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A Slaughter slab Survey of Contagious Bovine Pleuropneumonia Lesions in Slaughtered Cattle in Chavuma Districts, Northwestern Province, Zambia

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ABSTRACT

Objective: **To establish and estimate prevalence of contagious bovine pleuropneumonia (CBPP), using slaughter slabante-mortem examination and postmortem lesions as a diagnostic tool in slaughtered cattle in Chavuma, Northwestern province, Zambia.**

Methods: **Between August and December 2020, 364 cattle were slaughtered at six slaughter slabs in Chavuma district (Good Hope, Likola Levy, Supplies of Chavuma Boarding school, Formula Butchery, Grace of God, Never Lose Hope).The affected cattle had noisy breathing, nasal discharge and coughing. In addition, to yellow fluid in the chest cavity and lungs coated in yellowish substance lung lesions. This was according to the ante-mortem and postmortem reports.**

Results: **Hundred and five (28.8%) of the slaughtered cattle had gross lung lesions suggestive of CBPP. When compared to other slaughter slabs, the point prevalence of positive CBPP postmortem lesions was higher at Grace of God slaughter slab ($P<0.05$). Labored breathing (78%), dry cough (70%), and mucopurulent nasal discharge were the most common pneumonic signs (60%). The gross characteristic of CBPP postmortem lesions included the following; L/lung (45%), R/lung (28%), Both/lung (30%), P/adhesion (90%), L/pinkish (65%), Sequestra (20%) and Yellow fluid (60%). However, the frequently encountered was P/adhesion (90%), and pinkish lung (65%).**

Conclusions: **CBPP remains an issue in Chavuma district, and possibly Northwestern province as a whole, according to the findings of this study. Furthermore, since movement control is difficult in Chavuma district and herds are not restricted, testing and slaughter, even when combined with vaccination, may not be sufficient to control the disease. In the fight against disease, communities play a crucial role. To be effective, any government CBPP control systems must be thoroughly communicated to livestock farmers so that they are fully engaged, recognize the long-term benefits, and are willing to comply. Apart from that, a continued monitoring program is recommended, which involves regular checks of all cattle carcasses at the slaughter slab and subsequent epidemiological analysis of suspicious cases. As a result, concerted measures to eliminate the disease should be introduced as soon as possible, including improved cattle movement monitoring, strengthened epidemio-surveillance networks, and mass vaccination.**

Keywords- CBPP, Slaughter slab, Pleuropneumonia Lesions in Zambia.

I. INTRODUCTION

1.1 Background

The veterinary department under Chavuma District received reports of a strange cattle disease whose clinical manifestation was consistent with CBPP. Livestock farmers reported animals dying suddenly, some without exhibiting any symptoms prior to death. The symptoms included, noisy breathing, nasal discharge and coughing. Upon opening infected carcasses, farmers observed a yellow fluid in the chest cavity and lungs covered with yellowish material.

The possible source of the disease is believed to be Zambezi, as there is an interaction between the animals along the Chavuma/Zambezi boundary.

Mycoplasma mycoides subsp. mycoides causes contagious bovine pleuropneumonia (CBPP), an infectious respiratory disease that mostly affects cattle (Mmm). The disease is spread by infected cattle coming into direct or near contact with susceptible animals. Because of the significant financial losses associated with CBPP, it is a major constraint to cattle production in many areas, including Sub-Saharan Africa. The World Organization for Animal Health (OIE) has classified CBPP as one of the diseases that needs immediate outbreak reporting.

CBPP is a subtle disease with difficult control, and it continues to be a major issue in most cattle-

producing countries in Sub-Saharan Africa. The economic depressions of the 1980s and 1990s, which afflicted many African countries and the ensuing structural reform programs, have resulted in a reduction in funding for public veterinary services. As a result, CBPP surveillance and control in endemic areas is weak or non-existent. Other causes, such as pastoralists' decreased use of veterinary resources, the implications of movement regulation on pastoral livelihoods, and veterinary authorities' decreased implementation of animal health policies, have reduced the efficacy of disease prevention steps. As a result of all of this, the disease has become more prevalent in many areas of East, Central, and West Africa. With decreasing livestock and animal health budgets in Africa, most governments depend heavily on bilateral and multilateral international donor organizations for funding, which cannot be maintained indefinitely due to insufficient or non-existent financial contributions from recipient African governments, resulting in a complete lack of implementation of control measures.

Despite the difficulties, several attempts have been made to effectively regulate CBPP in Africa, with varying degrees of success. Past efforts include the Joint Project 28 (JP 28) in the 1970s and the Pan African Control for Epizootics in the 1980s (PACE). Regulation of CBPP was carried out during the implementation of JP 28 through a policy of compulsory mass vaccination campaigns in Africa's endemic foci, followed by quarantine, testing, and slaughter with reimbursement for contaminated cattle, and disease outbreak reporting. The mass vaccination, on the other hand, ran into serious problems, owing to pastoralists' reluctance to allow their animals to be vaccinated due to concerns about post-vaccination reactions. Unfortunately, the results of these efforts were not as positive as anticipated. Additionally, during the Pan African Rinderpest Campaigns (PARC), a combined control effort for Rinderpest and CBPP was carried out, with vaccine containing both Rinderpest and CBPP attenuated strains. While Rinderpest was eradicated in 1999, CBPP continues to exist despite a mass vaccination campaign against it, albeit epileptically using T1/44 CBPP vaccine. As a result, CBPP still exist on many cattle farms throughout the continent. Nonetheless, due to a lack of cooperation from herders, the PACE monitoring systems for transhumance cattle have not been widely successful, especially in Central and Western African countries.

