Mycobacterial safety of meat cuts from BCG-vaccinated African buffaloes (*Syncerus caffer*) experimentally infected with *Mycobacterium bovis*

By

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DECLARATION

I declare that this thesis, which I hereby submit for the degree Master of Science at the University of Pretoria, is my own work and it has not been previously submitted for a degree at any other tertiary institution.

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List of Abbreviations

AEC	Animal Ethics Committee
BCG	Bacillus Calmette and Guérin
bp	Base Pair
BSCL2	Biosafety Cabinet Level 2
BSL2+	Biosafety Laboratory Level 2+
bTB	Bovine Tuberculosis
CFU	Colony Forming Unit
CPC	Cetylpyridinium Chloride
cPCR	Conventional Polymerase Chain Reaction
DAFF	Department ofAgriculture, Forestry and Fisheries
DNA	Deoxyribonucleic Acid
EFSA	European Food Safety Authority
ELISA	Enzyme-Linked Immunoabsorbent Assay
GDP	Gross Domestic Product
HCl	Hydrochloric Acid
HHWRS	Hans Hoheisen Wildlife Research Station
HiP	Hluhluwe-iMfolozi Park
HIV	Human Immunodeficiency Virus
IM	Intramuscular
IV	Inactivated *M. bovis* Vaccine
IV-IM	Inactivated *M. bovis* vaccine administered Intramuscularly
IV-Oral	Inactivated *M. bovis* vaccine administered Orally
KNP	Kruger National Park
LJ	Löwenstein-Jensen
MIRU	Mycobacterial Interspersed Repetitive Units
MTBC	*Mycobacterium tuberculosis* complex
NaCl	Sodium Chloride
NaOH	Sodium Hydroxide
NTM	Non-Tuberculous Mycobacteria
PCR	Polymerase Chain Reaction
PPD	Purified Protein Derivative
qPCR	Quantative Polymerase Chain Reaction
REC	Research Ethics Committee
rRNA	Ribosomal Ribonucleic Acid
SC	Subcutaneous
SNPs	Single Nucleotide Polymorphisms
SOP	Standard Operating Procedure
TB	Tuberculosis
TST	Tuberculin Skin Test
VNTR	Varying Number of Tandem Repeat
WHO	World Health Organisation
WOAH/OIE	World Organisation for Animal Health

Summary

Title of dissertation: Mycobacterial safety of meat cuts from BCG-vaccinated African buffaloes (*Syncerus caffer*) experimentally infected with Mycobacterium bovis

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Degree: Master of Science Wildlife Health, Ecology and Management

Tuberculosis is a global disease that affects humans and animals, both wild and domestic. Bacteria, of closely related sub-species, from the *Mycobacterium tuberculosis* complex are what cause tuberculosis (TB). There are two main forms of the disease: the human disease, mainly caused by *Mycobacterium tuberculosis* and the animal disease, caused predominantly by *Mycobacterium bovis* and *Mycobacterium caprae*. The animal form of the disease can be zoonotic, particularly *M. bovis*.

The study was a qualitative study that set out to determine the safety of meat cuts (fillet, silverside, brisket, and rump) taken from vaccinated (inactivated *M. bovis* vaccine and BCG) and experimentally infected buffaloes with *M. bovis* in particular, for the presence of *M. bovis* and *M. bovis* BCG. The different cuts of meat were chosen due to their popularity in the consumer markets.

The animals were held at the Skukuza bomas and were euthanised and full post mortem examinations were conducted in October 2021 at the Skukuza abattoir. Meat samples were collected at the abattoir and biobanked until the samples were needed for a study. The meat samples were then processed at Hans Hoheisen Wildlife Research Station, Orpen Gate, Kruger National Park, in February 2023. The methodology involved the culture of samples from different meat cuts (fillet, brisket, silverside and rump), on mycobacteria-selective media. In this study, a total of 378 media slopes were produced of which only ten showed colony-like growth. Speciation by PCR was conducted on these ten media slopes. The data were analysed by creating pivot tables. Pivot tables enable large amounts of data to be summarised in an easy and understandable format.

The study concluded that the meat from vaccinated and experimentally infected buffaloes showed no positive results for *M. bovis* or *M. bovis* BCG. These results suggest that meat collected from vaccinated and experimentally infected buffaloes is likely to be safe for humans to consume, but larger sample sizes are needed to increase certainty.

Chapter 1 – Literature Review

General Introduction

Globalisation, climate change, other environmental changes and the ever-growing human population have contributed to the increased number of emerging zoonotic diseases (Ryser-Degiorgis, 2013). Diseases transmitted from animals to humans are known as zoonotic diseases. Most emerging diseases in humans are zoonotic (Mohamed, 2020; Escudero-Pérez et al., 2023). Tuberculosis (TB) is a global disease that affects animals, domestic and wild, and humans (Loiseau et al., 2020), and is endemic in many developing countries. TB can be a zoonotic disease. An estimated 10.6 million people were diagnosed and 1.6 million people died from TB in 2021 (World Health Organisation, 2022). TB is caused by bacteria belonging to the *Mycobacterium tuberculosis* complex (MTBC), of which seven lineages are human-adapted and many ecotypes are adapted to animals (Loiseau et al., 2020; Thomas et al., 2021). There are two main forms of the disease: the human disease, mainly caused by *Mycobacterium tuberculosis* and the animal disease, caused predominantly by *Mycobacterium bovis* and *Mycobacterium caprae*, both of which are important agents of disease in livestock (Hope & Villarreal-Ramos, 2008; Domingo et al., 2014; Michel et al., 2015; Loiseau et al., 2020). These two species (*M. bovis and M. caprae*) are estimated to cause approximately 12 500 deaths per year in humans (Loiseau et al., 2020). Bovine TB (bTB) has a zoonotic potential and is difficult to treat in humans due to its natural resistance to pyrazinamide (Loiseau et al., 2020).

The MTBC consists of closely related bacterial subspecies that have infected people and animals with tuberculosis for thousands of years (Wirth et al., 2008). The evolutionary and genetic history of *M. tuberculosis* strains may influence the pathogen's transmissibility and the ability of the pathogen to gain drug resistance (Gagneux et al., 2006). Worldwide, extremely drug-resistant and multi-drug resistant strains are emerging (Wirth et al., 2008; Sakamoto, 2012). Therefore, understanding the relationships between the different MBTC species and their hosts would help explain why tuberculosis has been so successful in spreading. (Wirth et al., 2008). Despite tuberculosis's global expansion, little was known about when it first began to evolve (Wirth et al., 2008). The limitations of the genetic markers currently in use (single nucleotide polymorphisms (SNPs)) are mainly to blame for this knowledge gap and the evolution of the MBTC has been based on universal mutation rates for bacteria (Ochman & Wilson, 1987; Wirth et al., 2008). In the 2000s, Wirth et al. (2008) used mycobacterial interspersed repetitive units (MIRUs). The varying number of tandem repeat (VNTR)

sequences found in MIRU loci make them effective genotyping markers (Wirth et al., 2008). Recent studies, using whole-genome sequencing, have shown that wild animals can act as reservoirs for *M. bovis* (Crispell et al., 2017; Orloski et al., 2018). In Africa, the main reservoir or maintenance species are the Kafue lechwe (*Kobus leche kafuensis*), the greater kudu (*Tragelaphus strepsiceros)*, and the African buffalo (*Syncerus caffer*) (Ayele et al., 2004; Renwick et al., 2007; Michel et al., 2015). Genetic sequencing of *M. bovis* has found that the bacteria most likely evolved in East Africa and that its evolutionary success can be linked to its effectiveness in infecting cattle (Loiseau et al., 2020).

The formation of the Mycobacterium genus is thought to have occurred about 150 million years ago (Barberis et al., 2017). Thus, since prehistoric times, species belonging to this genus have been responsible for illnesses (Sakamoto, 2012; Barberis et al., 2017). *M. tuberculosis* has survived over 70 000 years (Wirth et al., 2008; Barberis et al., 2017). Early hominids in East Africa may have been infected by a progenitor of *M. tuberculosis* as early as three million years ago (Gutierrez et al., 2005; Sakamoto, 2012). The modern ancestor of *M. tuberculosis* may have emerged around 20 000 years ago (Kapur et al., 1994; Brosch et al., 2002; Barberis et al., 2017). The findings from a study conducted by Wirth et al. (2008) indicate that MTBC consists of two main lineages that split off from the *Mycobacterium prototuberculosis* progenitor pool about 40,000 years ago.

Evidence of TB has been found in ancient Egyptian mummies but there are no illustrations of the disease in Egyptian papyri (Barberis et al., 2017). The first written descriptions of TB date back 3 300 and 2 300 years in India and China, respectively (Barberis et al., 2017). Descriptions of the disease have also been found in the Andean Region, Ancient Greece, Roman times, the Middle Ages, and during the Renaissance era in Europe (Barberis et al., 2017). The precise clinical and anatomical description of the condition was first illustrated in 1679 by Frances Sylvius (Barberis et al., 2017). During the 18th and 19th centuries, many European physicians worked on the disease. In 1720, Benjamin Marten, an English physician, conjectured, for the first time, the infectious origin of tuberculosis (Barberis et al., 2017). In the mid-19th century, Johann Lukas Schölein coined the term “tuberculosis” (Barberis et al., 2017). Consolidation, pleurisy, and lung cavitation were recognized by Theophile Laennac as pathognomonic symptoms of pulmonary or extrapulmonary TB (Barberis et al., 2017). He also described the different stages of the disease from the first appearance in the lungs (miliary form), progressing to larger tubercles containing a cheese-like substance (caseous form) and eventually forming

cavities and empyema (Barberis et al., 2017). A successful inoculation of material from a miliary tubercle into the liver and lungs of rabbits resulted in generalised tuberculosis in 1843, thanks to the work of the German surgeon Philipp Friedrich Hermann Klencke (Barberis et al., 2017). In 1867, Theodore Albrecht Edwin Klebs tried to isolate TB bacillus on egg white, stored in sterile flasks (Barberis et al., 2017). His experiments were successful as they caused disease in Guinea pigs. In 1882, following Paul Ehrlich's advice, Robert Koch used the methylene blue staining procedure to isolate tubercle bacilli. By inoculating laboratory animals with the bacillus, Koch was able to recognise, isolate, and grow the bacillus in animal serum and replicate the illness (Gradmann, 2001; Barberis et al., 2017). Koch also developed tuberculin, which is a glycerine extract of *M. tuberculosis* (Sakamoto, 2012). The Pirquet and Mantoux tuberculin skin tests, the Bacillus Calmette Guérin (BCG) attenuated vaccine strain, and antimycobacterial drugs such as streptomycin, isoniazid, rifamycins and pyrazinamide were another breakthrough in tuberculosis research, prevention and treatment (Sakamoto, 2012; Barberis et al., 2017).

The importance of ante-mortem diagnostics in livestock and wildlife is expanding, even if post-mortem investigation continues to be the cornerstone of tracking infectious diseases in wildlife (Didkowska et al., 2022). The need to protect threatened species, both in the wild and in captivity, and wildlife in general, has increased awareness and understanding of the role and threats to spillover and maintenance hosts. Therefore, a comprehensive approach is required to enhance medication therapy, diagnostic tools, and preventative measures because tuberculosis remains a persistent health issue for both humans and animals.

Mycobacteria

Taxonomy

There are currently over 160 species of Mycobacteria. Mycobacteria are acid-fast, aerobic, non-spore forming, nonmotile, weakly gram-positive, straight or slightly curved rods measuring 1 to 4 μm in length and 0.3 to 0.6 μm in width (Sakamoto, 2012). The genus *Mycobacterium* is broadly classified into two major groups namely non-tuberculous mycobacteria (NTM) and *Mycobacterium tuberculosis* complex (MTBC) (Porvaznik et al., 2016). Species belonging to the MTBC are obligate pathogens while species belonging to NTM do not cause tuberculosis but under certain conditions, members of NTM can cause TB-like symptoms (Musoke, 2016). The following species are included in the MBTC, *M. tuberculosis*,

M. bovis, *M. bovis* BCG, *M. pinnipedii*, *M. africanum*, *M. caprae*, *M. microti*, *M. canettii*, *M. mungi*, *M. orygis*, *M. suricattae*, and dassie bacillus (Rodriguez-Campos et al., 2014) but *M. bovis* and *M. tuberculosis* are the two most prevalent species infecting both people and animals.

Members of the MTBC are characterised by similarities exceeding 99.8% at the nucleotide level and 16S rRNA sequences that are almost identical (Rodriguez-Campos et al., 2014; Musoke, 2016). According to theory, the MTBC originated from a single ancestral *M. tuberculosis* that diverged due to random mutations and gradual genetic material loss (Brosch et al., 2002). Although the species within the MTBC are closely related with very little genetic differences, it is still important to specify the members and identify the different strains as they all have different epidemiological significance.

Mycobacterium bovis infection in animals

There are several animal species worldwide that are susceptible to *M. bovis* infection. In countries where the disease is endemic, control measures must be implemented indefinitely because total eradication is not practical (O'Reilly & Daborn, 1995).

Mycobacterium bovis infection in livestock

Bovine tuberculosis (bTB) is caused by *Mycobacterium bovis* and causes disease in livestock, many wildlife species and humans. *M. bovis* also has the widest host range of all the MBTC members. *M. bovis* was first isolated in cattle, hence the name bovine tuberculosis. Cattle are considered one of the key maintenance hosts for the bacterium. It is suspected that cattle have been domesticated twice in different events (Loiseau et al., 2020). Once in the Near East (*Bos taurus* cattle), which encompasses Egypt, Turkey, and East Asia and once in the Indus Valley (*Bos indicus* cattle), South Asia (Loiseau et al., 2020). Both species of cattle were introduced into Africa at different times and have subsequently interbred with indigenous breeds (Loiseau et al., 2020). Given the benefits of the burgeoning pastoralism in Africa, *M. bovis* may have genetically evolved after the introduction of the two cattle breeds, though the exact chronology of these events is uncertain (Loiseau et al., 2020). It is also possible that *M. bovis* originated in the Near East and travelled to Africa with cattle (Loiseau et al., 2020), but more genetic studies will need to be done to confirm the origin of *M. bovis*. However, the origin of *M. bovis* that is cited more often in the literature, is that *M. bovis* existed in the Mediterranean before records began. From northern Italy it spread to western Europe and the United Kingdom (Webb, 1936).

