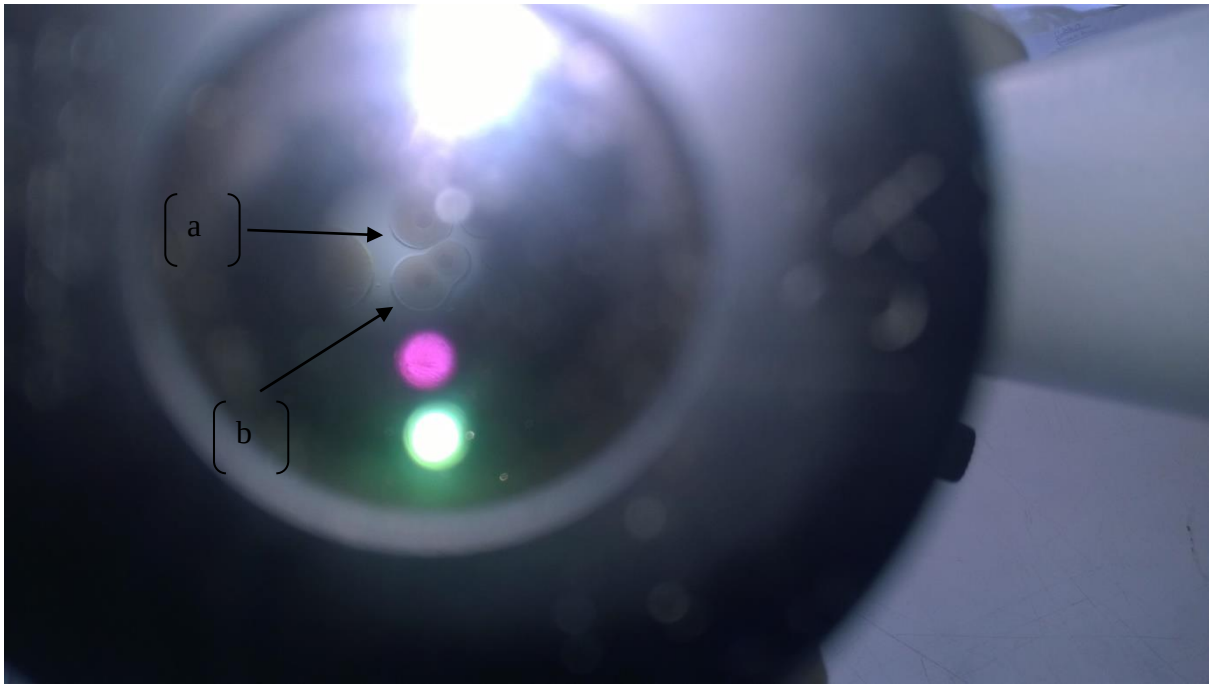

Plate 4.3: ***Mycoplasma mycoides mycoides*** **Small colony isolates a and b. Round with elevated centres, "fried-egg" morphology**