Due to militating factors such as problems with independent quality control by some manufacturers in Africa, insufficient handling of vaccines by inoculators during vaccination campaigns, such as poor cold chain management, and use of vaccines with sub-optimal amounts of Mmm strains, the quality of vaccines used to control CBPP has deteriorated in recent times. As a result, a few vaccinated cattle have developed post-vaccination reactions, and there have been a few deaths. Furthermore, some serological tests fail to detect vaccinated cattle, especially at the subclinical and chronic stages, resulting in ineffective sero-monitoring of effective and efficient vaccination coverage and herd immunity levels. As a result, a few vaccinated cattle have developed post-vaccination reactions, and they have been a few deaths. Furthermore, some serological tests fail to detect vaccinated cattle, especially at the subclinical and chronic stages, resulting in ineffective sero-monitoring of effective and efficient vaccination coverage and herd immunity levels. The study of CBPP epidemiology in endemic situations is problematic due to its insidious nature. This has resulted in poor understanding of its basic biology, immunology, pathogenesis, and distribution. In view of these challenges, effective surveillance and control of the disease will require understanding of its epidemiology and control strategies for good inferences to be made on such factors as herd immunity levels. Information deficits are often most visible in areas with large cattle populations, are hard-to-reach (remote), and have a high poverty level, as a result of declining resource allocation to public sector veterinary services in many African countries. Current challenges promoting livestock diseases such as CBPP in African livestock farming communities include expansion of the livestock production system, wars, terrorism, and dynamic ecological changes such as climate change and environmental degradation, as well as pastoralists' socio-cultural activities. These concerns are possible causes that could alter the relationships between hosts and disease agents, resulting in changes in CBPP distribution and prevalence. Identifying the Mmm strain lineages circulating in Africa will be critical for disease detection and control, as it will provide information on the status of the available strains for vaccine production. These concerns are possible causes that could alter the relationships between hosts and disease agents, resulting in changes in CBPP distribution and prevalence. Identifying the Mmm strain lineages circulating in Africa will be critical for disease detection and control, as it will provide information on the status of the available strains for vaccine production. CBPP is the leading cause of cattle deaths in Zambia's Western and North-western provinces (Muma et al. 2009; Anon 2008), and these outbreaks have harmed the livelihoods of many people who depend on cattle for survival. Owing to supply shortages and movement limits, the outbreaks have also impacted the availability of beef on the Zambian market, resulting in higher beef prices and leading to inflation. The aim of this analysis was to determine the extent of CBPP contamination in slaughtered cattle. In additional, to identify the most common pre-slaughter pneumonia symptoms, observe the ‘CBPP characteristic like lesion,' and estimate the prevalence’s of CBPP in slaughtered cattle from selected abattoirs/slaughter-slabs in Chavuma, Northwestern Zambia.

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1.2 Epidemiology

The Disease and Causative Agent

Mycoplasma mycoidess subspecies mycoides strain of the Class Mollicutes causes CBPP, a serious infectious transboundary disease of cattle. Fever, nasal discharge, cough, difficult breathing, extreme edema, and proliferation of interstitial tissues in the lungs, as well as diffused pneumonia and serofibrionous pleurisy, are all signs. At the outset of an outbreak, hyper-acute and acute forms of the disease predominate, while sub-acute and chronic clinical manifestations arise as the epidemic progresses. As a result of this, and the fact that certain infected animals may be carriers, clinical CBPP cases in endemic areas may be difficult to identify. When a CBPP outbreak occurs in a susceptible cattle herd that has never been exposed to the disease, the herd will experience up to 100% morbidity and nearly 50% mortality. During a natural infection, the incubation duration is uncertain. Until the end of 2004, it was the only bacterial disease among the 15 "List A" diseases that had to be reported to the World Organization for Animal Health (OIE) within 24 hours of occurrence or reoccurrence in any member country, with weekly follow-up and 6-monthly review. Surveillance and monitoring of CBPP in some African countries improved after the formation of the PACE in 1999, but sadly deteriorated after the program was completed. Mollicutes are the class that Mycoplasmas belong to. Ureaplasmas, Acholeplasmas, and Spiroplasmas are also members of this class.Previously, the Mycoplasmas cluster consisted of six closely related mycoplasmas made up of many ruminant pathogens, which included the following species or subspecies: *Mycoplasma capricolum*subsp. *capricolum, M. capricolum*subsp. *capripneumoniae, M. mycoides* subsp. *capri, M. mycoides* subsp. *mycoides* large-colony (LC), *M. mycoides* subsp. *mycoides* small-colony (SC), and *Mycoplasma* bovine biotype 7 now called *M. leachii. M. mycoides* subsp. *capri, M. mycoides* subsp. *mycoides* large-colony (LC), and *M. mycoides* subsp. *mycoides* small-colony (SC) are the best-known three species in the *Mycoplasma mycoides* cluster. However, *M. mycoides* subsp. *mycoides* large-colony (LC) is now considered as a serovar of *M. mycoides* subsp. *capri*and the nomenclature of *M. mycoides* subsp. *mycoides* small-colony (SC) has been changed to *M. mycoides* subsp. *mycoides* (*Mmm*). Mmm is also part of the M. mycoides cluster, which comprises five pathogenic mycoplasmas: M. mycoides subsp. mycoides, M. mycoides subsp. capri, M. capricolum subsp. capricolum, M. capricolum subsp. capripneumoniae, and M. leachii, all of which share several genotypic and phenotypic characteristics and cause diseases in ruminants. Mmm was divided into two epidemiologically distinct clusters in 1995: one containing strains isolated from various European countries, and the other containing strains isolated from Africa and Australia. CBPP outbreaks in Europe have been found to be less virulent than those in Africa, according to epidemiological and clinical reports. Mmm are pathogenic bacteria that are small (500–1500 bp), do not have a cell wall, are highly fastidious in vitro, and form center colonies on solid medium. Some Mmm are parasitic in nature, colonizing the mucosal epithelium and relying on the host for much of their nutritional needs, while the majority are commensals, but sometimes opportunistic, invading lung tissues in response to other bacterial or viral infections. Mycoplasmas are immune to antimicrobials that attack cell walls, and their lack of cell walls makes them vulnerable to the environment. They have a higher mutation rate than traditional bacteria, implying that they can gain resistance to antimicrobials such as oxytetracyclines and tylosin quickly.