Infected cattle from the Netherlands and the United Kingdom were brought with the colonial settlers and subsequently infected many other parts of the world (Webb, 1936; Renwick et al., 2007). Southern Europe, Western Asia, and East and North Africa have all contributed significantly to the domestication of cattle and the development of the *M. bovis* and *M. caprae* population structures (Loiseau et al., 2020). Bovine TB was first diagnosed in cattle in South Africa in 1880 (Paine & Martinaglia, 1929).

M. bovis is an important member of the MTBC and despite being predominantly known as an animal pathogen, it can also cause disease in humans (Bose, 2008). *M. bovis* infections generate large economic losses for farmers, due to the condemnation of carcasses at abattoirs (Michel, 2018). If there is a major outbreak, it can also affect the economy of the country because restrictions on cattle movement are then imposed (Hope & Villarreal-Ramos, 2008; Muñiz et al., 2022). Many developed countries have implemented their own control and eradication programmes for bTB (Muñiz et al., 2022) such as testing and slaughtering of infected animals. However, in many developing countries the bTB status in cattle is unknown due to the lack of control strategies. Due to *M. bovis* having the widest host range in the MTBC, it has established itself in many wildlife species, of which some are considered maintenance or reservoir hosts, and other species, spill-over hosts. The terms “maintenance” and “reservoir” hosts are generally used interchangeably in a single-host context (Nugent, 2011). By epidemiological definition, a maintenance host must be able to circulate the infection or perpetuate the disease without introductions from other sources (Michel et al., 2015). Maintenance and reservoir hosts can perpetually infect the ecosystem in which they live and can therefore spread the disease to other species that share this same environment. A spill-over host is defined by Wells and Clark, 2019 as “cross-species transmission of a parasite into a host population not previously infected”. Domestic species that are spill-over hosts include cats, dogs, goats, sheep, equines, and pigs (Amanfu, 2006; Pesciaroli et al., 2014).

The increased bTB incidence in cattle herds in developed countries, could be attributed to wildlife species that are a reservoir for *M. bovis* (Hope & Villarreal-Ramos, 2008). Some of the more notable species include the brush-tail possum (*Trichosurus vulpecula*) in New Zealand, and cervids in North America and Europe (Humblet et al., 2009). As many as 30% of cattle have been shown to shed *M. bovis* from their respiratory tracts (Costello et al., 1998). Results from epidemiological investigations suggest that during the early stages of infection, the disease is not readily spread to other cattle (Costello et al., 1998). During later stages of

infection, the bacteria can be shed into the environment where infected droplets can be inhaled by other cattle, which subsequently become infected (Rodwell et al., 2010). Recognition of macro- and microscopic lesions in cattle has aided in the understanding of the processes of the disease which has further resulted in helping develop plans for eradication from herds in some countries (Domingo et al., 2014). In developed countries, the risk of meat-borne transmission is considered negligible. However, recognising bTB during meat inspection at the abattoir is still relevant for the surveillance and monitoring of the disease in all countries (Domingo et al., 2014).

Mycobacterium bovis infection in wildlife

The role of wildlife hosts is dependent on different factors including the density of the host population, the transmission rate between animals of the same species and between different species, the effective contact rate between different species, the prevalence of bTB in the area, the longevity of the infected host, and the presence of *M. bovis* in the environment. Wildlife species that have been identified as maintenance hosts worldwide include the African buffalo (*Syncerus caffer*) and greater kudu (*Tragelaphus strepsiceros*) in South Africa (Michel et al., 2015), brush-tail possum (*Trichosurus vulpecula)* in New Zealand (Nugent, 2011), the European badger (*Meles meles*) in the United Kingdom (Delahay et al., 2001), the red deer (*Cervus elaphus*) and European wild boar (*Sus scrofa*) in France (Zanella et al., 2008), white-tailed deer (*Odocoileus vulpecula*) in North America and Canada (Brook et al., 2013) and the Kafue lechwe (*Kobus leche*) in Zambia (Renwick et al., 2007).

Spill-over or dead-end hosts are unable to maintain the infection in the absence of any maintenance host. Many different wildlife species can be spill-over hosts and South African examples include warthogs (*Phacochoerus aethiopicus*), chacma baboons (*Papio ursinus*), honey badger (*Mellivora capensis*), banded mongoose (*Mungos mungo*), lion (*Panthera leo*), leopard (*Panthera pardus*), and cheetah (*Acinonyx jubatus*), to name a few but there are still many others (Michel, 2002; Ayele et al., 2004; Renwick et al., 2007; Palmer, 2013; Michel et al., 2015). Factors such as resource utilisation patterns, disease susceptibility, transmission mechanisms, spatial distribution, and the ecology of the vector and host play a role in the maintenance and spread of the disease within non-maintenance hosts (Renwick et al., 2007). Therefore, it is becoming clear how important these spill-over species can be in disease transmission.

Mycobacterium bovis in South African wildlife

Paine and Martinaglia (1929) recorded the first cases of tuberculosis in wildlife in South Africa. The first two species in which *M. bovis* was recorded were the greater kudu and the common duiker (*Sylvicapra grimmia*) in the Eastern Cape (Paine & Martinaglia, 1929). Tuberculosis was first diagnosed in African buffaloes (*Syncerus caffer*) in 1986 in the Hluhluwe-iMfolozi Park (HiP) in northern Kwa-Zulu Natal (Michel et al., 2006, 2009). The introduction of bTB into African buffaloes is attributed to the sharing of communal grazing lands between buffaloes and cattle before the HiP was completely fenced (Michel et al., 2015). The Kruger National Park (KNP) is situated in the far north-east of South Africa bordering Mozambique. The first fatal account of mycobacteriosis in the KNP was identified in impala (*Aepyceros melampus*) in 1967 in the southern section, but the presence or species of mycobacteria was never confirmed as no cultures were grown from the carcasses (Michel et al., 2015). In July 1990, the first positive case of *M. bovis* was in a buffalo on the southwestern boundary of the KNP (Bengis et al., 2001). It is believed that *M. bovis* entered the KNP from a dairy herd located in the south, near Crocodile Bridge, in the late 1950s or early 60s (Michel et al., 2009, 2015). Regular monitoring and surveillance have taken place in the KNP since the 1990s and as a result, the incidences and prevalence of *M. bovis* have been systematically recorded. In 2006, bTB was recorded in the Pafuri region (far north) of the KNP for the first time (Michel et al., 2015). As the disease spread northward in the KNP, it also affected other species (spillover hosts).

Bovine TB has been recorded in 24 wildlife species throughout South Africa, making it a multihost pathogen and compared to other zoonoses, *M. bovis* has one of the largest and broadest host ranges (Michel et al., 2015; Renwick et al., 2007). It has the potential to cause large-spread infection and disease. Of the 24 affected species - two are considered the main maintenance hosts of bTB in South Africa: 1) African buffalo and 2) greater kudu (Grobler et al., 2002; Renwick et al., 2007; Michel et al., 2015; Thomas et al., 2021). The other 22 species are considered spill-over species which lack the potential to establish a persistent intraspecies infection but it is thought that the spill-over species may contribute to the persistence of the disease in free-ranging animals in the environment (Michel et al., 2015).

Epidemiology of bovine tuberculosis

Wildlife health assessment protocols are very similar to domestic evaluations with regard to objectives and methodology (Muñiz et al., 2022). Bovine TB is generally transmitted between animals through inhalation. It is a chronic granulomatous disease causing necrotising or caseous granulomas or tubercles in organs and tissues (Ayele et al., 2004; Domingo et al., 2014). The disease mainly affects the nasopharynx, lower respiratory system, lungs and the associated lymph nodes but can infect other organs and mucosa (Domingo et al., 2014). Recent investigations have drawn attention to the tonsils as a site of infection due to the interaction of the organ with inhalation and ingestion where the bacilli can enter the body (Domingo et al., 2014). Up to 20% of naturally infected cattle have bTB lesions in the palatine tonsils (Cassidy et al., 1999; Menzies & Neill, 2000).

There are different transmission routes, and the pathogenicity and infectious dosage needed to cause infection varies depending on how MTBC bacilli are delivered to a host (Musoke, 2016). There are different modes of transmission through which bacilli can enter the body. The most common routes of infection include ingestion (eating the bacilli) and inhalation (breathing in the bacilli), while infection via transplacental (through the placenta from mother to offspring), genital (through intercourse), and intramammary routes are infrequent (Menzies & Neill, 2000; Domingo et al., 2014; Borham et al., 2022). Depending on the mode of transmission, lesions are found in the associated organs and mucosa. However, the most common route of infection is through inhalation (respiratory route) or ingestion (oral route) of the bacilli.

The inhalation route uses the respiratory system, where the bacilli are inhaled in aerosol droplets. This route requires a very low infectious dose which could be as low as one colony-forming unit (CFU) (Dean et al., 2005; Musoke, 2016). Although one aerosol droplet can infect an animal, there are other factors that influence whether the animal will become infected, such as the immune system and virulence of the strain (Musoke, 2016). The ingestion route entails the bacilli going through the oral system. Therefore, the bacilli need to be consumed through contaminated food (milk, forage, etc), water or other materials. This mode of infection requires a high infective dose. According to reports, a few million bacilli are needed to cause infection through the oral and gastrointestinal route (Phillips et al., 2003; Musoke, 2016).

The implications of *M. bovis* transmission are farther reaching than just the wildlife as the health of humans and their livestock are also at risk due to the close proximity of rural communities to the borders of national parks. *M. bovis* is an important zoonosis that is of public health concern (Hope & Villarreal-Ramos, 2008; Thomas et al., 2021). However, in developing countries, there are often no control or eradication programmes for bTB. It is in these countries that the human populations are most at risk from infection. Humans can be infected with bTB either from their livestock that have been infected or from close interaction with infected wildlife, mainly through inhalation of the bacteria while handling infected carcasses rather than consuming the infected meat. Therefore, the implications of bTB are not isolated to only the wildlife in the KNP; other shared ecosystems are also at risk. The paternal bTB strain from the KNP has been found in Gonarezhou National Park in Zimbabwe, providing evidence of epidemiological links between the two national parks (de Garine-Wichatitsky et al., 2010).

There is also the possibility of spill-backs, where infected wildlife species can infect cattle and other livestock species. Potential spill-backs pose a zoonotic risk to farmers and their livestock at the wildlife-human-livestock interface (Michel et al., 2015). Interactions at the human-wildlife interface may be largely reduced in areas where there are fences used for conservation purposes. However, wildlife-livestock interactions cannot entirely be excluded because animal and human activities or flooding cause ongoing damage to the fences. This allows the different hosts to cross into farming areas, and livestock to cross into protected areas, thus maintaining the infection in livestock (Michel et al., 2015). There are some hosts, such as the greater kudu, which are able to breach intact fences.

Initially, wildlife species were most likely infected through the sharing of pasture lands or drinking points with cattle that were infected (Michel et al., 2015). The mode of transmission between different wildlife species can either be through the respiratory or oral route depending on the proximity of the different animals to one another. The environment can be contaminated either by aerosol droplets or through the saliva of the animals. Passing animals can then be infected from the environment. These animals can then either transmit the bacilli back into the environment or through the respiratory route to other individuals in their group. The respiratory route of infection requires relatively close proximity of individuals to one another.

It has been demonstrated that the primary transmission route in African buffaloes is via the respiratory tract. Buffaloes are gregarious animals with a loose social structure (Michel et al.,

2006) and exhibit very similar behaviour to that of cattle. Therefore, being gregarious, they form herds and the number of individuals in a herd can be in the thousands. The individuals are often in close contact with one another, which facilitates the transmission of bTB through aerosols and inhalation. Transmission between herds can also occur through fission/fusion behaviour (Michel et al., 2015). During the dry months, the larger herds split up into smaller ones for better foraging and in the rainy months, the smaller herds rejoin unrelated herds, increasing the risk of transmission (Michel et al., 2015). Together with the rejoining of herds in the rainy season, young puberty-aged heifers and bulls disperse from their natal herds, with the possibility of taking bTB with them (Michel et al., 2015).

In kudus, there can be a combination of transmission routes namely respiratory, oral and percutaneous (infection through a lesion on the skin) (Musoke, 2016). One of the first lesions ~~clinical symptoms~~ in kudus is fluctuating swelling in the parotid area which, when large enough, frequently ruptures with a thick, creamy exudate (Bengis et al., 2001; Musoke, 2016). This exudate contaminates the environment and spreads the disease.

Large carnivores can also contract tuberculosis. There are three main routes of infection that can occur, namely through ingestion of infected material (eg lymph nodes, infected organs), inhalation of the breath (respiratory route) of the prey species during suffocation, or through wounds sustained to the skin (percutaneous route) due to intraspecies aggression (Musoke, 2016). Miller et al. (2015) found *M. bovis* in tracheobronchial lavage samples from 8 of the 134 lions sampled. Although the study showed that this occurred in only 6% of the lions sampled, the implications are that lions can actively shed the bacilli into the environment.

Clinical signs of bovine tuberculosis

Bovine TB is manifested as a chronic but subclinical disease in buffaloes and cattle. Due to the subclinical nature of the disease in these two animals, clinical signs of the disease only show in the late stages of infection when emaciation is a constant finding (de Vos et al., 2001). Infected animals may not show any signs of disease for months or years, due to the slow-growing nature of *M. bovis* and may escape ante- and post-mortem detection (Michel, 2018). Clinical signs in cattle and buffaloes, are often non-specific and may only arise once the infected organ can no longer function properly (Domingo et al., 2014). Advanced cases of bTB are characterised by coughing, difficulty in breathing, enlargement of lymph nodes, sunken eyes and emaciation (Cousins et al., 2017; Michel, 2018). The disease may remain localised or

it may spread to other organs and tissues (Domingo et al., 2014). The lesions seen in buffaloes are similar to those seen in cattle. Bovine TB lesions are commonly found in the lymph nodes of the head, tonsils, and lungs (Michel et al., 2015).