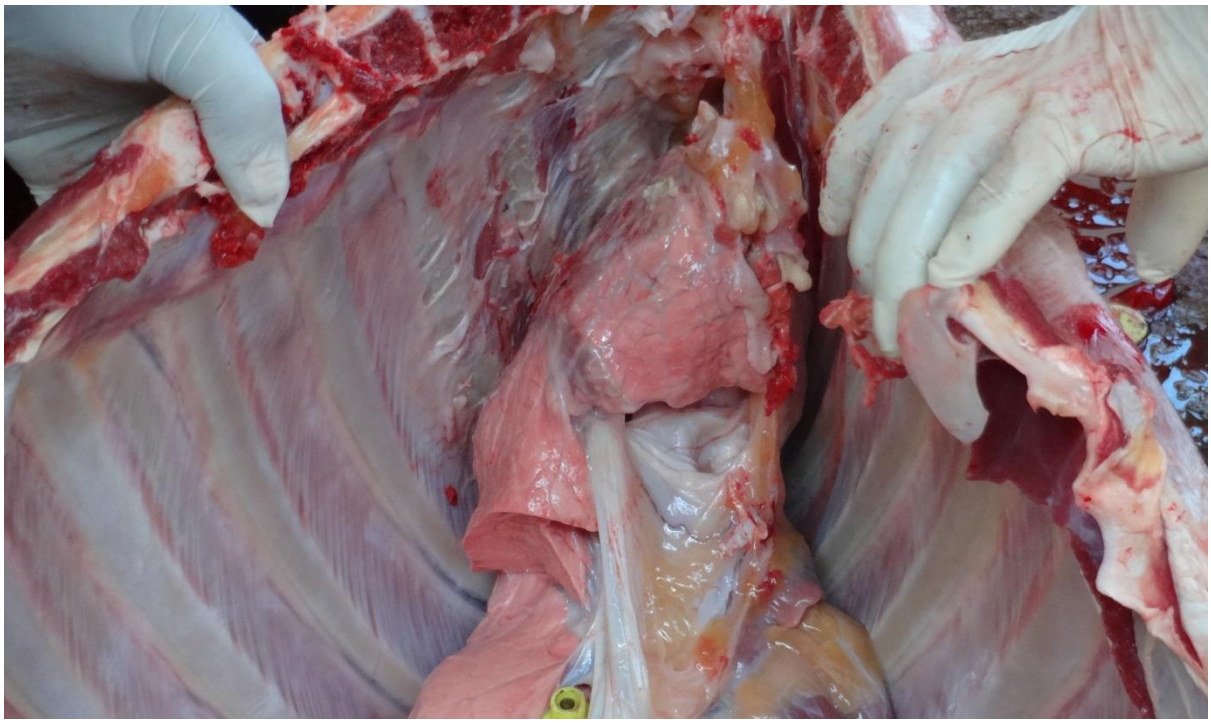

Plate 4.4: Thoracic tissue with adhesion of the lung to the thoracic wall

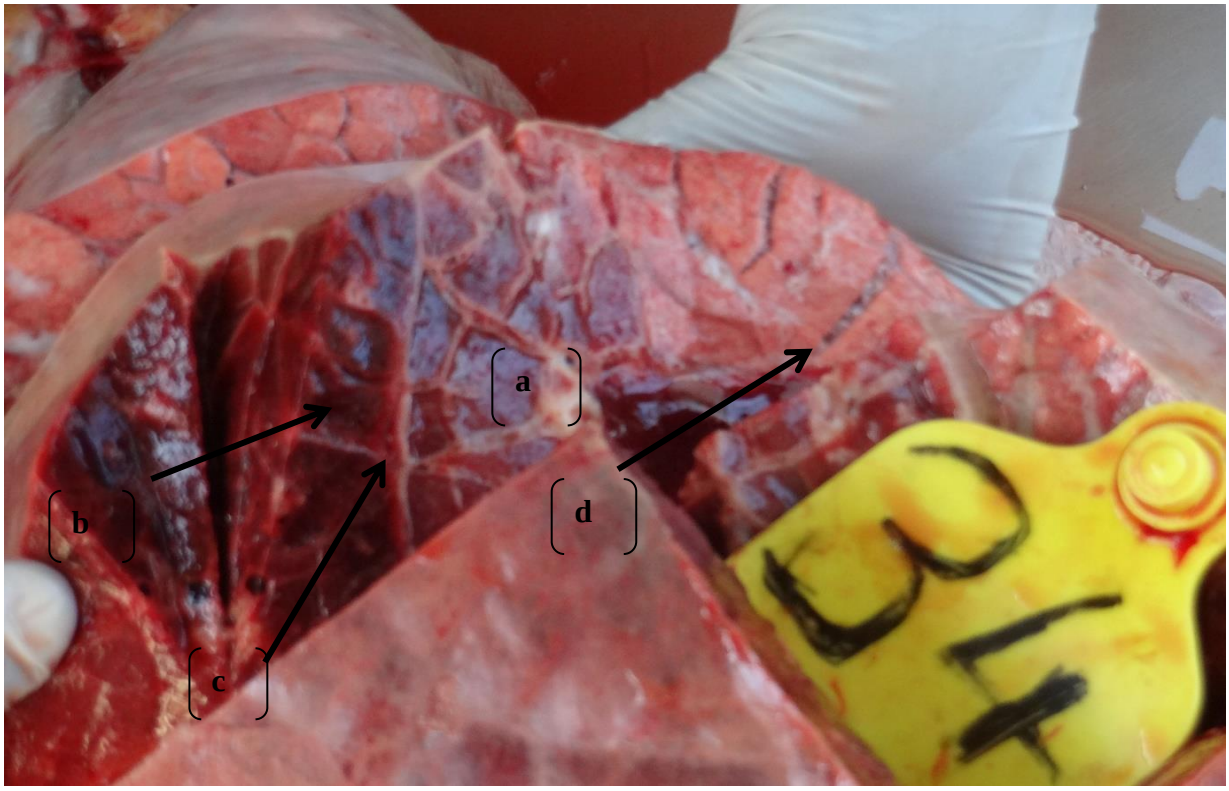

Plate 4.5: Lung tissue with areas of marbling, grey hepatinization (a) and red hepatinization (b) with hypertrophy interlobular septae (c and d)