1.3 Hosts and Transmission

Bos indicus and Bos taurus cattle are susceptible to Mmm in natural conditions, but breed susceptibility varies, with trypanotolerant breeds being more susceptible. Water buffaloes are also susceptible to Mmm, but domestic buffaloes are not. Asian yaks and American bison have been found to have CBPP, but African buffaloes have not (Synceruscaffer). The disease does not affect sheep or goats. CBPP is characterized by direct transmission through contacts, a long incubation period, and possibly early mycoplasma excretion during the course of the disease, which may take up to 20 days before clinical manifestations and up to 2 years in carriers (lungers). Different factors dominate CBPP epidemiology in Sub-Saharan Africa, including disease incidence in only cattle species, the absence of a reservoir host in wildlife, and near contact transmission between susceptible and infected cattle. In addition, cattle movements that are unregulated play a significant role in the maintenance of the CBPP in Africa. When inhaled aerosols, particularly those produced by cough, are inspired, disease transmission by close contact between infected and susceptible cattle occurs almost exclusively. CBPP transmission is aided by management practices such as kraaling (keeping cattle together in a small hut) at night and combining herds along stock routes and at watering points. Long-distance cattle trekking along stock routes aids disease spread and impact because it allows cattle to come into contact with one another within and between herds, and the dust caused by mass movement aids pathogen spread. Herdsmen's nomadic culture and tradition of transhumance have played a major role in the spread of CBPP in many African countries. Trans-placental transmission has been demonstrated by the isolation of Mmm from the foetus of an infected dam. Mmm has also been isolated from the urine of an infected cow in the acute stages of CBPP, with titers ranging from 102 to 108 Mmm per milliliter of urine. It was also removed from two bulls' sperm and sheath washings. However, the role of urine or sperm in Mmm transmission in

nature is unknown, although transmission through fomites and polluted fodder has been observed in laboratory settings. Outbreaks are more common in transit cattle, with incubation periods ranging from a few days to six months. The infection rate in susceptible cattle herds can be as high as 90%, with a 50% mortality rate, and 25% of infected cattle can recover and become carriers without showing clinical signs. The disease first arrived in Africa from Europe, but the epidemiology of the disease in the latter continent is still unknown. Despite the fact that water buffaloes are susceptible to CBPP, the disease's mode of transmission from buffaloes to cattle is unknown. Mmm has been epidemiologically identified as a large ruminant-specific pathogen, but it has also been isolated from small ruminants. Mmm has been isolated from caprine lungs in two strains (C305 and C425), and ovine milk in three strains (O326, O512, and O526). As a result, small ruminants can serve as potential Mmm reservoirs and should be included in Africa's CBPP surveillance and control program.

1.4 CBPP Distribution in Africa

CBPP has been found all over the world, with the exception of South America and Madagascar. Most pastoral cattle herds in Western, Southern, and Eastern Africa, as well as Angola and Northern Namibia, are infected with the disease. Cattle traded from India in the 19th century was thought to have contaminated herds in East, Central, and West Africa. CBPP was eradicated from the United States and the United Kingdom in the nineteenth century, but it still exists in many African countries, and it was common in 12 African countries between 2007 and 2008.

Due to annual combined vaccination campaigns from efforts to control Rinderpest, the geographical spread of CBPP was limited in the 1980s, and almost no outbreaks were observed. In the 1990s, the disease spread through Africa, re-invading countries like Botswana, Tanzania, and Rwanda that had previously been free of it. Botswana, on the other hand, was able to recover its independence after introducing stringent sanitary measures. CBPP is becoming more common in Africa, with at least 27 countries in equatorial, central, and southern Africa registering cases by the end of 1999.CBPP was thought to be present in all Sub-Saharan African countries in 2015. Due to physical barriers that prevent the disease from spreading, such as the Namibian veterinary cordon fence, the southern part of the continent is still considered free of the disease, but the Southern African Development Community (SADC) countries are clearly still at risk. Mmm is found in the majority of cattle herds in Sub-Saharan African countries, with the exception of Senegal and Gambia in West Africa, and Gabon and Congo Brazzaville in Central Africa, which do not report the disease. Except for Congo DR and Gabon, 18 African countries in the west, northern, east, and southern regions have been reporting CBPP to the African Union Inter-African Bureau for Animal Resources since 2006. (AU-IBAR). DRC and Gabon, on the other hand, began publishing in 2010.In the countries that reported the disease, the disease affected 304 epidemiological units, resulting in 16,836 cases and 3007 deaths, with an approximate case fatality rate of 17.9%. Ghana had the largest number of CBPP outbreaks (75), followed by the Central African Republic (43) and Ethiopia (29).CBPP is widespread in the majority of West, Central, and East African countries, with at least 24 (45%) recorded outbreaks every year for the past ten years. The disease is also spreading to new areas, with The Gambia announcing an outbreak for the first time in 2013 after 45 years of being free of the disease. CBPP has now been identified in a few Southern African countries (Angola, Namibia, and Zambia). The recorded rates of morbidity and mortality, as well as case fatality, have been inconsistent, and the outbreaks tend to have no specific seasonal trend. Many intrinsic and extrinsic factors affect the incidence and distribution of CBPP in cattle herds, including occasional diseases, age, genetic constitution, crowding, climatic conditions, and stress from transportation and handling. The final outcome of Mmm infection in herds is largely decided by these factors.