According to Keet et al. (2001), the greater kudu is the only species that show distinct clinical signs of the disease characterised by bilateral abscessation of parotid lymph nodes and the development of draining fistulae. Kudus are thought to be infected through the percutaneous route, and major lesions found at necropsy include granulomatous lymphadenitis of lymph nodes of the mesentery, thorax, neck and head (Bengis et al., 2001). In some cases of terminal tuberculosis, the affected lymph nodes are greatly enlarged with severe granulomatous pneumonia and tuberculosis pleuritis (Bengis et al., 2001). In animals that have apparently inhaled the disease, lesions are absent in the lymph nodes of the head and neck but are present in the lungs and the thoracic lymph nodes (Bengis et al., 2001).

Diagnosis of bovine tuberculosis

Diagnosis is essential for assessing the efficacy of vaccine trials as well as in disease control and management (Thomas et al., 2021). Many studies have looked into ways of conducting testing and surveillance of TB in wildlife (Thomas et al., 2021). However, there are challenges to diagnosing TB in wildlife due to capture and restraint, difficulty collecting samples from wildlife species and little knowledge about the true infection status (Ryser-Degiorgis, 2013). Some of the more frequently used methods of diagnosing tuberculosis are the tuberculin skin tests for live animals, post-mortem examinations, gamma interferon assay, necropsy, histopathology, bacteriological examinations, and enzyme-linked immunosorbent assay (ELISA) (Strain et al., 2011).

Capture and gamma interferon testing, used for non-lethal zonal surveillance, were conducted from 2000 to 2006 in 3 year intervals as well as in 2007 (Michel et al., 2015). From 2009 to 2011, in Gonarezhou National Park in Zimbabwe, similar ante-mortem tests were conducted, where for the first time in 2009, bTB was diagnosed in buffalo (de Garine-Wichatitsky et al., 2010). The non-lethal testing has allowed for surveillance and reporting to be conducted without unnecessarily culling large numbers of individuals, which may have tested negative for bTB (Michel et al., 2015). During non-lethal testing, a group of buffaloes is immobilised, while blood is drawn for testing, and each tested animal is marked and fitted with a radio collar

(Grobler et al., 2002). After 36 hours, animals that tested positive are euthanised and a full post-mortem examination is conducted (Grobler et al., 2002).

Culturing is the gold standard and the preferred method recommended for diagnosing bovine tuberculosis by the World Organisation for Animal Health (WOAH, previously known as Office International des Epizooties – OIE). However, bacterial and fungal contamination significantly reduces the efficacy of the culture systems (Kassaza et al., 2014). Culturing has a high sensitivity of greater than 90% and a specificity of 100%. Löwenstein-Jensen (LJ) pyruvate is the preferred growth media for culturing *M. bovis*. This is a selective media used for the isolation and growth of mycobacteria.

The growth characteristics of *M. bovis* colonies on LJ pyruvate media are smooth, sometimes rough, and off-white in colour (OIE Terrestrial Manual, 2022). These characteristics enable a presumptive diagnosis of *M. bovis* but each isolate needs to be confirmed (OIE Terrestrial Manual, 2022). Identifying the isolates to the MTBC can be performed by conducting polymerase chain reaction (PCR) tests.

The different tests that are currently available for testing bTB have, unfortunately, not been validated in wildlife except for the skin test which has been validated for buffaloes and lions in southern African reserves (Michel et al., 2015). Another test that has been validated for buffaloes in the KNP is the gamma interferon blood test, with the limitation that it does not provide consistent specificity and sensitivity values (Michel et al., 2011). This presents a very complex and difficult situation for disease monitoring and surveillance in South Africa since many of these tests have not been validated or are not reliable (Michel et al., 2015).

Tuberculin skin testing

Intradermal tuberculin tests (skin tests) are used to diagnose bTB in cattle. The skin test compares the host's delayed hypersensitivity response to the purified protein derivatives from *M. bovis* (and *M. avium*) before and after injection into the skin (Hope & Villarreal-Ramos, 2008). After 72 hours, bTB can be diagnosed by measuring the relative increase in skin thickness at the injection sites, (Hope & Villarreal-Ramos, 2008). If an individual cow or bull responds significantly to the skin test, they are slaughtered for control purposes (Hope & Villarreal-Ramos, 2008).

Tuberculin skin tests (TST) are the gold standard for ante-mortem diagnosis of bTB in cattle. The delayed hypersensitivity to mycobacterial tuberculo-protein is the foundation for TST. The skin thickness of the animal is measured before the injection of purified protein derivatives (PPDs) into the dermis. The PPDs are derived from *M. bovis* and *M. avium* growth and lysis products that have been heat-treated (Dominguez & Nodal, 2023). After 72 hours the skin thickness is measured again and any differences are recorded. Different types of TSTs are used for various reasons. However, the most commonly used method is the comparative intradermal tuberculin test between bovine PPD and avian PPD (Dominguez & Nodal, 2023). The two PPDs are injected into the skin of the neck at two different sites, about 10 cm apart. A difference measured at 4mm or greater is the recommended cut-off for classifying animals as positive for TB by the WOAH (Awah-Ndukum et al., 2016; Clarke, 2023). However, the performance of the test is influenced by external (environmental) and internal (the host's internal environment) factors. Thus, there is no perfect cut-off point, with each country and environment having slightly different cut-off points (Awah-Ndukum et al., 2016). Therefore, the interpretation of the skin test is subjective. The sensitivity, specificity, and prevalence of the disease in the population being tested determine how well the test will predict genuine positive disease status (Awah-Ndukum et al., 2016).

Skin tests are a convenient and cost-effective method but there are limitations such as a lack of standardisation in methodology and interpretation of results (Monaghan et al., 1994). These tests are difficult to conduct on wildlife and have only been validated for use in buffaloes. Wildlife species need to be sedated and immobilised so that the test can be conducted. Another major limitation in wildlife is that after three days the animals need to be recaptured for the results of the skin tests to be taken.

Implications of bovine tuberculosis

Bovine TB is a chronic and debilitating disease and the impact of bTB is not limited to the infected individual or species. There is also an economic cost to farmers if their livestock and wildlife are infected with bTB. Calculating the full socioeconomic costs of bTB is a challenging and complex exercise that calls for the assessment of several variables, including context (where the disease is occurring, in a developed or developing country), perspective (looking at the disease's effects through a social or business lens), the animal population involved (domestic livestock or wildlife), the zoonotic impact on human health, and more (WOAH, 2021). In developed countries, the costs of bTB are usually associated with trade restrictions

on live animals and animal products and the financial costs of implementing eradication programmes (WOAH, 2021). In developing countries, the prevalence of the disease is high. The cost of bTB in these countries is mainly related to production losses (WOAH, 2021), herd demographic changes, and trade restrictions for cattle farmers. Little is known about the cost implications in the wildlife industry.

The cost of bTB is not known but farmers and game reserves and parks spend a large amount of capital on physical barriers such as fences that are not impenetrable to wildlife. Elephants, as well as natural disasters, often damage these fences which then require more capital to fix and maintain the infrastructure. The damaged fences allow for other species to pass through the boundary, ultimately putting villagers at risk, especially those who are immunocompromised.

The diagnosis of bTB in wildlife areas has brought about movement restrictions of game species which affect both the national and international wildlife trade (Michel et al., 2006). A bTB diagnosis may also jeopardise other efforts to conserve endangered species and ensure genetic diversity.

Control of bovine tuberculosis in livestock

A major barrier to controlling or eradicating bTB is the ongoing transmission of *M. bovis* between wildlife and livestock. Brush-tail possums are a known maintenance host of bTB in New Zealand (Livingstone et al., 2015). Individuals terminally infected with bTB show changes in their behaviour, often erratic, that attract the attention of inquisitive livestock and other wildlife such as deer (Livingstone et al., 2015; Musoke, 2016). It has been reported that these inquisitive animals come into very close contact (sniffing, licking etc.) with the infected possums, increasing the risk of a spillback infection (Livingstone et al., 2015; Musoke, 2016). The same could occur in the United Kingdom where there is a close association between badger populations and livestock. In South Africa, there is both direct and indirect transmission between livestock and wildlife, which is difficult to control. Sichewo et al. (2020) investigated the genetic diversity of *M. bovis* isolates at the livestock-wildlife interface in KwaZulu Natal, South Africa. Their results suggest that there is transmission between herds and species based on spoligotyping and MIRU-VNTR types being shared between buffaloes and cattle at different dip tanks or farms. Spoligotyping and MIRU-VNTR are genotyping techniques.

Once bTB has been diagnosed, the dilemma is then what to do to control the disease (Michel et al., 2015). In South Africa, bTB is classified as a controlled and notifiable disease due to its economic importance and zoonotic implications in cattle. This has led to the implementation of a national control and eradication programme, beginning in 1969 (Cousins et al., 2017). The programme successfully reduced the incidence of bTB in cattle for a short while. Since the 1990s, the control programme has not been successful due to limited resources, with sporadic outbreaks occurring in cattle throughout South Africa (Hlokwe et al., 2014; Michel et al., 2015).

The test-and-slaughter method is the main control measure used for cattle. Skin tests are conducted on the animals. If there is a positive tuberculin skin test reaction, the whole herd needs to be repeatedly tested and the animals that test positive have to be slaughtered, until the herd tests negative (Arnot & Michel, 2020). Restrictions are then placed on the farm to prevent the movement of animals until further testing has shown the farm is clear of bTB. However, an early detection system of regular testing can result in quick actions being taken to control the disease with as little culling as possible (Michel et al., 2015). However, in developing countries, bTB is often endemic and there are often financial constraints for farmer compensation (if any) due to the lack of funding from the government (Meiring et al., 2018; Gutema et al., 2020). In South Africa, there is a severe lack of state veterinarians, as well as necessary resources, for the rigorous testing that is required for bTB (Meiring et al., 2018). There is also no compensation given to farmers in South Africa for culled animals. This has resulted in active resistance to testing as there is no incentive given to these farmers for their animals that have tested positive for bovine tuberculosis (Meiring et al., 2018). A good animal health surveillance scheme in livestock can aid in preventing spillover events when there is considerable overlap between livestock and wildlife.

Control of bovine tuberculosis in wildlife

Bovine tuberculosis is a notifiable disease according to the Animal Diseases Act (Act No. 35 of 1894) (Department of Agriculture, 1984), and management practices are required. In African ecosystems, *M. bovis* is regarded as an alien species as it is believed to have been introduced to Africa during the colonial era (Bengis et al., 2002). Harsh control measures have a negative impact on conservation objectives, endangered species, tourism, and trade (de Garine-Wichatitsky et al., 2010). Due to the slow growth of the bacterium, it is essential to take preventative measures to either control or stop the spread of the disease. Once bTB has been

introduced into a wildlife population it is extremely difficult to control (Michel et al., 2006; Munyeme et al., 2009) and the risk of spillback events increase.

Controlling bTB in wildlife is not simple or easy and comes with many challenges. There have been outbreaks of bTB in some provincial/national parks, such as HiP and the KNP, as well as private game reserves surrounding these areas. The Directorate of Veterinary Services has been and is currently implementing a countrywide programme to eradicate tuberculosis from South Africa (Meat Inspectors Manual - Game, 2007). The current management practice techniques for controlling bTB are based on the test-and-slaughter method, using the intradermal tuberculin test and recording the result after 72 hours, followed by slaughtering any positive animals (Arnot & Michel, 2020). Managers and researchers face difficult challenges, stemming from the cost and logistical constraints of disease control and surveillance. In addition, ante-mortem diagnostic testing is not specific or sensitive enough, making it difficult to determine the true disease status of the animal (Michel et al., 2015). Therefore, challenges arise regarding management and control actions. Decisions may need to be made on the best way to prevent, contain or eliminate the disease, which is nearly impossible with the tools that are currently available and compensation would need to be incorporated into any strategy used where animals are killed (Michel et al., 2015: Arnot & Michel, 2020). Areas most at risk from bTB introductions are conservation areas with overlapping communal farming areas and intensified game ranching systems where wildlife becomes a valuable economic commodity (Michel et al., 2015). Elimination (test-and-slaughter) of the disease may only be effective in small, contained game reserves (Michel et al., 2015). Decision-making needs to occur in a timeous manner to prevent the disease from spreading uncontrollably (Michel et al., 2015). The test-and-slaughter method in larger areas is impractical and can cause a threat to conservation strategies, with major ramifications (Michel et al., 2015; Thomas et al., 2021). The test-and-slaughter method is also not sufficient to control the disease in areas where bTB is endemic. However, the test-and-slaughter method has proven successful in reducing the prevalence of the disease in HiP, but it was never meant as an eradication programme (Michel et al., 2015). HiP have relatively small herds of buffalo where the entire herd can be tested and quarantined until culling or release depending on the results of skin tests (Michel et al., 2015; le Roex et al., 2016).

The construction of physical barriers (eg. game fences) to protect cattle and communal property from wildlife has required a significant investment in both labour and money (Musoke, 2016).

However, these fences do not provide total segregation. Natural disasters cause damage to the fences, leaving them penetrable, which can also facilitate the movement of animals. Movement control methods are therefore empirical in preventing the spread of disease. Quarantine and controlling the movement of livestock and other game species suspected of being infected with bTB will go a long way toward preventing unwanted introductions of bTB into new areas (Michel et al., 2015).

Current Management Practices

Intradermal tuberculin tests followed by the slaughter of positive animals are the methods used for controlling bTB in South Africa and therefore make up part of the current management practices.

The animals that have tested positive are then allowed to be transported, by prior arrangement and with a red-cross permit, to an abattoir. Once slaughtered, a meat inspector checks the carcasses for any lesions. If carcasses need to be condemned, a veterinarian comes to verify the carcasses marked for condemnation. Total condemnation of a buffalo carcass occurs if the animal is emaciated or the tuberculous lesions are seen in all the major organs targeted by *M. bovis*. However, if the rest of the carcass is otherwise healthy, only the parts that are affected need to be condemned (Meat Inspectors Manual - Game, 2007). This would be at the discretion of the meat inspector or the state veterinarian.