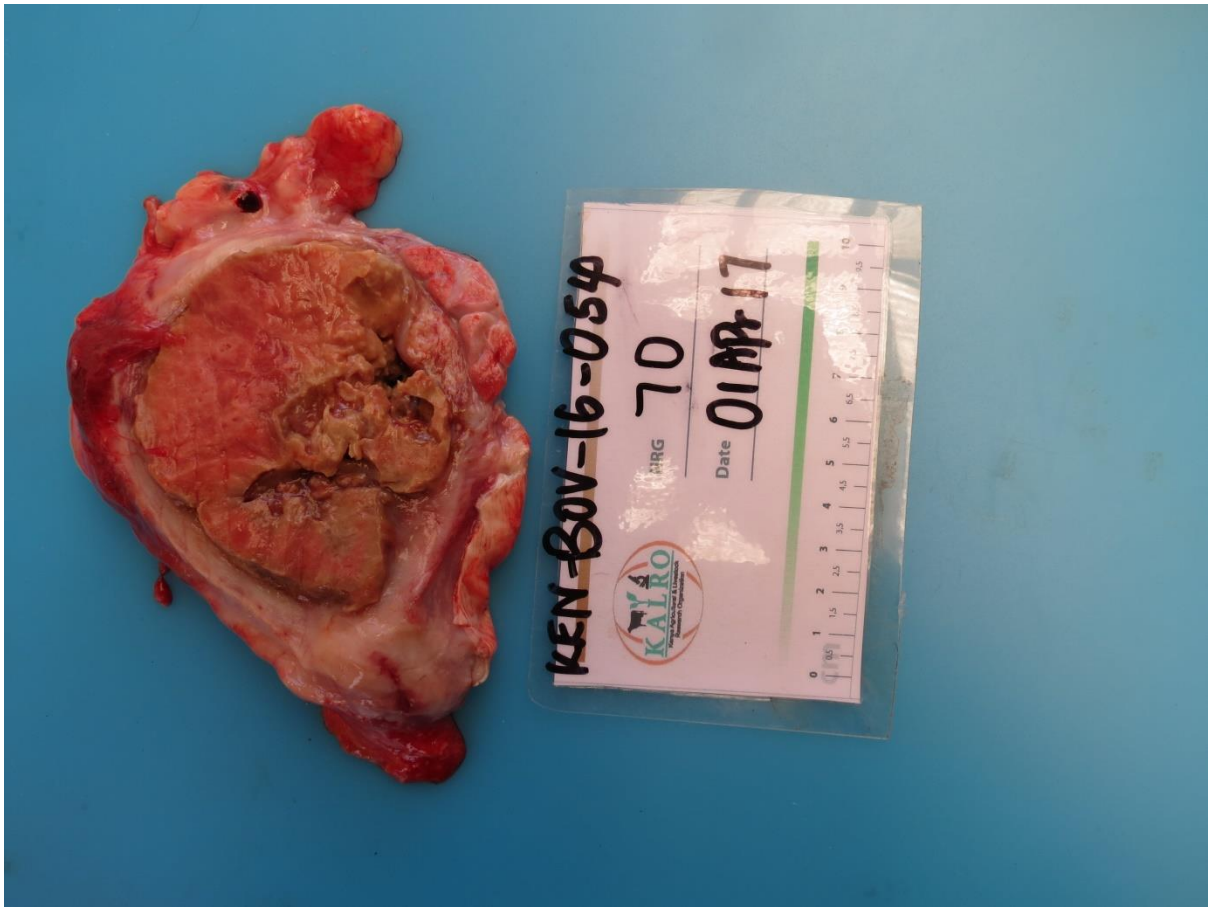

Plate 4.6: Lung tissue with encapsulated sequestra lesion

4.1.4 Serological results for the two groups

Cattle were infected with mycoplasma (section 4.1.1) and were treated with either Oxytetracycline or a placebo. In the control treatment group 14/15 (93%) cattle tested positive on CFT. The CFT titres ranged from 1/20 to 1/640. In the Oxytetracycline treated group 6/15 (40%) cattle tested positive on CFT with a titre of 1/20 to 1/80.

4.2 Potential for development of carrier status in Oxytetracycline treated group

When 5 each of uninfected/sentinel cattle were mixed with the Oxytetracycline treated group and the control group, none of the five in contact with tetracycline-treated cattle yielded *Mmm SC* at post-mortem, while one of the five in contact with the control cattle

yielded *Mmm SC* at post-mortem. The postmortem lesions observed in this control-in-contact animal included sequester, fibrin deposits and grey hepatisation.

Despite the small sample size, the fact that the tetracycline treated group did not infect any of the in-contact cattle suggests that Oxytetracycline treatment reduces the risk of developing carrier status. Details of the killings and isolation are given in Appendices 14 and 15.

5.0 DISCUSSION

The main antibiotics in treatment of CBPP and other respiratory diseases in pastoral communities in Kenya are Tylosin, tetracyclines and sulfadimidine (Muindi, 2014). This study assessed the efficacy of long-acting Oxytetracycline in treating contagious bovine pleuropneumonia in cattle and potential for development of carrier status of the disease after the treatment. The study was expected to contribute knowledge of antibiotic use in treatment of CBPP. In using an experimental approach, the day of infection, stage of disease at treatment was to be more accurately determined than in field trials or observational studies, and follow-up rate was higher than it would be in a field study. This was important in comparing outcome of the disease in antibiotic treated cattle, with consideration of stage of treatment. Separation of the treatment groups was to ease determination of source of infection by contact cattle after treatment of the infected cattle.

In this study, thirty cattle that had been infected with mycoplasma *(Mmm SC)* through contact transmission were divided into two groups and either assigned to Oxytetracycline treated group and a control group (given a placebo). The follow-up of the two groups for the 31 days post treatment included: assessment of clinical signs and of post mortem changes. Each group was later mixed with five (5) uninfected cattle to check for the possibility of carrier development. Both the clinical signs and the post mortem changes were significantly lower in severity in the Oxytetracycline treated group than in the control group. These results appear to suggest that Oxytetracycline treatment modifies the effects of CBPP in cattle.