1.5 Pathogenesis and Pathology

The occurrence of thrombosis in the pulmonary arteries, which may occur prior to the establishment of pneumonic lesions, is a hallmark of CBPP pathogenesis in susceptible animals. The exact mechanism of thrombosis formation is unclear, but it is thought to be regulated in part by cytokine induction. The disease results in a variety of pneumonias in the lung lobes, with prominent dilated inter-lobular septa caused by a high out-pouring of plasma and fibrin, giving the lung a "marbling" appearance. Bronchitis, bronchiolitis, and alveolitis were caused by the usual inflammatory reactions, while early inflammation in Mycobacterium tuberculosis was mainly caused by neutrophils and mononuclear cellular response. CBPP induces unilateral pulmonary necrosis, major sero-sanguineous fluid accumulation in the interstitia and pleura, and occasionally sequestration on a pathological level. Vasculitis, which occurs as exudation and pleurisy, is an essential component of the pathological shifts. Ischemic necrosis and lung infarctions may be caused by thrombosis. Cattle suffering from anoxia or toxemia are at risk of dying. Mollicutes developed a variety of substances that are essential to CBPP pathogenesis. They contain peroxide and superoxide, which can compromise the integrity of the host cell. Ureaplasma species contain urease, which can damage host tissues as a result of the ammonia released by urea hydrolysis. Mycoplasma phospholipases, which may play a role in pneumonia, may reduce the surface tension of alveolar surfactants, resulting in atelectasis. Mmm contains a galactan polymer that modulates and facilitates immune response dissemination.

1.6 Clinical Manifestations

CBPP can take four different forms: hyperacute, acute, subacute, and chronic. The hyperacute form of the disease appears at the start of outbreaks, can affect up to 10% of the infected herd, and sometimes results in sudden death without other clinical signs. Fever, self-isolation from the herd, anorexia, and difficult breathing that is labored and painful are observed in about 20% of the affected cattle during the acute form, which lasts 5 to 7 days and is characterized by fever, self-isolation from the herd, anorexia, and difficult breathing that is labored and painful. Abdominal breathing and "grunting" during expiration are two other signs to watch for. A shallow, dry, and painful cough can develop in affected cattle, which is frequently observed during exercise. When pressure is applied between the ribs, affected animals may protest and, in some cases, retaliate violently. In addition, in the extreme type, infected animals stand with their nostrils dilated, mouth open, and panting for air, with their head and neck extended, forelegs spread apart, frothy saliva accumulation in and around the mouth, and nasal discharge, often streaked with blood. In addition, some affected animals can develop throat swellings and dewlap at this time. Around 40 percent to 50 percent of the infected cattle have a subacute form with symptoms that are close to those seen in the acute form, but they may be less severe and have intermittent fever. Some cattle may reach the chronic stage right away, which is a normal progression from the acute and subacute stages. The clinical symptoms diminish with time, though infected cattle can still suffer fever, anorexia, and weight loss. Swollen, hot, and painful limb joints are common in young calves, resulting in lameness.

1.7 Diagnosis

Medical tests, post-mortem inspections, and laboratory studies using culture and isolation methods, as well as serological analyses, are used to diagnose CBPP. Molecular techniques based on proteins and nucleic acids have also developed and become more precise**.**

1.8 Clinical Examinations

When first introduced into a herd that has not been previously threatened with the disease, it causes high deaths of cattle. Few of them can die rapidly showing signs of only fever. Clinical signs, on the other hand, appear several days to months after Mmm infection, meaning that the disease may be present in a herd long before clinical signs appear, making tracing back difficult, particularly where long intervals between vaccination campaigns are practiced and antimicrobials are frequently used to treat clinical cases. Clinical signs are more useful for diagnosing CBPP in pastoral herds in Africa during the acute stage of the disease, when symptoms are evident, such as recurrent cough and respiratory discomfort due to pleuropneumonia in adult cattle and lameness due to arthritis in calves. The magnitude of the visible symptoms decreased in direct relation to the amount of time the disease was present in the herd. Many of the above clinical signs are not predictive of CBPP. As a consequence, it's important to rule out other potential diagnoses such as acute pasteurellosis, hemorrhagic septicemia, actinobacillosis, bovine tuberculosis, abscesses or hydatid cysts, or traumatic pericarditis throughout clinical evaluation.

1.9 Post-Mortem Examinations

Gross pathological lesions, such as sequestra, which are encapsulated necrotic pulmonary lesions, are often seen on post-mortem examinations. Fibrinous deposits on the parietal surfaces and interlobular spaces distension due to accumulation of straw-colored serofibrinous exudates characterize gross pathological lesions in the lungs in the acute stage. Gross pathological lesions, such as sequestra, which are encapsulated necrotic pulmonary lesions, are often seen on post-mortem examinations. In the acute stage, fibrinous deposits on the parietal surfaces and interlobular spaces distension due to accumulation of straw-colored serofibrinous exudates characterize gross pathological lesions in the lungs. The pleural cavity may contain large amounts of clear, yellow-brown fluid with fibrin fragments, whereas pathological lesions usually affect one lung and are frequently clustered in the diaphragmatic lobe with a distinctive marbling appearance. On palpation, lesions may be found, and incision reveals red and grey hepatization regions. In subacute cases, necrosis in the lobules and interlobular septa, as well as early sequestrum development, characterize gross lesions. In chronic cases, gross lesions are distinguished by well-defined sequestra surrounded by fibrous capsules, as well as adhesions linking thickened viscera and parietal pleura.