If an outbreak of bTB is confirmed, the affected farms are placed under veterinary quarantine until the outbreak has passed, but if the outbreak cannot be brought under control the quarantine period may last indefinitely (Arnot & Michel, 2020). In large open ecosystems, such as the KNP, it is almost impossible to apply the test-and-slaughter method (de Garine-Wichatitsky et al., 2010). However, in smaller parks, such as HiP, the test-and-slaughter method has been implemented with a reduction in the incidence, but not complete eradication, of the disease (Cooper, 2012).

Vaccines for bovine tuberculosis

Vaccines have been used and found to be promising for treating reservoir species and therefore may be a valuable complementary tool for bTB control (Arnot & Michel, 2020). Due to the increasing incidence of bTB in cattle, there is a renewed interest in effective vaccines due to the importance of TB in humans and animals, particularly livestock (Muñiz et al., 2022).

Vaccination programmes were previously not a viable management option (Michel et al., 2015). There are currently no bTB vaccines that are commercially available for cattle, despite extensive research on different vaccine candidates based on the *M. bovis* BCG strain (Muñiz et al., 2022). However, the BCG vaccine is available for *M. tuberculosis* and *M. bovis* infection in humans (Hope & Villarreal-Ramos, 2008). The BCG vaccine is thought to offer protection against mycobacteria but has not resulted in sufficient protection against the disease. It has shown great variability in its effectiveness, following infection from *M. bovis* (Hope & Villarreal-Ramos, 2008; Buddle et al., 2018; Marais et al., 2019; Muñiz et al., 2022).

In the future, the development of veterinary bTB vaccines that confer greater and more consistent protection against bTB may need to be considered for all species especially threatened maintenance species (Hope & Villarreal-Ramos, 2008; Michel et al., 2015). The use of the BCG strain vaccine is being considered in wildlife species that are regarded as reservoir hosts for *M. bovis* (Muñiz et al., 2022). However, the BCG vaccine is a live vaccine and may interfere with the skin tests used for diagnosing bTB (Muñiz et al., 2022). There are two novel vaccine candidates, MbΔmce2 and MbΔmce2-phoP, that are based on the deletion of two virulence genes (mce2 and phoP) from the *M. bovis* parental strain (Muñiz et al., 2022). The RD1 locus, which encodes for virulent factors, is retained in the two candidate vaccines to allow the establishment of a robust and specific immune response (Muñiz et al., 2022). Candidate vaccine – MbΔmce2 - performed better in cattle than the BCG vaccine, showing significantly lower histopathological scores (Muñiz et al., 2022). The candidate vaccines should not be capable of transmitting the vaccine strains to other individuals or the environment (Muñiz et al., 2022). Unfortunately, the two candidate vaccines have only been tested in cattle and the effect of these candidate vaccines in wildlife is unknown.

The game meat industry provides opportunities for land-use planning and environmental management but also provides and supports economic growth and food security and contributes to the Gross Domestic Product (GDP) for South Africa (DFFE, 2022). Buffalo ranching and associated hunting activities in South Africa generate a supply of buffalo meat. In some smaller game reserves, buffalo populations have grown and are overcrowding the reserve and thus the populations need to be managed. This provides an opportunity for sustainably using buffalo meat, but it raises questions regarding meat safety if these buffaloes are infected with *M. bovis* or are vaccinated.

Research aim and objective

This study aimed to determine the safety of meat obtained from experimentally infected buffaloes post-vaccination; in particular for the presence of *M. bovis* and *M. bovis* BCG in meat cuts used for human consumption.

The objective was to perform mycobacterial cultures and PCR speciation on isolates obtained from meat samples collected at post-mortem examination from buffaloes that were part of a vaccine trial. Vaccination was performed with the BCG and inactivated *M. bovis* vaccines 12 months before the sample collection, followed by experimental *M. bovis* infection which allowed the specific confirmation or exclusion of *M. bovis* or *M. bovis* BCG in muscle tissue.

Benefits arising from this research project

There are several benefits arising from this study. It will provide scientific evidence regarding the safety of meat samples. If the meat samples test negative for live BGC and virulent *M. bovis*, the meat may be safely consumed by humans. This results in less wastage of protein and it contributes to food security. A benefit relating to the live BCG vaccine would be that the absence of live *M. bovis* BCG in meat from vaccinated buffaloes demonstrates its safety for human consumption. Therefore, there would be no concern if vaccination was considered, which has been a public health concern for people who are human immunodeficiency virus (HIV) positive consumers.

It could also be determined whether live *M. bovis* BCG transmission between individual animals is possible. In the vaccine trial, animals with different vaccine treatments were kept in the same boma, which could lead to the transmission of the live BCG vaccine and therefore may or may not be present in the meat of individuals that were not vaccinated using the BCG vaccine.

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Chapter 2 – Materials and Methods

Study animals

Bovine TB negative buffalo (n=21) were captured from Phinda and Manyoni Game Reserves, in Kwa-Zulu Natal. The animals were transported to Skukuza, Kruger National Park, where they were placed in three bomas. The vaccination and experimental infection were done under controlled conditions in the bomas.

Study design

As part of the vaccination trial, of which this study formed a part, the 21 study buffaloes were divided into four different treatment groups: 1x control group, 3x vaccinated groups (Table 1: Treatment groups in the vaccination trial.Table 1). The vaccinated groups were divided into 1x group receiving the live BCG vaccine and 2x groups receiving inactivated (IV) *M. bovis* vaccines via different routes of administration, orally and intramuscularly (IM). The animals were challenged with *M. bovis* more than seven months post-vaccination. The vaccine trial was terminated five months after the animals were challenged with *M. bovis*. During the vaccine trial study period, the buffaloes were kept in mixed groups (controls and vaccinated together) divided between four separate bomas. Each animal was assigned a tag and a microchip number at the start of the vaccine trial. Experimental infection of *M. bovis* occurred in May 2021 in all 21 buffaloes in the boma. The vaccine trial study was terminated with post-mortem examinations in October 2021.

Table 1: Treatment groups in the vaccination trial.

Vaccine Groups		Vaccine Dose	Challenge Dose of *M. bovis*	Number of Animals
Control Group		N/A	N/A	4
Vaccinated Groups	Live BCG Vaccine	$x10^6$	$x10^6$	5
	Inactivated Vaccine (Oral)	$x10^7$	$x10^6$	6
	Inactivated Vaccine (Intramuscular)	$x10^6$	$x10^6$	6

Sample collection

The animals were processed at the Skukuza abattoir. The euthanasia and the dressing of the buffalo carcasses followed the same procedures used in the cattle industry. The buffaloes were taken from the boma to the abattoir, where two teams work - the dirty team and the clean team. The buffaloes were mechanically stunned in the head and immediately bled out. The animals were then eviscerated, where the entrails were removed, and skinned. The meat inspection team then received the carcasses where the animals were inspected and all the relevant information and samples were taken for research. Routine hygiene procedures were followed for the slaughter and skinning of the buffalo which prevents any risk of contamination to the meat.

The meat samples from each animal were freshly collected, following the trimming of the carcass halves. Four different meat samples (fillet, silverside, brisket, and rump; Figure 1) were taken from each animal. Each collected sample could be linked to a specific individual because of the assigned number to each individual. The four meat samples were chosen because they are the most popular meat cuts for human consumption.

A total of 84 meat samples were made available from the vaccine trial. Each meat sampled weighed between 200-300g.

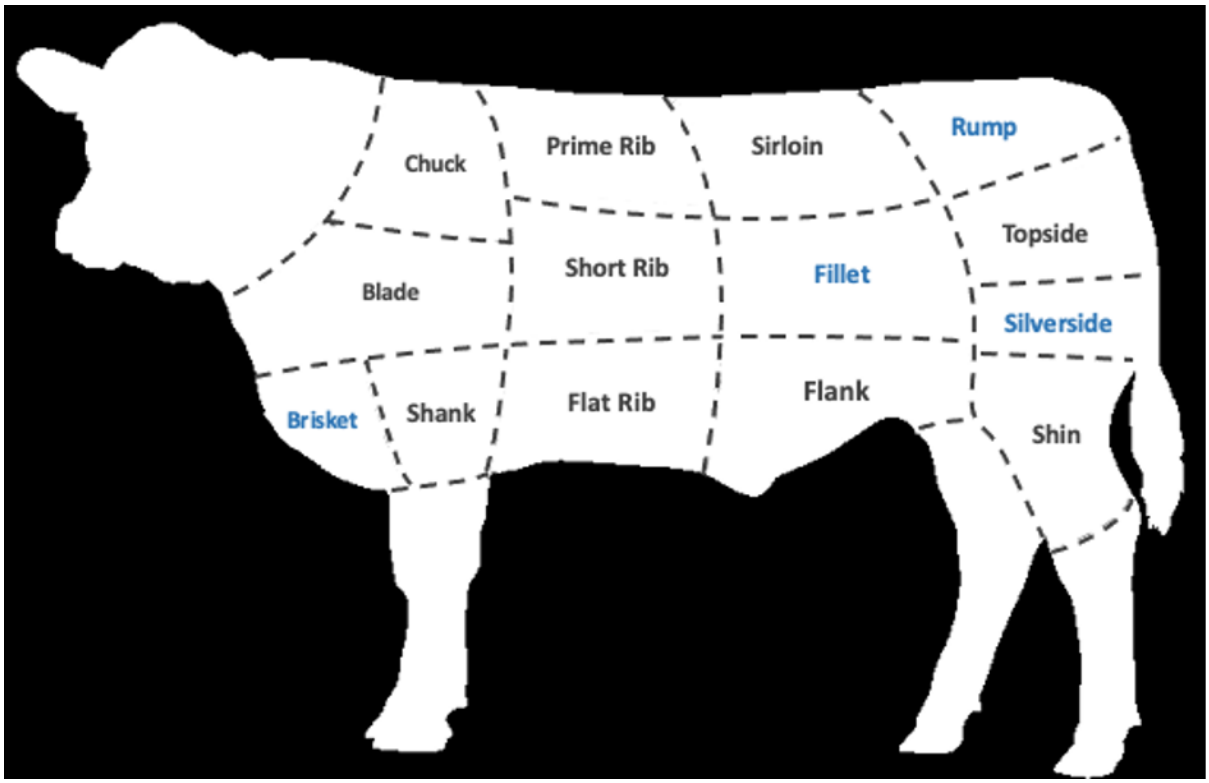

Figure 1: Meat Cuts within a bovine, as used in this study (Fillet, Silverside, Brisket, Rump). Modified from: https://bluffmeatsupply.co.za/cuts-of-meat/.

Storage

All 84 samples were stored in a designated Hisense freezer (FC130D4BW) in the biosafety laboratory level 2+ (BSL2+ laboratory) at Hans Hoheisen Wildlife Research Station (HHWRS), Orpen Gate. The samples were stored here for 16 months before they were processed.

Sample Testing

Hans Hoheisen Wildlife Research Station is part of the Department of Veterinary Tropical Diseases of the University of Pretoria's Faculty of Veterinary Science. HHWRS is where all the samples for this study were processed. This laboratory was used because it is part of the University of Pretoria and it falls within the infected zone of South Africa. South Africa has three veterinary disease control zones, namely the infected zone, buffer zone, and inspection zone. These zones exist to prevent controlled diseases from spreading from an infected animal to other susceptible animals in the rest of South Africa. HHWRS is the only laboratory within the infected zone with a Department of Agriculture, Land Reform, and Rural Development approved BSL2+ laboratory.

Test Methods

Sample Processing

The microbiological tests were conducted approximately 16 months after the meat samples were collected. However, being kept in a biobank at -80ºC the viability of the organisms as well as the sample quality were adequately maintained. The processing of the samples was conducted in line with the Standard Operating Procedure (SOP): Processing of tissue for the isolation and identification of *Mycobacterium bovis* and *Mycobacterium tuberculosis* (QA/BS/SOP BSL 008, Version 7).

The below-mentioned method was used on all 84 samples, but the explanation is based on the processing of one meat sample.

Four meat samples (samples from one animal) were taken out of the Hisense freezer (FC130D4BW) and placed in an Engel Fridge-Freezer (Model: MT45F-G4-S) in the BSL2+ laboratory to defrost overnight. Samples were then processed throughout the course of the next day.

The equipment that was used in the biosafety cabinet, per sample, included a centrifuge tube rack, 2x50ml centrifuge tubes, 1x15ml centrifuge tube, a 250ml Erlenmeyer flask filled with 4% sodium hydroxide (NaOH), a 250ml Erlenmeyer flask filled with 1% cetylpyridinium chloride (CPC), a 250ml Erlenmeyer flask filled with sterilised, distilled water, a 1ℓ container filled half full with F10 Ready to Use disinfectant, a sachet of K1020 Loops ($10\mu l$, blue), scissors, sterile sea sand, and a permanent marker (Artline725). The 15ml and 50ml centrifuge tubes were placed in the centrifuge tube racks. The 15ml centrifuge tubes were used for the reference samples. 1x50ml centrifuge tube was used for one decontamination method and the other 50ml centrifuge tube used for the other decontamination method.

The 15ml centrifuge tube was labelled with the laboratory sample number, reference, and the date the sample was placed in the tube. The 50ml centrifuge tubes were labelled with the laboratory sample number, decontamination method and the date. The decontamination methods used were 4% NaOH and 1% CPC.

Sixteen Löwenstein Jensen Pyruvate (LJ pyruvate) culture media slopes (Mast Rediprep® - Löwenstein Jensen with pyruvic acid) were brought into the BSL2+ for the four meat samples to be processed throughout the day. All the slopes were labelled with the laboratory sample number and date. Half of the slopes were labelled with NaOH and the other half labelled with CPC. Four slopes were used per meat sample; 2xNaOH and 2xCPC.

The bag containing the meat sample was cut open. A sterile pestle and mortar and sterile scissors and forceps were then opened. The meat sample was placed into the sterile mortar using the sterile forceps before undergoing a visual examination. The meat sample was then cut up using the sterile scissors and forceps and examined for any abnormalities, including the presence or absence of lesions. A representative sample of approximately 20 grams, including any lesions, was taken from each meat sample. The remaining parts of the meat sample were put back into the bag that the sample originally came from and then placed in an autoclave bag in the biosafety cabinet level 2 (BSCL2), ready for disposal when the bag was full.