The positive effect of Oxytetracycline treatment on the clinical course of CBPP is consistent with a similar study by Niang *et al.* (2010) where, of the 26 untreated animals, 13 died with significant findings of acute lung lesions. Thirteen of the untreated and all the treated animals survived. Results of the current study are also in conformity with a twin trial that

was done in Zambia by Muuka *et al.* (2019). In addition, results of the current study are in agreement with preliminary reports of a study that showed that Oxytetracycline reduced inflammation at vaccine injection site and reduced severity of the lung lesions associated with CBPP (Yaya *et al*., 2003). However, like in the current study, results of the two other studies are not conclusive as they were conducted in the confines of a research station and not in the animal's natural environment. Results of such studies would be more relevant if they were conducted in animal's natural environment (Dohoo *et al.,* 2010). An example of a study conducted in a naturally infected herd was that of Muuka *et al.* (2017). They worked with 500 cattle; of which, eighty-six were treated with Oxytetracycline at rate of 20mg/Kg for five days, after 3 of them had died, after showing difficulties in breathing. In this study, there was no separation of the treated from the untreated, in the field, and treatment was only given to those that showed difficulty in breathing. Confirmatory diagnosis was not done until 112 days after the three deaths, when 92 other cattle had shown signs of illness. Over a period of 3 months, 57.4% (39) of the treated had died of CBPP and 42.6% (29) survived. Since treatment was only given to those that showed difficulty in breathing it is possible that some clinical cases were missed; the stage or extent of disease could not be accurately estimated as confirmatory diagnosis was done long after treatment. This particular study demonstrates that the stage of the disease determines the outcome of the disease in treated cattle.

One of the reasons for discouraging the use of antibiotics for treatment of CBPP infected cattle is the fear of development of a carrier status, since the antibiotics may not kill the *Mycoplasma* organisms; the carriers would end-up shedding the organisms and contaminating the environment (Thiacourt *et al.,* 2004). However, there are studies that have recorded contrary results – that the antibiotic treated animals did not transmit the disease to contact susceptible cattle. This was seen in a case where naturally infected cattle were treated with Danofloxacin at 2.5mg/Kg body weight and had less isolates than the untreated ones

(Huebschle *et al.,* 2006). Using Oxytetracycline, there was a significant difference in the postmortem lesions with 76.92% and 11.11% of the untreated and treated presenting a sequestrum according to a study by Niang *et al.* (2010). In the study by Muuka *et al.,* (2017), at post mortem, 16.6% of lesions observed were from an Oxyteracycline treated group while 83.4% were from the untreated group. Fibrotic lesions, a sign of healing, were equally observed in both groups (45.8% and 52.2%, respectively). These findings support what was found in the current study in demonstrating benefits of using Oxytetracycline in treating CBPP; it contributed to reduced lesions resulting in improved carcass score at meat inspection. The twin study in Zambia (Muuka *et al.,* 2019) also supported the benefit of treatment with respect to post mortem changes, and hence carcass value. It is, however, important to note the difficulty in cross-infection from cattle that had recovered from artificial infection of the disease to healthy uninfected ones; this was demonstrated by Windsor and Masiga (1977). Putting the two in close contact, there was no transmission despite exposing the recovered animals to stress, splenectomy and injection with corticosteroid to reactivate the old CBPP lesions. However, the relevance of these studies to the animals' natural environment remains unclear and needs to be confirmed or otherwise in a controlled trial.

In this study, none of the five sentinel cattle exposed to the Oxytetracycline treated group yielded *Mmm SC* at post mortem. On the other hand, one cow yielded *Mmm SC* after being exposed to the control group. These results appear to suggest that Oxytetracycline treatment of CBPP infected cattle does not lead to the development of carrier status. These results are supported by other similar studies that showed that *Mmm SC* organisms are not transmitted to susceptible animals exposed to experimentally infected cattle and treated with long acting Oxytetracycline (Niang *et al.,* 2007; Niang *et al.,* 2010; Muuka *et al.,* 2019). Like in the current study, these studies were done in confined research conditions and thus the results

should be interpreted with caution as their relevance in the real world is unknown. As suggested earlier, there is need for controlled trials in the animals' natural environment particularly in pastoral areas where CBPP is endemic.

If in the future the results of this study, especially the development of carrier status in cattle with CBPP, are confirmed by larger studies and the use of Oxytertacycline is recommended, there may be problems particularly in pastoral areas. This is because in Kenya the use of veterinary antibiotics is indiscriminate, where drugs are easily accessed over-the-counter. In such circumstances under-dosing of animals is common and for a disease like CBPP, development of chronic carrier status may need to be considered. The case for under dosing veterinary drugs was demonstrated in a study by Noreen *et al.* (2008). In the study. 85.7% of cattle farmers underestimated the animals with up to 46.9% of the live weight, the animal health workers had better estimate with 76.6% being within 20% of the real live weight. Veterinary authorities will have to be stringent on who is to access such antibiotics to avoid such problems as well as drug resistance.