1.10 Serological Analysis

The OIE recommends the Complement Fixation Test (CFT) and competitive Enzyme Linked Immunosorbent Assay (c-ELISA) for herd-level serological diagnosis, and they are widely used in Africa for disease investigation. The sensitivity of the CFT to Mmm infection varies depending on the clinical stage of CBPP manifestation, which is highest in the acute stage due to the high level of circulating complement fixing immunoglobulin. A research on CBPP outbreaks in Botswana reported a sensitivity of 98 percent using post mortem lung lesions as a gold standard. However, a study conducted elsewhere in Italy found a lower sensitivity of 64%, indicating that the disease had a relatively low prevalence due to an eradication program.

Many herds in Africa have used a c-ELISA with a particular monoclonal antibody targeting Mmm antigens. There were no cross-reactions with other Mycoplasma bacteria, and the sensitivity and specificity were 96 percent and 97 percent, respectively.

Cattle vaccinated with live attenuated CBPP vaccine produced immune responses that were detected by CFT and c-ELISA. However, sero-prevalence research on vaccinated cattle herds in some African

countries found that CFT and c-ELISA had weak agreement.

Attempts to use serological approaches to evaluate the efficacy of vaccination in disease-vaccinated cattle have failed because vaccination with T1/ 44 or T1-SR vaccine strains does not always result in measurable antibody responses. As a result, depending solely on CFT or cELISA to determine vaccine efficacy is not recommended. Since post-vaccination antibodies do not survive after three months, CFT or c-ELISA can be used to detect natural infections in cattle herds in Africa even in areas where vaccination campaigns are not routinely conducted. Both tests are only useful for diagnosing the disease in herds, not for identifying individual infected cattle. Lpp Q is a 48 kDa protein that has been used in Mmm field strains in Europe, Africa, and Australia. The protein has been used to detect Mmm in experimentally infected cattle using an Immunoblotting procedure, and it may also be used to diagnose CBPP in Africa under natural conditions.

1.11 Molecular Analyses

Biochemical and antigenic methods, as well as culture and identification, have historically been used to isolate mycoplasmas, including Mmm. Due to cross-reactivity of antigenic determinants from closely related organisms, bacterial contamination, and time and labor intensive laboratory procedures, these techniques have been restricted in their sensitivity and specificity. Important advances have been made since the advent of polymerase chain reaction (PCR) in 1994, because it allows for a faster and more sensitive diagnosis of CBPP. Mmm's identification has been difficult due to its close phylogenetic association to other members of the M. mycoides cluster, such as M. mycoides subsp. mycoides biotype wide colony (now a serovar of M. mycoides subsp. capri), M. mycoides subsp. capri, M. capricolum subsp. capricolum, M. capricolum subsp. capripneumoniae, Due to shared genetic, immunologic, and biochemical features, it has also been difficult to distinguish Mmm from other members of the "mycoides cluster." These are some of the difficulties that African molecular laboratories face.

PCR has developed effective diagnostic procedures for the precise identification of Mmm strains, as well as robust quick detection, identification, and differentiation of M. mycoides cluster members, despite the challenges. The use of nested PCR systems, which are sensitive for detecting Mmm in cultures and clinical materials with very low numbers of target organism, is of particular interest.

The African, Australian, and European lineages of Mmm have been identified in studies on the molecular epidemiology of Mmm in Africa. An notable feature, insertion sequence IS 1296, has eased the earlier difficulty associated with species differentiation. Within the Mmm subspecies, distinct strains of African, Australian, and European origins were identified through the use of a DNA probe against the IS element. The difference between the two strains was discovered to be due to the absence of an 8.84-kb deletion in European strains, but its presence in African and Australian strains. IS1296 was inserted upstream of this deletion region and found to be stable enough for species typing, so it was used as a molecular marker in Mmm strains' restriction fragment length polymorphism (RFLP) study. Furthermore, the maker's position was used to create a PCR assay that precisely classified and distinguished Mmm from these geographical backgrounds.

Wide genomic rearrangements in bacteria, such as deletions, insertions, and inversions, may be caused by insertion sequences (ISs), which are useful molecular markers for the diagnosis and epidemiological studies of bacterial pathogens. IS elements are mobile DNA segments with structural and organizational similarities that are less than 2.5 kb in size. They have one or two Open Reading Frames (ORFs), with three or more on rare occasions. These genes code for proteins that help IS elements transpose, such as a transposase. In transposition reactions, the termini of IS elements usually form inverted repeats (IRs), which serve as recognition and cleavage sites for the IS-encoded transposase. Mmm has been connected to only two IS elements: IS1296 and IS1634. Mmm strains were clustered into two large epidemiologically distinct geographical classes during IS typing with an IS1296-specific probe.

1.12 Immunity Against Mmm Infections

Because stamping-out and movement control are difficult to implement in most disease-endemic countries in Africa, the use of attenuated T1/44 live strain vaccination remains the most effective method of choice for CBPP control. According to field observations, naturally recovered cattle develop immunity to Mmm but are susceptible to re-infection. However, there is no information available on what is meant by “infected and recovered” cattle in herds. Although the widely used T1/44 attenuated live vaccine is known to provide immunity for up to one year, it is unable to prevent the formation of gross pathological lesions in challenged animals, implying that it only induces limited immunity. However, no study has found that Mmm infections consistently induce solid immunity in infected cattle. In the immunology of CBPP, the main protective mechanism against disease is unknown. Although the exact nature of the protective response against Mmm is still unknown, there is a theory that immune responses are involved in protection against Mmm during a primary infection and that this contributes to a reduction in disease severity due to acquired immunity induced after vaccination.