The representative samples were homogenized using a pestle, mortar, sterile sea sand, and a small volume (~2ml) of distilled, sterilised water. Another small volume (~2ml) of distilled water was added to the mortar to ensure the homogenate was thoroughly mixed. 5ml of the homogenate was added to each of the 2x50ml centrifuge tubes and 14ml, or what was left in the mortar, was added to the 15ml tube as the reference sample.

To both 50ml tubes, 15ml of distilled water was added to the 5ml of homogenate to get a volume of 20ml. To the tube labelled NaOH, 20ml of NaOH was added and 20ml of CPC 1% was added to the tube labelled CPC. The final volumes of the 50ml tubes were 40ml.

The positive and negative controls were processed in the same way as the meat samples but only at the end of the week. The positive control was processed as the second last sample of the day and the negative control was processed last. Five positive and negative controls were used during the study as the processing of the meat samples took five weeks.

The positive controls used were:

1. A consolidated caseous lung sample from a male buffalo (field case),
2. Lesions from the right cranial lung lobe of buffalo 5,
3. Lesions from the right cranial lung lobe of buffalo 23.

4. Mixed granular lesions from the left caudal lung of buffalo 26 (Positive Control #4 and #5)

A total of 20 culture slopes were produced for the positive controls. Positive control #1 produced four culture slopes, two were decontaminated with hydrochloric acid (HCl - replaced with 1% CPC) and the other two were decontaminated with NaOH. Positive control #2 produced two culture slopes from NaOH. Positive control #3 produced four culture slopes, two from NaOH and two from 1% CPC. Positive controls #4 and #5 (buffalo 26) produced five culture slopes each, with NaOH used for three slopes and two slopes used for 1% CPC.

The negative controls included samples from the parotid lymph node of buffalo 21, the sub-iliac lymph node of buffalo 20, and store-bought beef rump.

Decontamination Methods

Hydrochloric Acid (HCl) Decontamination Method

HCl was used for four samples at the beginning of the study. Volumes of 5ml of homogenate, 15ml of HCl, and 25ml of distilled water were added to the labelled 50ml centrifuge tube. The centrifuge tubes were left to incubate at room temperature for 10 minutes before being centrifuged at 4000rpm for 10 minutes. After the first centrifuging, the supernatant was poured off into the liquid waste disposal flask. 3ml of sterilised, distilled water was added back into the centrifuge tube and mixed together with the back end of an inoculation loop. Once the water was incorporated into the pellet, the centrifuge tube was filled to the 50ml mark and centrifuged again at 4000rpm for 10 minutes. Once this was complete, the supernatant was once again poured into the liquid disposal flask. Approximately 1ml of sterilised distilled water was added to the second pellet. An inoculation loop was used to break up the pellet. The same inoculation loop was used to inoculate two LJ pyruvate slopes.

Sodium Hydroxide (NaOH) Decontamination Method

The tube containing the NaOH was left to incubate for 10 minutes at room temperature before being centrifuged for 10 minutes. After the initial centrifuging, the supernatant was poured into a liquid waste disposal flask. Three millilitres of distilled water was added back into the 50ml centrifuge tube, containing a pellet, before being mixed together with the back end of an inoculation loop. Once the water had been incorporated into the pellet, the centrifuge tube was filled to the 50ml mark and centrifuged again for another 10 minutes at 4000 rpm. This was to neutralise the NaOH. After this round of 10 minutes, the supernatant was again poured off. The

remaining pellet had 1ml of distilled water added to it. This was to soften the pellet just enough so that the inoculation loop could be used to break up the pellet. Once the pellet had been broken up, the same inoculation loop was used to inoculate two LJ pyruvate slopes.

Cetylpyridinium Chloride (CPC) Decontamination Method

The 50ml tube labelled with the CPC which contained the CPC decontaminant was treated slightly differently to the NaOH method. The 50ml tube containing the CPC was left to incubate at room temperature for a minimum of 30 minutes and a maximum of 60 minutes. The average time left for the CPC to incubate was 40 minutes. During these 40 minutes, the tube was shaken regularly to ensure the solution had as much surface area with the homogenate as possible. After 40 minutes, the 50ml tube was centrifuged. The supernatant was poured off. The remaining pellet had 2ml of distilled water added to it and was allowed to sit for 2 minutes to soften up the pellet. After 2 minutes the pellet was soft enough to be broken up with the back end of an inoculation loop. A sterile inoculation loop was then used to inoculate two LJ pyruvate slopes.

Incubation

Once all the medium slopes for the one sample had been inoculated, they were placed in IncoTherm (Labotec) Incubators (Serial Numbers: 9296R01 and 9296R02) at 37^{o}C in the BSL2+ laboratory for ten weeks with no humidification because the medium is in a sealed bottle.

Growth Monitoring and Identification

All medium slopes were checked for growth on a weekly basis for ten weeks. The positive and negative controls were monitored in the same way as the meat samples. The monitoring process followed the SOP: Monitoring and evaluation of cultures in the BSL2+ laboratory (QA/BS/SOP BSL013, Version 2). A symbol chart was used (Appendix A) to record the status of all the cultures, meat samples and the controls.

After ten weeks, all culture slopes were checked for growth. At the end of the monitoring period, the culture slopes with no growth were discarded into autoclave bags. These discarded slopes were autoclaved and placed in the walk-in fridge ready for collection by the disposal company to be disposed of correctly.

The culture slopes that had growth or suspected growth were placed into an empty incubation tray on the workbench and then stored in the incubator before DNA extraction and PCR testing. The culture slopes were ranked from samples with the most to least growth. They were placed into batches and labelled accordingly. The culture slopes were put into groups of seven. A total of ten culture slopes were used for PCR and visualised using gel electrophoresis.

DNA Extraction

Nine 2ml microcentrifuge tubes were labelled with the laboratory number of the sample and the decontamination method. 1ml of distilled water was pipetted into each microcentrifuge tube using a 100-1000μl Eppendorf Reference Pipette.

One colony was removed from each growth media using 10μl K1020 Loops (blue), taking a new loop for every new media and placed in the corresponding microcentrifuge tube. Once all the microcentrifuge tubes had a colony placed in them, they were put in an Eppendorf Thermomixer Comfort at 95ºC for 25 minutes. Thereafter, the tubes were placed into a container which was disinfected with double strength F10 in order to be taken out of the BSL2+ laboratory.

The microcentrifuge tubes were used as crude Deoxyribonucleic acid (DNA) templates in the conventional polymerase chain reaction (cPCR).

Conventional PCR

Distilled water was used as the negative control. Positive Control #4 (Left caudal lung lesion from buffalo 26) was used as the control from the study as it had the most growth out of all the controls.

The different reagents used for the PCR targeted different regions of mycobacterial DNA including rpoB forward and reverse (rpoB-F and rpoB-R), RD1 forward and reverse (RD1-F and RD1-R), RD8 forward and reverse (RD8-F and RD8-T-R), RD8 deleted forward and reverse (RD8-F and RD8-B-R). The rpoB primers are used to facilitate the identification of all *Mycobacterium* species (Lee et al., 2000; Kim et al., 2013). The RD1 reagents are used to detect virulent MTBC strains; *M. tuberculosis*, *M. africanum*, and *M. bovis* (Kim et al., 2013). *M. bovis* BCG, *M. microti*, and NTMs produce no RD1 products (Kim et al., 2013). The RD8

reagents are used to determine the presence of *M. tuberculosis* and *M. africanum* (Kim et al., 2013). The RD8 deleted reagents are used to determine the presence of *M. bovis* and *M. bovis* BCG (Kim et al., 2013). *M. microti* and NTMs produce no RD8 products.

Each PCR reaction contained $2\mu l$ of crude DNA, $12.5\mu l$ of 2x Phusion Flash PCR Master Mix, $0.5\mu l$ of each primer, and made up to $25\mu l$ with nuclease-free water.

The PCR was conducted in an automated thermal cycler (2720 Thermo Cycler, Applied Biosystems). The cycling parameters are based on Kim et al. (2013) with slight modifications (QA/Mol/SOP 4.1 Version 1). The amplification was initiated by 1 cycle of a denaturation step set at 98ºC for 10 minutes followed by 35 cycles of 95ºC for 15 seconds, 68ºC for 30 seconds, and 72ºC for 30 seconds. The final elongation step was set at 72ºC for 7 minutes. Sterile, distilled water was used as the negative control.

The PCR amplicons were stained with $2\mu l$ of Gel Loading Dye Purple (6x) (#B7024S: New England BioLabs).

The PCR amplicons were electrophoretically fractionated in 2% agarose with ethidium bromide at 100V for 30 minutes. $5\mu l$ of 100 base pair (bp) DNA ladder (New England BioLabs) was used to reference the specific PCR band sizes. Visualisation occurred on a FotoDyne Imagine System.

Based on the detection of bands of various sizes, the outcome of speciation was interpreted. The different primers, their sequences, and the base pairs are shown in Table 2. All *Mycobacterium* species yield a single 518bp product with the rpoB-F and rpoB-R primers (Kim et al., 2013). *M. tuberculosis*, *M. africanum,* and *M. bovis* yield a single 254bp product with the RD1-F and RD1-R primers, while *M. microti* and *M. bovis* BCG do not produce any products with these primers (Kim et al., 2013). *M. tuberculosis* and *M. africanum* strains also produce a single 150bp product with the RD8-F and RD8-T-R primers, indicating the presence of the RD8 sequence in these species (Kim et al., 2013). *M. bovis* and *M. bovis* BCG strains yield a single 360bp product with the RD8-F and RD8-B-R primers, which indicated the deletions of the RD8 sequence in *M. bovis*, including *M. bovis* BCG (Kim et al., 2013).

Table 2: PCR primer sequence and corresponding regions of difference with the number of base pairs

Description of Target	Primer Names	Primer Sequence	Base Pairs
rpoB	rpoB-F rpoB-R	5' GCTGGACATCTACCGCAAGCTGC 3' 5' CAGCGGGTTGTTCTGGTCCATG 3'	518
RD1	RD1-F RD1-R	5' CGAGGGGAAGCAGTCCCTGA 3' 5' AGGTCGAACTCGCCCGATCC 3'	254
RD8 Present	RD8-F RD8-T-R	5' GTCGAAGCGGGGCGCTCT 3' 5' GCGCAACGGATTTCCATCGT 3'	150
RD8 Deleted	RD8-F RD8-B-R	5' GTCGAAGCGGGGCGCTCT 3' 5' GGTTCTTGGCGTCTTGGAAGG 3'	360

Primer sequences according to Kim et al., 2013

Data Analysis

All the data was entered into an Excel spreadsheet (Appendix B). Pivot tables were used for analysing the data.

Ethical Approval and Section 20

Approval to perform this project was granted by the Animal Ethics Committee (AEC) of the University of Pretoria and the Faculty Research Ethics Committee (REC; AEC and REC Certificate Number: REC163-22, Appendix C).

The samples used in this study were collected during the larger vaccine trial conducted by Dr Jennie Hewlett. The AEC granted the approval of that project (Certificate Number: REC148-19; Appendix D) and the collection was done in line with the Department of Agriculture, Forestry and Fisheries (DAFF) Section 20 application (Certificate Number: 12/11/1/1/6; Appendix E). The processing of the samples was done in line with the DAFF Section 20 application that was obtained for this project (Certificate Number: 12/11/1/1/8 (2868PM); Appendix F).

Ethical Considerations

This research project is linked to a larger trial investigating the efficacy of anti-tuberculosis vaccines in African buffalo (REC 148-19 – Appendix D). The welfare of the animals is covered by REC148-19.

Reference List

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Chapter 3 - Results

Samples

Four different meat cuts from 21 buffaloes were used in this study. These meat cuts were fillet, silverside, brisket and rump. Overall, 84 samples were processed for this study.

Macroscopic examination

During the processing of the samples, two pathological findings were observed. One pathological finding was found on the rump cut of buffalo 3 (IV-IM) under a layer of fat. It burst and was filled with pus. The other pathological finding, from the brisket cut of buffalo 7 (IV-Oral), presented as an encased spherical mass of approximately 0.4 x 0.4 x 0.4cm in dimension. The contents of the spherical mass were chalky in texture.

Culture Results

Colony morphology

The colony morphology observed in the positive controls was round (1 - 2mm diameter), cream-coloured, opaque, and raised with some areas having a rough texture.

The colony morphology on the study meat samples varied in colour from clear (no pigmentation) to opaque white. The colonies were very small in size (1mm). The surfaces of the colonies were smooth and not irregularly raised.

Culture Controls

All the negative controls showed no growth at the end of the 10 weeks.

All the culture slopes inoculated with the positive control samples grew substantial colonies. Positive Control #4 was chosen for PCR amplification. The specific band sizes for the positive control were 518bp, 360bp, and 254bp.

A total of 20 culture slopes were produced for the positive controls. Table 3 shows the source of the positive control tissues. 50% ((10/20)*100) were from the left caudal lung of buffalo 26, 20% ((4/20)*100) were from the lung sample of an infected male buffalo (field case) not part of the vaccine trial, 10% ((2/20)*100) were from the right cranial lung lobe and consisted of mixed lesions from buffalo 5, 20% ((4/20)*100) were from a right cranial lung from buffalo 23 (Table 3).

Table 3: The source of the positive control tissues and the corresponding buffalo number.

Sample Type	Number (x/20)	Percentage (%)
Lung lesions from buffalo 26	10	50
Lung lesion from a field case	4	20
Lung lesions from buffalo 5	2	10
Lung lesions from buffalo 23	4	20

Suitability of decontamination agents

During processing, each sample was treated with two combinations of decontamination methods. One consisted of HCl and NaOH and the other of 1% CPC and NaOH. Gel formation was observed when using HCl as a decontamination method. Due to the gel formation, the inoculum was not picked up by the loop, making the inoculation of the culture slope impossible. A small amount of liquid with the final pellet is required for the inoculation loop. No pellet was formed after neutralising the HCl, thus *M. bovis* could have been suspended anywhere in the gel which increased the chance of it being missed when using the inoculation loop. HCl was substituted with 1% CPC as the alternate decontaminant. The 1% CPC did not turn the homogenate to a gel-like consistency.