Cattle in the Oxytetracycline treatment group and the control group were compared with respect to loss of body condition. Cattle in the control group fared badly compared to the other group. This has some implication on the sale value of their carcasses at slaughter. According to the Meat Control Act (cap 356) of Kenya, cattle in the control group would have had more condemnations because of emaciation. On the other hand, cattle in the Oxytetracycline group would be passed except maybe for the lungs which would be condemned. This would save the farmer some salvage value for their cattle. It must be stressed that the Oxytetracycline treatment of the cattle should be by a veterinarian to reduce the general abuse of a potentially useful drug in treatment of CBPP in endemic areas of Kenya.

In conclusion this study has shown that CBPP infected cattle, treated with Oxytetracycline, have less severe clinical signs and postmortem lesions. There was no evidence of the development of carriers in such cattle. However, the results should be interpreted with caution as the experiment was conducted in confined laboratory conditions. The results should be confirmed or otherwise in controlled experiment in the animals' natural environment.

6. CONCLUSIONS AND RECOMMENDATIONS

6.1 CONCLUSIONS

The following conclusions were drawn from the study:

- Experimental transmission of contagious bovine pleuropneumonia (CBPP) was successfully introduced through close contact. The most prominent clinical signs exhibited included depression, respiratory distress and cough. The most prominent post-mortem changes included hepatisation and adhesions of the lungs to the ribs. The clinical signs and the post mortem lesions were consistent with those of CBPP infection

- Results of this study showed that Oxytetracycline treatment of CBPP- infected cattle reduces the severity of the clinical signs as well as pathological changes

- Although the mean score for lung lesions (10/14; 71%) was higher in the control group than in the Oxytetracyline- treatment group (4/15; 27%) the difference was not significant ($p<0.05$). However, the use of Oxytetracycline appeared to reduce the severity of the lesions caused by CBPP in the lungs

- On post mortem examination, 57% (8/14) of the cattle in the control group yielded mycoplasma on culture of the various tissues, while only 6.7% (1/15) of those in the Oxytetracycline group yielded mycoplasma. This is an indication that Oxytetracycline treatment reduces *Mycoplasma* infection

- With respect to testing for CBPP using CFT, 93% (14/15) of cattle in the control group tested positive for CBPP on CFT while only 40% (6/15) in those in the Oxytetracycline group tested positive for CBPP

- Of the 5 naïve cattle exposed separately to both the control and the Oxytetracycline treated groups, 1/15 (20%) in the former group and none in the latter group yielded mycoplasma at post mortem. This appears to suggest that Oxytetracycline treatment may not lead to the development of carrier status

6.2 RECOMMENDATIONS

- The results of this study should be treated as preliminary and not conclusive since it was conducted under very controlled laboratory conditions. Thus, the results may not be reflective under field condition. There is therefore need to confirm these results by conducting field studies in the animal's environment, most preferably in pastoral areas where CBPP is endemic

- Oxytetracycline treatment of CBPP-infected cattle can still be done but under the supervision of veterinarians. This would prevent farmers losing money due to emaciated carcasses at slaughter

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APPENDICES

Appendix 1: Study approval

KENYA AGRICULTURAL & LIVESTOCK RESEARCH ORGANIZATION
VETERINARY RESEARCH INSTITUTE
P.O. BOX 32 - 00902 KIKUYU, KENYA
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When replying please quote:
Our ref: COMTE/45/34

The chairperson,
Animal Care and Use committee,
Veterinary Sciences Research Institute
Date: 7th October, 2016

Dr. Hezron O. Wesonga
Principal investigator,
GALVmed antimicrobial project

RE: APPROVAL OF A PROJECT ENTITLED "EXPLORATORY CONTROLLED CLINICAL STUDY TO DETERMINE EFFICACY AND SAFETY OF TULATHROMYCIN AND GAMITHROMYCIN IN THE TREATMENT AND METAPHYLAXIS OF *Mycoplasma mycoides* subspecies *mycoides* INFECTION IN CATTLE" KALRO-VSRI/ IACUC010/07102016

The animal care and use committee of Veterinary Sciences Research Institute, KALRO-Muguga North, met on 7th July, 2016 where the project proposal was presented and deliberated upon. The committee members found that the proposed experiment met the requirements needed to comply with animal welfare and use in research experiments.

The project implementers outlined an experimental protocol that had inbuilt animal welfare at the core of the experimental design and assured that welfare of animals will be protected during all stages of implementation of the study. The committee therefore approved the project to be conducted at the Veterinary Research Centre, Muguga North and was given an approval code **'KALRO-VSRI/ IACUC010/07102016'**

Yours sincerely,

Dr. James C. Njanja
Chairperson,
VSRI, Animal Care and Use Committee (IACUC)

Appendix 2: Infective material and its preparation

Challenge material, *Mycoplasma mycoides mycoides Small Colony* (*Mmm*SC) strain Afadé, was sourced from International Livestock Research institute. The *Mmm* SC strain Afadé was grown in Gourlay's medium. The medium was stored at 4°C and used within 14 days. One ml aliquot of the *Mmm SC* strain Afade master seed culture was thawed for 30 minutes at room temperature and inoculated into Bijou bottles containing fresh Gourlay broth pre-warmed at 37°C. Ten-fold dilutions were made into Bijou bottles containing the broth and apart of these, dilutions were streaked on Gourlay agar plates containing 20% heat-inactivated pig serum, 0.25 mg/ml penicillin, and 0.025% thallium acetate. These were then incubated at 37°C in humidified incubator containing 5% CO_2 for 48 hours. Colonies were screened for the typical fried egg appearance of *Mmm* SC. Cultures were upscaled every 24 hours, ensuring that mycoplasma were always kept in log phase. The cultures were pooled and aliquoted in samples of 50 ml (approximately 10^{10} CFU/ml) and stored at −80ºC to provide a standardized source of inoculum