The role of CD4+ T-cells in protecting animals against Mmm during its primary infection has been studied extensively. During primary infection, a link has been discovered between high numbers of mycoplasma-specific IFN-secreting CD4+ T lymphocyte subsets and a mild form of the disease. However, the protective role

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of CD4+ T-cells against Mmm in vaccinated cattle is still debated. Natural killer and natural killer T-cells produce 117 IFN as part of the innate immune response, and CD4 Th1 and CD8 cytotoxic T lymphocyte effector T-cells produce it when antigen-specific immunity develops. It activates macrophages with aberrant expressions associated with auto inflammatory and autoimmune diseases, which have immunostimulatory and immunomodulatory effects, and it plays an important role in innate and adaptive immunity against viral and intracellular bacterial infections. A study found that total depletion of CD4+ T-cells in infected animals resulted in a dramatic increase in CBPP severity and mortality during Mmm primary infection, indicating that IFN secreting CD4+ T-cells play a protective role.

It would be difficult to attribute defense of infected cattle to high antibody titers against Mmm during primary infection since no research has found a clear link between antibody titers and disease severity in infected animals. The only hypothesis was that innate responses may play a significant role in defense. However, studies have shown that CD4+ T-cells, in combination with antibody development after vaccination or during secondary infection, can defend against Mmm. Since CBPP is often associated with high mortality in naive herds, there is a risk that the mediated immune responses are inadequate or too late to adequately defend against the infection. All of these claims suggest that acquired CD4+ T-cell responses play a minor protective role against primary Mmm infection. Furthermore, the low cell-mediated immune response elicited during infection may be linked to the weak immunity defense given after vaccination with live T1/44 attenuated vaccine. As a result, the role of innate, acquired cell-mediated, and humoral immunity in providing protection to Mmm-infected cattle is still being researched. Even after vaccination, however, the pathological lesions caused by Mmm infection are essential indicators of an immunopathological phase.

Finally, the diagnosis of CBPP should be based on the presence of lesions as well as the presence of Mmm in infected tissues, and serological tests should be used at the herd level to determine herd immunity. Control of CBPP in Sub-Saharan Africa is still constrained by a number of factors, including a decline in the quality of veterinary services, a lack of financial resources for diagnostic and long-term control programs, and the lack of policies on cattle movement control within and between countries. These issues also aided the spread of the disease in much of Africa. Effective CBPP control in Africa is possible, but only with stronger political commitments that prioritize the disease among the continent's major diseases of high economic value to the livestock industry. CBPP-affected countries must persuade regional agencies, international bodies, and funding partners to invest in CBPP control as a foundation for improving food security, livelihoods, and people's overall well-being.

II. MATERIALS AND METHODS

2.1 Study slaughter slabs:

The study was carried out on sixslaughter slabs, Good Hope, Likola Levy, Formula Butchery, Supplies Chavuma Boarding school, Grace of God, Never Lose Hope. The criteria for selection included availability of slaughter slab facility, qualified meat inspectors and willingness to join the study.

Likola Levy, and Never Lose Hopeslaughter slab receives trade livestock mostly from Lingundu veterinary camp . Good Hope slaughter slab receives trade livestock mostly from Kahokola, which is found in Lingundu veterinary camp. The slaughter slab that Supplies Chavuma Boarding school receives trade livestock mostly from Chilonga, which belongs to Lukolweveterinary camp. Grace of God slaughter slab receives trade livestock from Nyakutemba, which is part of Nyatanda veterinary camp.

The cattle presented to the slaughter slab came from various livestock traders and individual livestock farmers.

2.2 Data collection and pre-slaughter examination of animals

The age determination was not done hence the slaughtered stock were not grouped into age categories.

The questionnaire was designed as a one-paged document and had four major components: slaughter slab profile, source/destination of slaughtered stocks, pre-slaughter pneumonia signs and post mortem features. In order to improve and maximize the precision of responses, more closed ended questions were used after pre-testing. Each slaughter slab was visited (by our respective meat inspector officers) daily for a month, between the period of May and December 2020. Records of numbers slaughtered, source of slaughter stock and the lesion(s) observed were noted down in the questionnaire by our meat inspectors in these slaughter slabs. All animals presented for slaughter were physically observed and examined a day or shortly prior to slaughter. Inspection of the animals was made while at rest or in motion for any obvious signs of the disease. Special attention was made to the respiratory related signs like breathing pattern (labored/distressed), coughing (dry/moist), standing posture (nostril dilated, neck extended).

2.3 Post slaughter examination of animals

The postmortem slaughter examination involved a visual examination of all carcasses and its visceral organs including palpation and incision of tissues/organs. Gross pathological lesions on each of the diseased lungs were recorded. Samples (sera and tissue) for detailed laboratory investigation could not be collected due to the logistic and capacity of the local regional Solwezilaboratory which is more than six hundred kilometers from Chavuma district.

2.4 Data analysis

Our monthly prevalence of CBPP was calculated using the following formula: Proportion of the stock affected by one or more lung lesion(s) to the total (monthly) number of slaughtered stock. Data files for the studied parameters were edited, developed and analyzed using both Epi-Info version 7.1 and Statistix 8.1 .

III. RESULTS

A total of 364 cattle were slaughtered and examined in the selected slaughter slabs during the survey period. Of the slaughtered and examined cattle, 28.85% cattle had lesions suggestive of CBPP. Two hundred and fifty nine (71.15%) showed no gross lesion suggestive of CBPP. The proportions of slaughters and stocks with CBPP in each category of each variable investigated during the study are shown in Table 2. The prevalence of lesions was significantly (P<0.05) higher at Good Hope butchery/ slaughter slab compared to other slaughter slabs.