Meat samples

A total of 340 culture slopes were produced for the meat samples. Colony-like growth was observed on ten culture slopes from seven animals. No typical *M. bovis*-like colonies were observed.

Contamination rate

Growth, other than those with typical *M. bovis* characteristics, was observed on the culture media from 11 animals of which five animals (buffaloes 5, 6, 10, 16, and 22) had growth on more than one sample type (meat cut). Of these five animals, growth was observed on the media of the brisket and silverside of buffalo 5, the brisket and rump of buffaloes 6 and 16, the

rump and silverside of buffalo 10, and the brisket and silverside of buffalo 22. Growth was only observed on one sample type for the remaining six animals.

A total of 378 culture slopes were produced during this study. Of the 378 culture slopes, 340 culture slopes came from the test samples (meat samples), 20 culture slopes were positive controls, and 18 culture slopes were negative controls. Of the 378 slopes, 30 showed contamination resulting in a contamination rate of 7.94%. Of the 7.94%, 16.67 % ((5/30)*100) showed contamination of more than 50% of the medium slope, 66.67% ((20/30)*100) showed contamination on less than 50% of the medium slope, 16.67% ((5/30)*100) showed contamination of the entire slope.

Decontamination Methods

Of the 378 culture slopes, NaOH was used in the decontamination of homogenates inoculated onto 190 culture slopes. HCl was used in the decontamination of homogenate inoculated onto 24 slopes and 1% CPC was used in the decontamination of homogenate inoculated onto 164 slopes.

Polymerase Chain Reaction (PCR)

In total, ten culture slopes presented with colony-like growth; this excluded all the positive controls, which were further investigated using PCR. The cultures were evaluated according to the colony morphology of the growth. These ten culture slopes were processed for *Mycobacterium* speciation by PCR.

No *M. bovis*, *M. bovis* BCG or *Mycobacterium* spp. were detected in any of the meat samples by PCR (Figure 2).

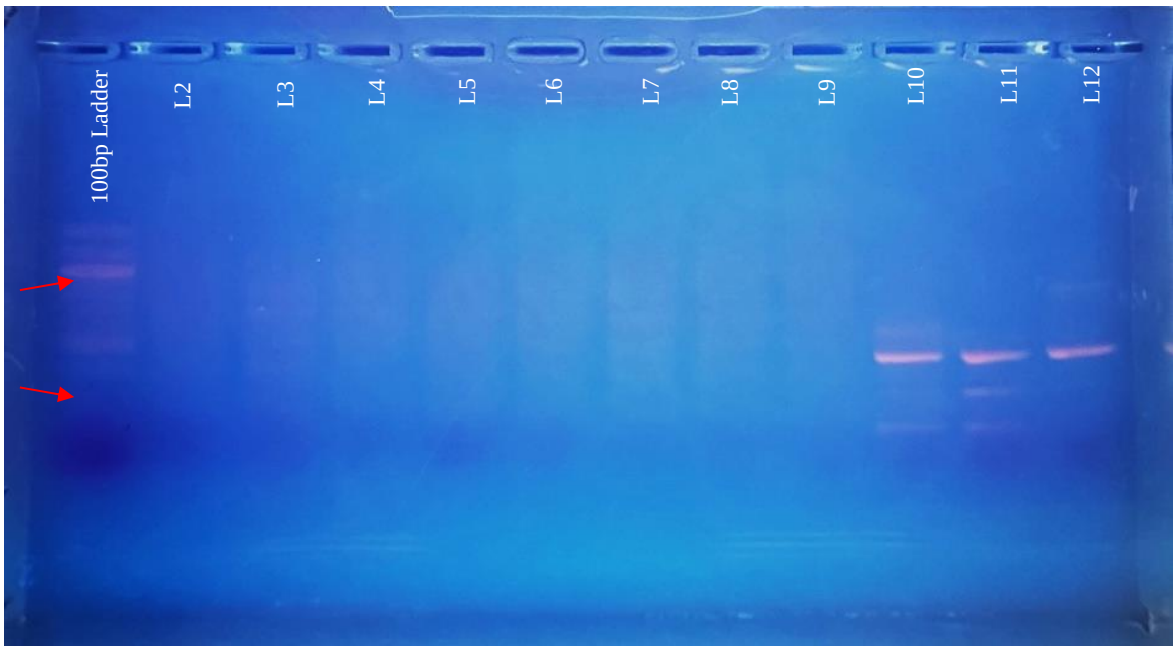

Figure 2: A 2% agarose gel showing the electrophoretic fractionation of the PCR products. L2: Negative Control; L3: 319C NaOH; L4: 313A NaOH; L5: 316D NaOH; L6: 325B NaOH; L7: 326B 1%CPC; L8: 318B NaOH; L9: 320A NaOH; L10: Positive Con #4 NaOH; L11: Field Sample of M. bovis, L12: ATCC 6841.

Chapter 4 - Discussion

While meat must be safe for human consumption, the base of this study was to determine whether or not *M. bovis* and/or *M. bovis* BCG were present in the meat samples. This study set out to determine the meat safety of meat cuts taken from vaccinated (inactivated *M. bovis* vaccine and live BCG vaccine) buffaloes experimentally infected with *M. bovis*. *M. bovis* was not isolated in the meat cuts 154 days post-experimental infection.

The different meat cuts (fillet, silverside, brisket and rump) for this study were chosen for their popularity for human consumption. The results of this study showed that no *M. bovis* or *M. bovis* BCG were detected in the meat samples from these animals. Therefore, as shown in this study, the specific meat cuts from buffaloes infected with *M. bovis* or meat from buffaloes vaccinated with *M. bovis* BCG may be considered safe for human consumption. Despite the high sensitivity of culture as a detection method, a small chance remains that, in severe or widespread instances of BTB, there may be a low concentration of M. bovis in the muscle tissue. This possibility cannot be eliminated by culture-negative results.

Bovine TB continues to be a global health problem, both for animals and humans. One of the epidemiological concerns that remains to be verified about zoonotic tuberculosis caused by *M. bovis* is the potential for humans to become infected through the consumption of meat (Clausi et al., 2021). The risk of human infection is linked to the persistence of tubercular infection in the organs and lymph nodes of cattle and wildlife.

There is huge economic potential in South Africa's game meat industry. It is therefore important that research is conducted on aspects such as meat safety to prove that meat from animals (such as buffaloes) , potentially infected or infected with bovine tuberculosis, is safe for human consumption. The game meat industry faces a variety of challenges, such as the informality of the industry, the complexities regarding regulations that are deemed too complex, the high level of non-compliance with regulations within the industry, and the major impact of diseases, such as tuberculosis and foot and mouth disease. These challenges affect meat exports from South Africa to the world (DFFE, 2022). Not only do diseases affect our export markets but they negatively affect the South African economy. One of South Africa's biggest economic sectors is agriculture and with continuous disease outbreaks the growth prospects for the sector are decreased, while prices continue to rise for consumers within South

Africa. Ensuring that the game meat industry is more compliant with international regulations will allow for increased food security and sustainable economic growth within the country, while also enabling the export of game meat to the rest of the world. Therefore, research into the meat safety of wildlife would ensure that a relatively untapped resource in South Africa could be used to not only grow the GDP of the country but also allow for increased food security and a decreased public health risk for those who already consume game meat.

Concerning food safety, there are limited studies that have researched the risk of transmission of *M. bovis* to humans through the consumption of meat from known hosts and species affected by the disease. The European Food Safety Authority (EFSA) has reported, based on scientific findings, that the possibility of human infection from the consumption of meat and meat-derived products from animals that reacted positively to the intradermal tuberculin tests is relatively low or even absent (EFSA, 2004, 2013). Drieux (1957) reviewed studies of the isolation of *M. bovis* from skeletal muscle (EFSA, 2004, 2013). The majority of these studies were either unable to extract *M. bovis* from skeletal muscle or only seldom succeeded in doing so through culture (EFSA, 2004, 2013). However, two studies isolated *M. bovis* from skeletal muscle tissue in high proportions in the cases studied (EFSA, 2004).

After extensive literature searches, there are very few studies that focus on natural contamination of game meat with members of the MTBC. From the literature searches, the meat samples were spiked with *M. bovis* or *M. bovis* BCG in the studies that concerned food safety.

Clausi et al. (2021) spiked the meat (loin steak and minced meat for sausages) of 15 wild boars (*Sus scrofae*). The results found in the study showed that even after being spiked, only two loin samples were PCR positive while all the cured sausage samples were positive. Although Clausi et al. (2021) did not explicitly state the exact location of their loin, their results can be compared to the fillet results in the current study, as the fillet is also known as the tenderloin. This result implied that the meat from wild boar artificially infected with *M. bovis* poses a risk to consumers.

To distinguish between living and dead MTBC species in venison, a study by Dorn-In et al. (2019) used quantitative PCR (qPCR) amplification along with Propidium Monoazide (PMA™

Dye) dye to create a rapid and precise method for quantifying and differentiating living and dead MTBC cells. In the German Alpine region, where bTB infection in red deer is highly prevalent, venison (red deer) was purchased from nearby butchers. The presence of *Mycobacterium* species was evaluated in fifty samples. *M. bovis* BCG was inoculated into meat samples to be used as controls to detect dead vs living cells. They observed only one suspicious colony from the 50 samples. The gene sequence (16S rRNA) was amplified with universal primer pairs and the results showed a 99% similarity to a DNA sequence of *M. avium* provided by GenBank. The monitoring methods were similar to the monitoring methods used for this meat safety study but the culture media was different. No *Mycobacterium* species were observed in this study and only one colony was observed by Dorn-In et al. (2019). Therefore, although these two studies differ in terms of how the meat was infected, the results from the two studies are comparable.

The contamination rate of culture media in the current study was 7.94%. The contamination rate in this study is higher than 5% which is considered normal in tuberculosis diagnostic laboratories. However, this is a small percentage compared to a study conducted by Kassaza et al. (2014). The study conducted by Kassaza et al. (2014) used sputum (mucus made in the lungs) while the current study used meat samples. Kassaza et al. (2014) had a contamination rate of 32.1%. Sputum samples are expected to have a higher contamination rate due to having more contaminants in the samples. Meat tissue is considered a clean tissue type, compared to sputum and lymph nodes, which filter harmful substances and waste products from the body.

The contamination observed on the 30 culture slopes could have occurred for different reasons. The most likely explanation for the media becoming contaminated, is from the meat sample containing traces of bacteria, despite decontamination. The meat samples were collected from the abattoir under non-sterile conditions, therefore the meat samples may have been contaminated through the environment, ultimately showing up on the culture media. It is unlikely that the source of the contamination came from within the media. Manufacturing companies carry out sterility tests on their media to ensure the media is free of microbes. Therefore, the contamination of the media was random.

From the extensive literature searches, no other study was found to differentiate between the different cuts of meat when culturing for mycobacteria. The reason for this paucity of studies is due to research being focussed mainly on the dissemination of *M. bovis* to the respiratory

system and the associated lymph nodes. There are two stages by which tuberculosis spreads within the body: the main (primary) complex and post-primary dissemination (Domingo et al., 2014; Borham et al., 2022). The term "main or primary complex" refers to this dual infection of the initial site of infection and the new site of infection being the draining lymph nodes associated with the initial infection site (Domingo et al., 2014). Depending on whether both lesions are present or if the lesion at the site of entry is absent, the infection is typically classed as full or incomplete. When bacteria inside the lesion remain dormant and do not proliferate, it is characterised as the non-replicative persistence phase of infection, causing a latent infection that may last for years (Borham et al., 2022). Chronic or post-primary tuberculosis occurs when the immune system is unable to stop the spread of infection or when the dormant pathogen is reactivated and the initial small granulomatous lesion becomes larger (Domingo et al., 2014; Borham et al., 2022). Tissue damage progresses to large caseous necrotic lesions that have become mineralised and fibrotic (Domingo et al., 2014). Generalisation can occur during both the initial infection and the post-primary phase, known as early and late generalisation respectively. As tubercles enlarge and erode small blood or lymphatic arteries, the mycobacteria spread haematogenously or lymphatically, resulting in generalisation (Domingo et al., 2014). However, there is a general absence of literature referring to *M. bovis* in musculature and the dissemination of *M. bovis* into muscle tissue.

In populations with an unknown *M. bovis* prevalence, the culture method (assuming 100% sensitivity and 100% specificity) is considered to detect infection with 95% confidence if the prevalence is at least ~3.5%. We are confident that this study would have detected *M. bovis* if present in the meat samples since 100% of the buffaloes were classified as infected by culture, histopathology, or immunological tests (Hewlett, unpublished data).

Decontamination methods are required to avoid the overgrowth of unwanted micro-organisms that are also present in the meat sample (OIE Terrestrial Manual, 2022), even with the use of a selective growth media. Decontamination methods and techniques are used throughout the entire process from sample collection to processing. The decontamination methods used in this study are based on the methodology recommended by the WOAH with slight adjustments. The preparation of samples for culture requires a decontamination process. The decontamination ensures that other micro-organisms are substantially reduced in number or killed before inoculation, allowing the propagation of the slowly-growing mycobacteria and reducing the

potential for contamination of the culture media. The decontaminants generally used for *Mycobacterium* species are NaOH and HCl.

NaOH was used throughout the study. The use of HCl was discontinued. Once the decontamination and neutralization process, with HCl, was complete, the homogenate had a gel-like consistency. It was difficult to collect onto the inoculation loops. Gelation is the process that occurred in these meat samples. Wong (2018) defines protein gelation as "an aggregation of denatured molecules with a certain degree of order, resulting in the formation of a continuous network". Gelation can occur physically or chemically. There are different types of chemical gelation, but for this study, acid-induced gelation is the chemical process that occurred to form the gel-like consistency. Acid-induced gelation is common in some food products (Totosaus et al., 2002). HCl lowered the pH of the small meat sample in solution, denaturing the myosin to cause spontaneous interactions followed by the formation of a network structure (Totosaus et al., 2002).