Appendix 3: Infection/intubation process

Distilled water was dispensed into a beaker, agar low temperature melting agar-agarose was then added to make 1.5% solution to help keep the culture in the lungs. This was stirred while bringing to boiling point. The solution was left to cool until pre-setting stage.

The culture was prepared, stored frozen at -80^{o}C in aliquots of 60 ml, ready for use, in single use syringes. On the day of intubation, the syringes were taken out of the freezer and placed at room temperature for one hour, then transferred to water baths at 37 ^{0}C.

Cattle were placed in a crush. They were individually given intramuscular injection of 20% Xylazine according to their pre- determined weights by use of a weigh band, at dose rate of 0.25mm/100Kg body weight. An intranasal tube of 5mm diameter and 1 metre length was lubricated using K-Y jelly. To a standing bull the intranasal tube was introduced through the nostril. A piece of cotton wool was placed near the opening of the intranasal tube to confirm breathing through the tube. Through the tube 60ml of culture aliquoted in syringes was inoculated into the animal. This was the followed with of 30 ml of 1.5% agar. Then, lastly 30mls of phosphate buffer solution. The tube was then gently withdrawn and flushed with hot water.

Appendix 4: Modified Hudson and Turner (1963) pathological scoring method

The pathology score is calculated using a) a lesion score, b) a score of 2 is added to the lesion score whenever mycoplasma is isolated from the lung lesion and c) The total is multiplied by a factor determined by the average diameter of the lesion.

a) For a lesion score, i) the presence of a resolving lesion with only fibrous tags or pleural fibrous adhesions only is rated 1; ii) If other types of lesions are present, namely consolidation, acute, necrotic or sequestrated, these lesions are rated 2;
b) As an addition, if *Mmm*SC is isolated a score of 2 is added.
c) A factor of between 1 and 3 is used to multiply with the lesion score (above). This factor is determined as follows: i) 1 is used if the lesion size is under 5cm; ii) 2 is used if the lesion size is over 5 and under 20cm) and iii) 3 is used if the lesion size is 20cm and above.

Hence, the maximum pathology score is (2+2)3=12 for each animal. There is a variation between the method described here and that of the published Hudson

and Turner method (1963). Isolation by Hudson and Turner was carried out from all organs. Here culture from the different lobes of the lung only were considered in the pathological scoring

Appendix: 5 : General Health Observations

Date: _____ **Pen:** _____
(DDMMMYY)

Are all animals normal? * ☐ Yes ☐ No

For each animal of abnormal health, complete entry below:**

Animal (ID)	**Pen^**	**Comment^^**

* If no, give brief comment for each abnormal animal in the group. N.B. Animals NOT entered MUST have normal general health.

** If for any individual animal there are any abnormal health observations recorded then the animal should be given a clinical examination by a veterinarian and DCF 14 (Veterinary Report) completed.

^ Indicate group (cage number, pen number, room number or other identification of group) of animals examined.

^^ Observations will include, but not limited to, general physical appearance, signs of illness, ill thrift or significant trauma, demeanour.

Explanation of "cross-out" corrections:

Observed by: _____ Date: _____
(DDMMMYY)

Recorded by: _____ Date: _____
(DDMMMYY)

Appendix: 6 : Rectal Temperature

Date: _____ **Temperature Units:** _____ **Thermometer ID:** _____
(DDMMMYY) (°C)

Pen: _____

Animal (ID)	**Rectal Temperature**	**Animal (ID)**	**Rectal Temperature**

Explanation of "cross-out" corrections:

Measured by: _____ **Date:** _____ (DDMMMYY)

Recorded by: _____ (if different to measured by) **Date:** _____ (DDMMMYY)

Appendix 7: Clinical Observations (by Veterinarian)

Date: _____ (DDMMMYY) **Pen:** _____

Animal ID (ear tag number)	**General Condition**	**Depression**	**Respiratory Distress**	**Cough**	**Nasal Discharge**	**Appetite**	**Diarrhoea**	**Time*** (HH:MM 24h Clock)	**Comment**

Score 0 to 3 according to the scoring guide in Appendix G of the Protocol: 0=absent or normal, 1=mild, 2=moderate and 3=severe.
*Time only to be recorded on the day T0 animals are endobroncheally challenged.