Table 1: Detected cases of CBPP at butcheries in Chavuma

S/N	NAMEOF TRADER	NAME OF BUTCHERY	CBPP CASES	CAMP OF ORIGIN	OWNEROF KRAAL
1	Kanyangulu Given	Good Hope	23	Kahokola	SaviyeMutondo
2	LufundaSandonji	Likola Levy	12	Lingundu	LufundaSandonji
3	Christopher Luneka	Supplies Chavuma Boarding	17	LukolweChilonga	Christopher Luneta
4	Logan Kakhoma	Formula Butchery	10	Chitembi	Logan Kakhoma
5	Douglas Mbilikita	Grace of God	22	Nyakutemba	Douglas Mbilikita
6	KakhomaMbilikita	Never Lose Hope	21	Lingundu	KakhomaMbilikita
	TOTAL CASES		**105**		

From table 1: One hundred and five slaughtered cattle had lesions suggestive of CBPP.

Table 2: Prevalence of CBPP by abattoir location in the district

Abattoir	Number of slaughtered	Number of affected [*n* (%)]
Good Hope	35	23 (6.32)
Likola Levy	20	12 (3.30)
Supplies Chavuma Boarding	25	17 (4.67)
Formula Butchery	90	10 (2.75)
Grace of God	60	22 (6.04)
Never Lose Hope	134	21(5.77)
Overall	364	105 (28.85)

From table 2: Out of three hundred and sixty four slaughtered and examined cattle, one hundred and five (28.85%) cattle had lesions suggestive of CBPP. Two hundred and fifty nine (71.15%) showed no gross lesion suggestive of CBPP.

Table 3: Observed ante-mortem signs in bovine

SIGNS	PERCENT
dry cough	70
labored breathing	78
mucopurulent nasal discharge	60
Fever	56
moist cough	45
weight loss	28

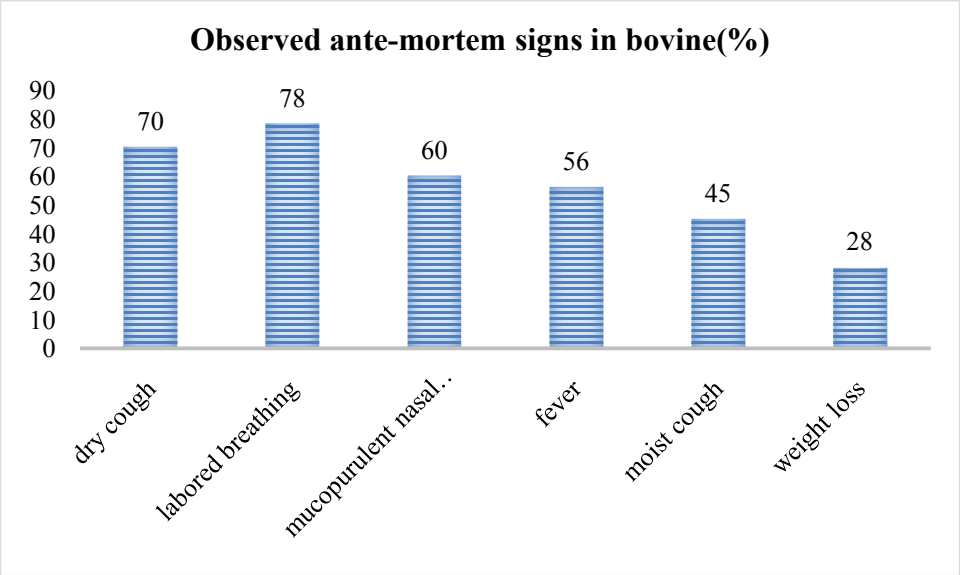

Figure 3: The most observed ante-mortem 'pneumonia like, signs in bovine during Jan-Dec 2020.

L/breathing: Labored breathing; N/discharge: Mucopurulent nasal discharge.The results in Figure 3. shows that of the 105 cattle affected, 70%, 78% and 60% had dry cough, labored breathing and mucopurulent nasal discharge, respectively.

Table 4: Observed post-mortem lung lesions

SIGNS	PERCENT (%)
L/lung	45
R/lung	28
Both/lung	30
P/adhesion	90
L/pinkish	65
Sequestra	20
Yellow fluid	60

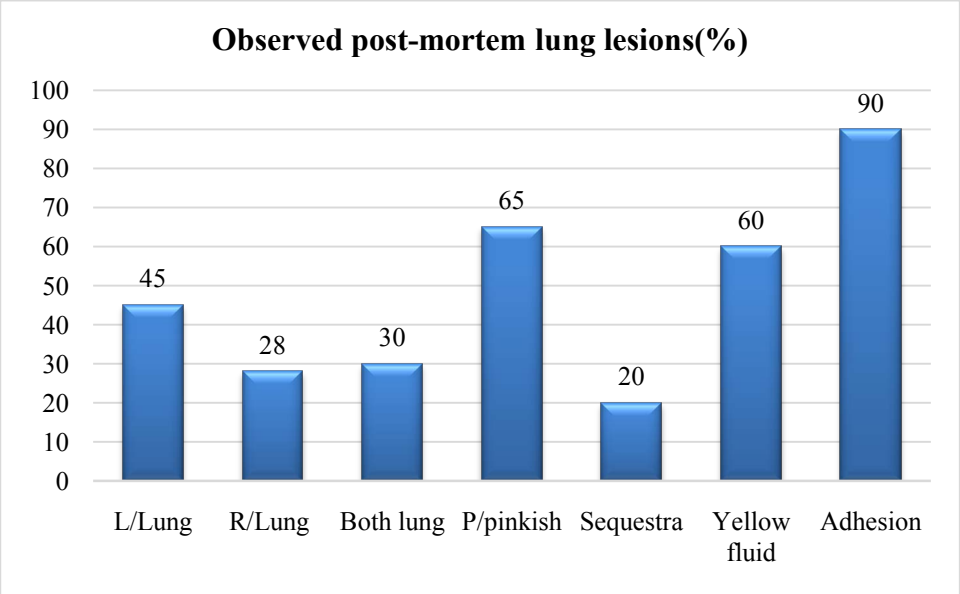

Figure 4: The most observed post-mortem lung lesion in Bovine: Jan-Dec 2020.