Cetylpyridinium chloride (CPC) was used as a replacement for HCl. CPC is a quaternary ammonium surfactant. The preferred concentration is 1% CPC which was used in this study. It is a broad-spectrum antimicrobial agent widely used as an antiseptic and disinfectant. Examples include mouthwashes and a cleaning solution for food items (Nasila et al., 2021). Smithwick et al. (1975) showed that CPC is effective at liquifying and decontaminating sputum specimens. 1% CPC and 2% sodium chloride (NaCl) were used to decontaminate raw milk (Sichewo et al., 2020). A study conducted by Radomski et al. 2010) used 0.1% CPC on surface water samples for the detection of nontuberculous mycobacteria (NTM). Although the study by Sichewo et al. (2020), Radomski et al. (2010) and the current study cannot be directly compared, it showcases that CPC can be used in the decontamination of a variety of samples from milk and meat to water in varying concentrations.

Given the significance of tuberculosis in both humans and animals, there is a renewed focus on developing effective vaccines in response to the rising frequency of bTB in cattle. Despite intensive research on many vaccine candidates based on the *M. bovis* BCG strain, there are presently no commercially licensed bTB vaccinations for cattle. Nevertheless, the live BCG vaccine is available for *M. bovis* and *M. tuberculosis* infection in humans. The BCG vaccine is thought to offer protection against mycobacteria but has not resulted in enough protection against the disease and showed great variability in effectiveness, following infection from *M.*

bovis (Hope & Villarreal-Ramos, 2008; Buddle et al., 2018; Marais et al., 2019; Muñiz et al., 2022).

In a subset of human patients with primary immunodeficiency illnesses, the BCG vaccination, which is given to all infants in countries with a high prevalence of tuberculosis, may cause serious complications ranging from local disease (known as BCGitis) to widespread disease (BCGosis) (Movahedi et al., 2010). A few case studies show that children vaccinated with the BCG vaccine at a very early age and who are immunocompromised have been shown to develop BCGiosis (Movahedi et al., 2010; Yamazaki-Nakashimada et al., 2020). Therefore, the BCG vaccine can revert to virulence within humans.

The BCG vaccine has been shown to reduce the severity of disease in White-tailed deer in the United States, but vaccine persistence in the tissues of animals has also been observed (Palmer et al., 2010). This study used oral and subcutaneous (SC) BCG vaccines. Interestingly, the study found that SC BCG is disseminated from the site of injection to sites such as the hepatic and bronchial lymph nodes, suggesting systemic spread via the lymphatic or vascular channels. At no point in the study was BCG isolated from any meat samples, both from the oral and SC vaccine groups. The lymphoid organs are usually avoided for human consumption and muscle meat is generally cooked before consumption. Therefore, the potential for human exposure through consumption is low but cannot be disregarded.

The vaccines used in the larger vaccine trial during which the samples for this study were collected included the live, attenuated BCG vaccine and an inactivated *M. bovis* vaccine. The inactivated *M. bovis* vaccine is heat-inactivated and contains dead *M. bovis* cells (Garrido et al., 2011). The inactivated vaccine was administered either orally (IV-Oral) or intramuscularly (IV-IM). The inactivated *M. bovis* vaccine, regardless of the administration route, will not pose a public health risk because it is inactivated.

Examining the longevity of viable BCG in the anatomical tissues of vaccinated animals and determining whether or not vaccinees may excrete viable BCG were two of the objectives of the Williams et al. (2022) investigation. According to their findings, no BCG was grown from calves' saliva or from raw milk samples taken from lactating cows who had received a BCG vaccination. Therefore, the likelihood of BCG infection in humans from drinking raw milk from BCG-vaccinated cows is very low. The results from this study show that no *M. bovis* or

M. bovis BCG were found in these meat cuts and therefore do not pose a public health risk and can be safely consumed.

African buffaloes are not used for milk production but the results from Williams et al. (2022) can be extrapolated to buffaloes concerning the results on saliva. From the extensive literature searches, there are no reports of humans becoming infected with the *M. bovis* BCG vaccine administered to animals. However, the public health risk of *M. bovis* BCG returning to virulence in humans and animals cannot be overlooked. Individuals whose immune systems are compromised are more likely to have *M. bovis* BCG return to virulence as compared to healthy individuals. Due to no *M. bovis* BCG being found in the meat cuts from this study, we can negate the risk of *M. bovis* BCG returning to virulence in meat tissues.

Limitations

Sample size

The small sample size and the fact that all samples were sourced from a group of experimentally infected animals is a limitation. However, since the meat samples came from a controlled animal study, the infection status of all animals was confirmed which is not always the case when carcasses for similar studies are harvested from infected herds.

This study aimed to assess the safety of the meat for consumption. While the sample size was limited, the strength of this study is that, for all experimentally infected animals tested, multiple replicates (n = 4 for each animal) were screened for the presence of *M. bovis* or *M. bovis* BCG. Therefore, assuming a 100% specificity and sensitivity of the test, the presence of the pathogen would likely be detected, even if it occurred at a low prevalence. The fact that the pathogen was not detected indicates that these samples are not likely to contain *M. bovis* or *M. bovis BCG* and are therefore safe for human consumption.

Culture

Culturing is the gold standard and preferred method recommended for diagnosing bovine tuberculosis by the WOAH. Culturing has a high sensitivity of greater than 90% and a specificity of 100%. However, the specificity and sensitivity of culturing are linked to organs and other tissue types, not to cuts of meat. Therefore, the sensitivity and specificity can be biased due to the tissue samples used in this study.

Conclusions

The consumption of venison in South Africa, and worldwide, is on the rise. There are very few studies that focus on the contamination of muscle meat from *Mycobacterium bovis*. However, from studies in which meat was artificially infected, there is evidence that *M. bovis* can survive in muscle tissue. Therefore, the safety of the meat from known infected carcasses, without being spiked, has not previously been determined. Thus this is a novel, baseline study and will allow for future research in this field.

In conclusion, this study showed that meat from vaccinated and experimentally infected buffaloes showed no contamination of *M. bovis* BCG approximately 13 months after vaccination and no contamination of *M. bovis* five months after infection. The absence of *M. bovis* and *M. bovis* BCG from the different meat cuts insinuates that, although *M. bovis* had ample time to colonise the entire animal, it was not detected in the selected meat cuts. A corollary to this is that as no *M. bovis* or *M. bovis* BCG was detected, the meat could be considered safe for human consumption.

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Appendices

Appendix A: Symbol chart used during the growth and monitoring of the culture samples in the incubator

Symbol	Description
0	No Growth
d	Medium Dried Out
+	Typical Growth Comprising of More Than 5 Colonies
1, 2, 3, 4, 5	Number of Colonies Observed if 5 or Fewer Colonies were seen
0/c	Less than 50% of the Medium Surface is Contaminated
c/0	More than 50% of the Medium Surface is Contaminated
C	The Whole Medium Surface is Contaminated
*****	One or More Pigmented Colonies
Big colony	Atypical Growth
?	Unsure
Clear	The growth on the media was clear and not pigmented
Egg Yellow	The growth on the media was egg yellow in colour
White	The growth on the media was opaque.

Appendix B: Excel Spreadsheet of Raw Data

Buffalo_Number	Sample_Type	Vaccine_Treatment	Sample_(Lab)_Number	Date_Processed	Treatment	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9	Week 10	Final_Growth	PCR_Final_Result	Comments
1	Fillet	IV-Oral	313A	17/01/2023	HCl	0	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. The homogenate that was neutralised the HCL treated specimen turned to have a jelly-like consistency with a pH of 2.
1	Fillet	IV-Oral	313A	17/01/2023	HCl	0	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. The homogenate that was neutralised the HCL treated specimen turned to have a jelly-like consistency with a pH of 2.
1	Fillet	IV-Oral	313A	17/01/2023	NaOH	0	0	0	0	0	0	0	0	0	1	Yes	Culture_Suspect_PCR_Negative	No abnormalities were found on first inspection. The homogenate that was neutralised the HCL treated specimen turned to have a jelly-like consistency with a pH of 2. The growth colony formed was opaque white.
1	Fillet	IV-Oral	313A	17/01/2023	NaOH	0	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. The homogenate that was neutralised the HCL treated specimen turned to have a jelly-like consistency with a pH of 2.
1	Fillet	IV-Oral	313A	18/01/2023	HCl	0	0	0	0	1*-Yellow	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. The homogenate that was neutralised the HCL treated specimen turned to have a jelly-like consistency with a pH of 2.
1	Fillet	IV-Oral	313A	18/01/2023	HCl	0	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. The homogenate that was neutralised the HCL treated specimen turned to have a jelly-like consistency with a pH of 2.
1	Silverside	IV-Oral	313B	17/01/2023	HCl	0	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. The homogenate that was neutralised the HCL treated specimen turned to have a jelly-like consistency.
1	Silverside	IV-Oral	313B	17/01/2023	HCl	0	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. The homogenate that was neutralised the HCL treated specimen turned to have a jelly-like consistency.
1	Silverside	IV-Oral	313B	17/01/2023	NaOH	0	0	0	0	?	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. The homogenate that was neutralised the HCL treated specimen turned to have a jelly-like consistency.
1	Silverside	IV-Oral	313B	17/01/2023	NaOH	0	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. The homogenate that was neutralised the HCL treated specimen turned to have a jelly-like consistency.
1	Silverside	IV-Oral	313B	18/01/2023	HCl	0	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. The homogenate that was neutralised the HCL treated specimen turned to have a jelly-like consistency.
1	Silverside	IV-Oral	313B	18/01/2023	HCl	0	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. The homogenate that was neutralised the HCL treated specimen turned to have a jelly-like consistency.
1	Brisket	IV-Oral	313C	19/01/2023	HCl	0	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. The HCl treated homogenate worked better than the previous attempts (5ml meat, 20ml water, 25ml HCl).
1	Brisket	IV-Oral	313C	19/01/2023	HCl	0	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. The HCl treated homogenate worked better than the previous attempts (5ml meat, 20ml water, 25ml HCl).
1	Brisket	IV-Oral	313C	19/01/2023	NaOH	0	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. The HCl treated homogenate worked better than the previous attempts (5ml meat, 20ml water, 25ml HCl).
1	Brisket	IV-Oral	313C	19/01/2023	NaOH	0	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. The HCl treated homogenate worked better than the previous attempts (5ml meat, 20ml water, 25ml HCl).
1	Rump	IV-Oral	313D	19/01/2023	HCl	0	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. The HCl treated homogenate turned to jelly with the above-mentioned method.
1	Rump	IV-Oral	313D	19/01/2023	HCl	0	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. The HCl treated homogenate turned to jelly with the above-mentioned method.
1	Rump	IV-Oral	313D	19/01/2023	NaOH	0	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. The HCl treated homogenate turned to jelly with the above-mentioned method.
1	Rump	IV-Oral	313D	19/01/2023	NaOH	0	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. The HCl treated homogenate turned to jelly with the above-mentioned method.

Buffalo_Number	Sample_Type	Vaccine_Treatment	Sample_(Lab)_Number	Date_Processed	Treatment	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9	Week 10	Final_Growth	PCR_Final_Result	Comments
2	Fillet	Control - No Vaccine	314A	19/01/2023	HCl	0	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. Changed the volume of the meat for the for the HCl treatment to approximately 3ml, 22ml water, 25ml HCl. This method produced a very soft jelly-like consistency which was easier to work with.
2	Fillet	Control - No Vaccine	314A	19/01/2023	HCl	0	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. Changed the volume of the meat for the for the HCl treatment to approximately 3ml, 22ml water, 25ml HCl. This method produced a very soft jelly-like consistency which was easier to work with.
2	Fillet	Control - No Vaccine	314A	19/01/2023	NaOH	0	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. Changed the volume of the meat for the for the HCl treatment to approximately 3ml, 22ml water, 25ml HCl. This method produced a very soft jelly-like consistency which was easier to work with.
2	Fillet	Control - No Vaccine	314A	19/01/2023	NaOH	0	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. Changed the volume of the meat for the for the HCl treatment to approximately 3ml, 22ml water, 25ml HCl. This method produced a very soft jelly-like consistency which was easier to work with.
2	Silverside	Control - No Vaccine	314B	20/01/2023	HCl	0	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. The HCl treated homogenate was a soft jelly. 3ml of meat sample were used.
2	Silverside	Control - No Vaccine	314B	20/01/2023	HCl	0	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. The HCl treated homogenate was a soft jelly. 3ml of meat sample were used.
2	Silverside	Control - No Vaccine	314B	20/01/2023	NaOH	0	0	0	0	1*-Egg Yellow	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. The HCl treated homogenate was a soft jelly. 3ml of meat sample were used.
2	Silverside	Control - No Vaccine	314B	20/01/2023	NaOH	0	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. The HCl treated homogenate was a soft jelly. 3ml of meat sample were used.
2	Brisket	Control - No Vaccine	314C	20/01/2023	HCl	0	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. The HCl treated homogenate was a soft jelly. 3ml of meat sample were used.
2	Brisket	Control - No Vaccine	314C	20/01/2023	HCl	0	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. The HCl treated homogenate was a soft jelly. 3ml of meat sample were used.
2	Brisket	Control - No Vaccine	314C	20/01/2023	NaOH	0	C	C - Discarded							C	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. The HCl treated homogenate was a soft jelly. 3ml of meat sample were used.
2	Brisket	Control - No Vaccine	314C	20/01/2023	NaOH	0	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. The HCl treated homogenate was a soft jelly. 3ml of meat sample were used.
2	Rump	Control - No Vaccine	314D	20/01/2023	HCl	0	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. The HCl treated homogenate was a soft jelly. 3ml of meat sample were used.
2	Rump	Control - No Vaccine	314D	20/01/2023	HCl	0	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. The HCl treated homogenate was a soft jelly. 3ml of meat sample were used.
2	Rump	Control - No Vaccine	314D	20/01/2023	NaOH	0	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. The HCl treated homogenate was a soft jelly. 3ml of meat sample were used.
2	Rump	Control - No Vaccine	314D	20/01/2023	NaOH	0	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. The HCl treated homogenate was a soft jelly. 3ml of meat sample were used.