Explanation of "cross-out" corrections:

Observed by: _____ (Examining Veterinarian) _____ (DDMMMYY)

Recorded by: _____ (if different to observed by) _____ (DDMMMYY

Appendix 8 : Clinical Observations Scoring Guide

Score	General Condition	Depression	Respiratory Distress	Cough	Nasal Discharge	Appetite	Diarrhoea
Absent **0**	Clinically normal condition	Bright, alert, responsive Normal behaviour	Normal respiratory effort and rate	No coughing	No nasal discharge or small amount of clear secretione (is considered normal)	Normal appetite	Absent Normal faeces
Mild **1**	Slightly abnormal condition	Reduced responsiveness Otherwise normal behaviour	Slightly increased respiratory effort and rate	Occasional cough	Intermittent watery-mucoid nasal discharge present	Slightly decreased appetite	Pasty/soft faeces
Moderate **2**	Moderately abnormal condition	Depressed Separates from group Extended resting periods, reluctant to stand, lethargic	Obvious abdominal breathing	Frequent coughing	Persistent mucoid-mucopurulent nasal discharge	Clearly decreased appetite Not eating concentrate feed	Watery faeces
Severe **3**	Severely abnormal condition	Unable to stand without assistance	Severe abdominal breathing Mouth breathing	Very frequent coughing with prolonged episodes	Severe nasal discharge (persistent purulent or haemorrhagic discharge)	Not eating Anorexia	Bloody diarrhoea

Appendix 9: Sample Collection and Identification DCF

Date (of sample collection)**:** _____ (DDMMMYY) **Nostril**: ☐ Left ☐ Right (if NPS) **Pen:** _____

Animal	**NPS swab Collected?**	**NPS Sample ID[1]**	**Time[2]** (HH:MM 24hr Clock)	**Blood Sample Collected?**	**Blood Sample ID***	**Time[2]** (HH:MM 24hr Clock)
_____	☐ Yes ☐ No	NPS/054/ _____	_____	☐ Yes ☐ No	SE/054/ _____	_____
_____	☐ Yes ☐ No	NPS/054/ _____	_____	☐ Yes ☐ No	SE/054/ _____	_____
_____	☐ Yes ☐ No	NPS/054/ _____	_____	☐ Yes ☐ No	SE/054/ _____	_____

1 Number must state: **sample type/3-didgit study number/ animal number/ date of sample collection** (e.g. SE/054/001/30SEP16; NPS/054/024/01OCT16)

2 Time only to be recorded on the day of endobroncheal challenge and on the days of co-mingling groups.

Explanation of "cross-out" corrections:

Collected by: _____ Date: _____ (DDMMMYY)

Recorded by: _____ Date: _____
if different to collected by)

Appendix 10: Necropsy DCF

Animal (ID): _____ Date: _____ (DDMMMYY)

Site / Tissue / Organ		**Gross Lesions Present***	**Tissue Collected?**	**Comment**
External	(Ext)	☐ Yes ☐ No	☐ Yes ☐ No	_____
Peribronchial Lymph Node (right)	(PBRi)	☐ Yes ☐ No	☐ Yes ☐ No	_____

Peribronchial Lymph Node (left)	(PBLe)	☐ Yes ☐ No	☐ Yes ☐ No	
Caudal Mediastinal Lymph Node	(CMLN)	☐ Yes ☐ No	☐ Yes ☐ No	
Lung	(Lu)	☐ Yes ☐ No	☐ Yes ☐ No	
Pleural Cavity	(Pleu)	☐ Yes ☐ No	☐ Yes ☐ No	
Pericardial Cavity	(PeCa)	☐ Yes ☐ No	☐ Yes ☐ No	
Abdomen	(Ab)	☐ Yes ☐ No	☐ Yes ☐ No	
Musculoskeletal	(Msk)	☐ Yes ☐ No	☐ Yes ☐ No	
Other (Specify)		☐ Yes ☐ No	☐ Yes ☐ No	
		☐ Yes ☐ No	☐ Yes ☐ No	
Lung Lavage	(BAL)			
Lung Swab	(LuSw)		☐ Yes ☐ No	

*If yes, provide a brief comment **Provisional Diagnosis:**

Explanation of "cross-out" corrections:

Examined by: _____ Date: _____ (DDMMMYY)

Recorded by: _____ Date: _____ (DDMMMYY)
(if different to examined by)

Appendix 11: Descriptive stages of lesion development

Date: _____ **Animal:** _____ **P en:** _____
(DDMMMYY) (ear tag)

	Engorged/ Hyperaemia	***Red Hepatisation.***	***Marbling***	***Necrosis***	***Sequestra(ø*** mm)	***Grey Hepatisation***	***Resolution/ Remission***	***Pleuritis***	***Fibrinous adhesion***	***Fibrous adhesion***
left apical	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No	Ø	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No
right apical	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No	Ø	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No
left cardiac	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No	Ø	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No
right cardiac	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No	Ø	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No
accessory lobe	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No	Ø	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No
left diaphragmatic	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No	Ø	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No
right diaphragmatic	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No	Ø	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No
Pleural	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No	Ø	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No

Explanation of "cross-out" corrections:

Described _____ **Date:** _____

by: _____ _____
(DDMMMYY)

Recorded by: _____ **Date:** _____
(if different to described by) (DDMMMYY)