Adhesion ;P/Pinkish; Yellow fluid; L/lung;

The results in Figure 4. shows that out of 105 cases of lung infection, 28% and 45% affected the right and left lungs, respectively while 30% was bilateral. Pleural adhesion and presence of yellowish fluid in the thoracic cavity was observed in 90% and 60% respectively of all cattle that had lung lesions. Sequestra formation was associated with 20% of all animals slaughtered.

IV. DISCUSSION

From the pre and post slaughter signs and lesions recorded in this study, there is indication that CBPP is present in the district as seen by105 (28.85%) positive cases. Epidemiological data about our country also support this observation. In 2020, the department of veterinary services under Chavuma District received reports of a strange cattle disease whose clinical manifestation was consistent with CBPP. The department managed to collect lung tissue and whole blood from one of the affected animal that died in Lukolwe veterinary camp. Samples were forwarded to CVRI for further diagnosis. The test results for the lung tissue came back positive for CBPP. This lead to Surveillance at slaughter slabs and butcheries to be strengthened.364 cattle were slaughtered at the slabs under the review period, 105 (28.8%) of the slaughtered cattlehad gross lung lesions suggestive of CBPP. The point prevalence of positive CBPP postmortem lesions ($P<0.05$) was higher at Grace of Godslaughter slab compared to others. The most observed pneumonic signs included labored breathing (78%), dry cough (70%) and mucopurulent nasal discharge (60%). The gross characteristic of CBPP postmortem lesions included the following; L/lung (45%), R/lung (28%) Both/lung (30%), P/adhesion (90%), L/pinkish (65%), Sequestra (20%) and Yellow fluid (60%). However, the frequently encountered was P/adhesion (90%), and pinkish lung (65%). In the district, the disease has been reported in all the nine veterinary camps and the slaughter slabs at the butcheries. During the review period, Good hope slaughter slab recorded 23 positive cases out of 35 total number of cattle slaughtered at this slab accounting for 6.32% of all the positive cases. Likola Levy slaughter slab recorded 12 positive cases out of 20 total number of cattle slaughtered at this slab accounting for 3.30% of all the positive cases. The slaughter slab Supplying Chavuma Boarding school recorded 12 positive cases out of 20 total number of cattle slaughtered at this slab accounting for 4.67% of all the positive cases. Formula Butchery slaughter slab recorded 10 positive cases out of 90 total number of cattle slaughtered at this slab accounting for 2.75% of all the positive cases. Grace of God slaughter slab recorded 22 positive cases out of 60 total number of cattle slaughtered at this slab accounting for 6.04% of all the positive cases . Never Lose Hope slaughter slab recorded 21 positive cases out of 134 total number of cattle slaughtered at this slab accounting for 5.77% of all the positive cases. In all this study, 364 cattle were recorded to have been brought to the slaughter slab. The estimated point prevalence was 28.85%,which is very high as compared to prevalence of 3.60% which has been reported in Zambia (Phiri, 2006). The prevalence of CBPP based on gross lesions does not give a true picture of the disease as compared to culture and molecular diagnosis. This is because many other diseases are responsible for gross pathological lesions on lung tissues. Although the overall prevalence of CBPP was high, stratifications of prevalence by slaughter slabs reveals that Good Hope slaughter slab have recorded significantly high number of CBPP infected cattle than any other slaughter slabs in the district. The reason for high point prevalence rate might be difficult to explain, however, detailed tracing of source of cattle indicate majority to originate from Kahokola, which is found in Lingundu veterinary camp. The second highest is Grace of God slaughter slab which receives trade livestock from Nyakutemba which is part of Nyatanda veterinary camp. These areas warrant close attention. The control of CBPP has not been given the adequate attention as is the case in western province of Zambia. This is probably because of the insidious nature of CBPP. Majority of the cases remain sub clinical and affected animals becoming carriers due to the encapsulation of the lesions in the lungs. The present study was only restricted to six slaughter slabs where animals are brought for slaughter from within the district. Therefore, this cannot be treated as a true representation of the Northwestern province of Zambia . However, this survey gives an insight to the status of CBPP in the district/province and warrants measures for its control.

It is concluded from this study that CBPP remain a problem in Chavuma district and probably Northwestern province as a whole. Coordinated efforts to eradicate the disease through improvements of cattle movement control, strengthening epidemio-surveillance networks and mass vaccination should urgently be implemented. Tracing the source of infected cattle detected at a slaughter slab and enforcement of strict rules for livestock movement can aid in the control of the disease in our district. However, in Chavuma district, movement control is difficult and herds are not enclosed, test and slaughter even when coupled with vaccination may not adequately control the disease. Hence, implementation of control measures should therefore be holistic to ensure that all key factors involved in the spread and perpetuation of the disease are curtailed. Since traditional practices play a key role in the epidemiology of CBPP there is need for qualitative anthropological studies, to investigate how some of these traditional practices could be modified to incorporate disease control measures. Communities play a key role in disease control. To ensure success, it is essential that any government CBPP control programmes are fully explained, so that livestock farmers are fully involved

and understand the longer term benefits and are willing to comply.

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