Appendix 12: Pre-treatment output of rectal temperature analysis

Day of infection	Control group rectal men temp	Treatment group rectal mean temp	T statistic	P value
1.	37.73	37.23	1.73	0.098
2.	38.45	37.96	2.5	0.022
3.	37.63	37.41	1.24	0.225
4.	37.71	37.41	1.28	0.211
5.	37.84	37.71	0.73	0.472
6.	37.53	37.53	0.0	1
7.	37.67	37.67	0.05	0.961
8.	37.76	37.79	-0.21	0.836
9.	37.56	37.25	1.8	0.083
10.	37.89	37.91	0.1	0.919
11.	37.53	37.75	1.49	0.148
12.	37.73	37.62	0.81	0.429
13.	37.73	37.56	1.19	0.243
14.	37.69	37.59	0.55	0.509

15.	37.45	37.63	0.94	0.357
16.	37.33	37.53	0.09	0.931
17.	37.49	37.14	1.66	0.108
18.	36.93	36.92	0.03	0.978
19.	37.37	37.46	0.46	0.648
20.	36.93	36.89	0.39	0.699
21.	37.22	36.93	1.70	0.099
22.	37.09	37.03	0.39	0.703
23.	37.98	36.73	1.06	0.297
24.	36.63	36.25	1.51	0.143
25.	36.92	37.31	-2.03	0.055
26.	36.87	37.05	-0.9	0.374
27.	36.85	36.81	0.28	0.778
28.	37.92	37.80	0.42	0.68
29.	37.44	37.36	0.9	0.34
30.	37.09	37.11	0.12	0.906

31.	37.23	36.91	1.9	0.068
32.	37.04	36.94	0.53	0.602
33.	36.82	36.85	0.09	0.926
34.	37.0	36.91	0.34	0.734
35.	37.16	37.24	-0.31	0.762
36.	37.53	37.37	0.47	0.641
37.	37.4	37.35	0.18	0.857
38.	37.29	37.46	0.54	0.596
39.	38.62	38.77	-0.49	0.629
40.	38.62	38.77	-0.49	0.629
41.	38.44	37.73	-0.89	0.38
42.	37.62	38.78	-0.40	0.692

Appendix 13: Post-treatment output of rectal temperature analysis

Day of infection	Control group rectal men temp	Treatment group rectal mean temp	T statistic	P value

1.	38.21	38.18	0.21	0.835
2.	37.63	37.75	-0.6	0.552
3.	37.40	37.05	1.49	0.148
4.	37.44	37.25	0.79	0.435
5.	37.40	36.73	1.54	0.135
6.	37.65	37.31	1.21	0.236
7.	37.78	37.13	2.26	0.032
8.	38.36	37.64	3.02	0.007
9.	38.28	37.41	3.09	0.005
10.	38.15	37.63	2.11	0.051
11.	37.93	37.53	1.35	0.196
12.	38.10	38.25	3.79	0.001
13.	37.65	37.31	1.05	0.309
14.	37.85	36.98	2.85	0.011
15.	37.56	37.01	1.8	0.090
16.	37.71	37.11	2.36	0.03

17.	37.49	37.19	0.97	0.344
18.	37.69	37.49	0.66	0.522
19.	37.60	37.07	1.78	0.097
20.	37.27	36.84	1.36	0.192
21.	37.68	36.91	1.97	0.066
22.	37.44	37.21	0.61	0.552
23.	37.69	37.19	1.6	0.131
24.	37.52	37.75	-0.77	0.456
25.	37.62	37.13	1.70	0.108
26.	37.24	37.33	-0.32	0.756
27.	37.42	37.32	0.33	0.745
28.	37.51	37.49	0.11	0.913
29.	37.21	37.71	-2.04	0.063
30.	37.67	37.42	0.84	0.415
31.	37.71	37.76	-0.25	12.81

Appendix 14: Post mortem lesions in the lungs and bacterial isolation in the treatment group

Animal Identity	Day of killing	Size of lesion	Post mortem Lesions score	Isolation	Pathology score
2	43	>20	2	Negative	6
16	20	>20	2	Negative	6
22	18	>20	2	Positive	12
26	15	>20	2	Negative	4
36	13	>20	3	Negative	9
83	74	>20	3	Negative	9
87	67	<5	1	Negative	1
101	148	Nil	0	Negative	0
110	147	Nil	0	Negative	0
112	146	<5	1	Negative	1
114	26	<5	1	Negative	1
123	145	Nil	0	Negative	0
132	145	<20	1	Negative	3
142	144	<5	2	Negative	2
1326	142	Nil	0	Negative	0

Appendix 15: Post mortem lesions in the lungs and pathology score in the control group

Animal Identity	Day of killing Post treatment	Size of lesion	Post mortem Lesions Score	Isolation	Pathology score
1	26	>20	2	Negative	6
7	97	>20	2	Negative	6
14	42	>20	2	Positive	12
20	101	>20	2	Negative	6
34	26	>20	2	Positive	12
50	95	Nil	0	Negative	0
58	100	Nil	0	Positive	2
63	33	Nil	0	Positive	2
70	101	<20	2	Negative	6
82	95	>20	3	Negative	9
109	99	<20	2	Missing	6
143	95	Nil	0	Negative	0
149	27	<20	2	Positive	8
1359	99	<5	2	Negative	2