Buffalo_Number	Sample_Type	Vaccine_Treatment	Sample_(Lab)_Number	Date_Processed	Treatment	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9	Week 10	Final_Growth	PCR_Final_Result	Comments
3	Fillet	BCG	315A	02/02/2023	CPC 1%	0	0	?	?	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. CPC was used. It didn't turn the homogenate to jelly but the end pellet was harder than the other treatments. (20ml of 10% CPC was used).
3	Fillet	BCG	315A	24/01/2023	NaOH	0	0	0	?	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. CPC was used. It didn't turn the homogenate to jelly but the end pellet was harder than the other treatments. (20ml of 10% CPC was used).
3	Fillet	BCG	315A	24/01/2023	NaOH	0	0	0	?	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. CPC was used. It didn't turn the homogenate to jelly but the end pellet was harder than the other treatments. (20ml of 10% CPC was used).
3	Silverside	BCG	315B	02/02/2023	CPC 1%	0	0	?	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. CPC was used. It didn't turn the homogenate to jelly but the end pellet was harder than the other treatments. (20ml of 10% CPC was used).
3	Silverside	BCG	315B	02/02/2023	CPC 1%	0	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. CPC was used. It didn't turn the homogenate to jelly but the end pellet was harder than the other treatments. (20ml of 10% CPC was used).
3	Silverside	BCG	315B	24/01/2023	NaOH	0	0	1*-White	2*-Clear	2*-Clear	2*-Clear?	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. CPC was used. It didn't turn the homogenate to jelly but the end pellet was harder than the other treatments. (20ml of 10% CPC was used).
3	Silverside	BCG	315B	24/01/2023	NaOH	0	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. CPC was used. It didn't turn the homogenate to jelly but the end pellet was harder than the other treatments. (20ml of 10% CPC was used).
3	Brisket	BCG	315C	02/02/2023	CPC 1%	0	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. CPC was used. It didn't turn the homogenate to jelly but the end pellet was harder than the other treatments. (20ml of 10% CPC was used).
3	Brisket	BCG	315C	02/02/2023	CPC 1%	0	0	0	1*-Clear	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. CPC was used. It didn't turn the homogenate to jelly but the end pellet was harder than the other treatments. (20ml of 10% CPC was used).
3	Brisket	BCG	315C	24/01/2023	NaOH	0	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. CPC was used. It didn't turn the homogenate to jelly but the end pellet was harder than the other treatments. (20ml of 10% CPC was used).
3	Brisket	BCG	315C	24/01/2023	NaOH	0	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. CPC was used. It didn't turn the homogenate to jelly but the end pellet was harder than the other treatments. (20ml of 10% CPC was used).
3	Rump	BCG	315D	02/02/2023	CPC 1%	0	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	A lesion was found on first inspection under a layer of fat. The lesion burst. It had a puss-like consistency. Part of the lesion was placed into the sample to be cultured.
3	Rump	BCG	315D	02/02/2023	CPC 1%	0	0	1*-Clear	1*-Clear	0	0	0	0	0	0	No	Not_Applicable_Not_Done	A lesion was found on first inspection under a layer of fat. The lesion burst. It had a puss-like consistency. Part of the lesion was placed into the sample to be cultured.
3	Rump	BCG	315D	24/01/2023	NaOH	0	0	0	0	?	0	0	0	0	0	No	Not_Applicable_Not_Done	A lesion was found on first inspection under a layer of fat. The lesion burst. It had a puss-like consistency. Part of the lesion was placed into the sample to be cultured.
3	Rump	BCG	315D	24/01/2023	NaOH	0	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	A lesion was found on first inspection under a layer of fat. The lesion burst. It had a puss-like consistency. Part of the lesion was placed into the sample to be cultured.

Buffalo_Number	Sample_Type	Vaccine_Treatment	Sample_(Lab)_Number	Date_Processed	Treatment	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9	Week 10	Final_Growth	PCR_Final_Result	Comments
4	Fillet	IV-IM	316A	09/02/2023	CPC 1%	0	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. 20ml of 10% CPC was used. It didn't turn the homogenate to jelly but the end pellet was harder than the other treatments.
4	Fillet	IV-IM	316A	09/02/2023	CPC 1%	0	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. 20ml of 10% CPC was used. It didn't turn the homogenate to jelly but the end pellet was harder than the other treatments.
4	Fillet	IV-IM	316A	24/01/2023	NaOH	0	0	0	1	1	1	1?	0	0	1/c	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. 20ml of 10% CPC was used. It didn't turn the homogenate to jelly but the end pellet was harder than the other treatments.
4	Fillet	IV-IM	316A	24/01/2023	NaOH	0	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. 20ml of 10% CPC was used. It didn't turn the homogenate to jelly but the end pellet was harder than the other treatments.
4	Silverside	IV-IM	316B	09/02/2023	CPC 1%	0	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. 20ml of 10% CPC was used. The end pellet was hard and rubber-like. A 1000ml pipette with the blue attachement was used to break up the pellet.
4	Silverside	IV-IM	316B	09/02/2023	CPC 1%	0	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. 20ml of 10% CPC was used. The end pellet was hard and rubber-like. A 1000ml pipette with the blue attachement was used to break up the pellet.
4	Silverside	IV-IM	316B	24/01/2023	NaOH	0	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. 20ml of 10% CPC was used. The end pellet was hard and rubber-like. A 1000ml pipette with the blue attachement was used to break up the pellet.
4	Silverside	IV-IM	316B	24/01/2023	NaOH	0	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. 20ml of 10% CPC was used. The end pellet was hard and rubber-like. A 1000ml pipette with the blue attachement was used to break up the pellet.
4	Brisket	IV-IM	316C	09/02/2023	CPC 1%	0	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. 20ml of 10% CPC was used. The end pellet was hard and rubber-like. A 1000ml pipette with the blue attachement was used to break up the pellet.
4	Brisket	IV-IM	316C	09/02/2023	CPC 1%	0	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. 20ml of 10% CPC was used. The end pellet was hard and rubber-like. A 1000ml pipette with the blue attachement was used to break up the pellet.
4	Brisket	IV-IM	316C	25/01/2023	NaOH	0	1*-Clear	?	1*-Clear	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. 20ml of 10% CPC was used. The end pellet was hard and rubber-like. A 1000ml pipette with the blue attachement was used to break up the pellet.
4	Brisket	IV-IM	316C	25/01/2023	NaOH	0	1*-Clear	?	1*-Clear	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. 20ml of 10% CPC was used. The end pellet was hard and rubber-like. A 1000ml pipette with the blue attachement was used to break up the pellet.
4	Rump	IV-IM	316D	09/02/2023	CPC 1%	0	1	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. 20ml of 10% CPC was used. The end pellet was hard and rubber-like. A 1000ml pipette with the blue attachement was used to break up the pellet.
4	Rump	IV-IM	316D	09/02/2023	CPC 1%	0	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. 20ml of 10% CPC was used. The end pellet was hard and rubber-like. A 1000ml pipette with the blue attachement was used to break up the pellet.
4	Rump	IV-IM	316D	25/01/2023	NaOH	0	0	0	0	0	0	0	0	0	1	Yes	Culture_Suspect_PCR_Negative	No abnormalities were found on first inspection. 20ml of 10% CPC was used. The end pellet was hard and rubber-like. A 1000ml pipette with the blue attachement was used to break up the pellet. The colony formed was opaque white
4	Rump	IV-IM	316D	25/01/2023	NaOH	0	0	1*-Clear	1*-Clear	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. 20ml of 10% CPC was used. The end pellet was hard and rubber-like. A 1000ml pipette with the blue attachement was used to break up the pellet.

Buffalo_Number	Sample_Type	Vaccine_Treatment	Sample_(Lab)_Number	Date_Processed	Treatment	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9	Week 10	Final_Growth	PCR_Final_Result	Comments
5	Fillet	IV-Oral	317A	10/02/2023	CPC 1%	0	?	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. 20ml of 10% CPC was used. The end pellet was hard and rubber-like. A 1000ml pipette with the blue attachement was used to break up the pellet.
5	Fillet	IV-Oral	317A	10/02/2023	CPC 1%	0	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. 20ml of 10% CPC was used. The end pellet was hard and rubber-like. A 1000ml pipette with the blue attachement was used to break up the pellet.
5	Fillet	IV-Oral	317A	25/01/2023	NaOH	0	0	?	?	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. 20ml of 10% CPC was used. The end pellet was hard and rubber-like. A 1000ml pipette with the blue attachement was used to break up the pellet.
5	Fillet	IV-Oral	317A	25/01/2023	NaOH	0	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. 20ml of 10% CPC was used. The end pellet was hard and rubber-like. A 1000ml pipette with the blue attachement was used to break up the pellet.
5	Silverside	IV-Oral	317B	10/02/2023	CPC 1%	0	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. 20ml of 10% CPC was used. The end pellet was hard and rubber-like. A 1000ml pipette with the blue attachement was used to break up the pellet.
5	Silverside	IV-Oral	317B	10/02/2023	CPC 1%	0	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. 20ml of 10% CPC was used. The end pellet was hard and rubber-like. A 1000ml pipette with the blue attachement was used to break up the pellet.
5	Silverside	IV-Oral	317B	25/01/2023	NaOH	0	0	0	?	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. 20ml of 10% CPC was used. The end pellet was hard and rubber-like. A 1000ml pipette with the blue attachement was used to break up the pellet.
5	Silverside	IV-Oral	317B	25/01/2023	NaOH	0	0	0	?	0	0	0	0	0	3/c	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. 20ml of 10% CPC was used. The end pellet was hard and rubber-like. A 1000ml pipette with the blue attachement was used to break up the pellet.
5	Brisket	IV-Oral	317C	10/02/2023	CPC 1%	0	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. 20ml of 10% CPC was used. The end pellet was hard and rubber-like. A 1000ml pipette with the blue attachement was used to break up the pellet.
5	Brisket	IV-Oral	317C	10/02/2023	CPC 1%	0	?	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. 20ml of 10% CPC was used. The end pellet was hard and rubber-like. A 1000ml pipette with the blue attachement was used to break up the pellet.
5	Brisket	IV-Oral	317C	25/01/2023	NaOH	0	0	1*-Clear	2*-Yellow	0	0	0	0	0	2/c	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. 20ml of 10% CPC was used. The end pellet was hard and rubber-like. A 1000ml pipette with the blue attachement was used to break up the pellet.
5	Brisket	IV-Oral	317C	25/01/2023	NaOH	0	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. 20ml of 10% CPC was used. The end pellet was hard and rubber-like. A 1000ml pipette with the blue attachement was used to break up the pellet.
5	Rump	IV-Oral	317D	10/02/2023	CPC 1%	0	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. 20ml of 10% CPC was used. The end pellet was hard and rubber-like. A 1000ml pipette with the blue attachement was used to break up the pellet.
5	Rump	IV-Oral	317D	10/02/2023	CPC 1%	0	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. 20ml of 10% CPC was used. The end pellet was hard and rubber-like. A 1000ml pipette with the blue attachement was used to break up the pellet.
5	Rump	IV-Oral	317D	26/01/2023	NaOH	0	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. 20ml of 10% CPC was used. The end pellet was hard and rubber-like. A 1000ml pipette with the blue attachement was used to break up the pellet.
5	Rump	IV-Oral	317D	26/01/2023	NaOH	0	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. 20ml of 10% CPC was used. The end pellet was hard and rubber-like. A 1000ml pipette with the blue attachement was used to break up the pellet.

Buffalo_Number	Sample_Type	Vaccine_Treatment	Sample_(Lab)_Number	Date_Processed	Treatment	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9	Week 10	Final_Growth	PCR_Final_Result	Comments
6	Fillet	IV-IM	318A	13/02/2023	CPC 1%	0	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. 20ml of 10% CPC was used. The end pellet was hard and rubber-like. A 1000ml pipette with the blue attachement was used to break up the pellet.
6	Fillet	IV-IM	318A	26/01/2023	NaOH	0	0	1/c-White	1/c-White	0/c	0/c	0/c	0/c	0/c	0/c	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. 20ml of 10% CPC was used. The end pellet was hard and rubber-like. A 1000ml pipette with the blue attachement was used to break up the pellet.
6	Fillet	IV-IM	318A	26/01/2023	NaOH	0	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. 20ml of 10% CPC was used. The end pellet was hard and rubber-like. A 1000ml pipette with the blue attachement was used to break up the pellet.
6	Silverside	IV-IM	318B	13/02/2023	CPC 1%	0	1*-Clear	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. 20ml of 10% CPC was used. The end pellet was hard and rubber-like. A 1000ml pipette with the blue attachement was used to break up the pellet.
6	Silverside	IV-IM	318B	13/02/2023	CPC 1%	0	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. 20ml of 10% CPC was used. The end pellet was hard and rubber-like. A 1000ml pipette with the blue attachement was used to break up the pellet.
6	Silverside	IV-IM	318B	26/01/2023	NaOH	0	0	0	1?	1?	1?	1?	1?	1?	1?	Yes	Culture_Suspect_PCR_Negative	No abnormalities were found on first inspection. 20ml of 10% CPC was used. The end pellet was hard and rubber-like. A 1000ml pipette with the blue attachement was used to break up the pellet. The colony formed was clear.
6	Silverside	IV-IM	318B	26/01/2023	NaOH	0	0	0/c-Black	1?	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. 20ml of 10% CPC was used. The end pellet was hard and rubber-like. A 1000ml pipette with the blue attachement was used to break up the pellet.
6	Brisket	IV-IM	318C	13/02/2023	CPC 1%	?	?	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. 20ml of 10% CPC was used. The end pellet was hard and rubber-like. A 1000ml pipette with the blue attachement was used to break up the pellet.
6	Brisket	IV-IM	318C	13/02/2023	CPC 1%	0	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. 20ml of 10% CPC was used. The end pellet was hard and rubber-like. A 1000ml pipette with the blue attachement was used to break up the pellet.
6	Brisket	IV-IM	318C	26/01/2023	NaOH	0	0	1*-White	1*-White	1*-White	1*-White	1*-White	1*-White	1*-White	1/c	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. 20ml of 10% CPC was used. The end pellet was hard and rubber-like. A 1000ml pipette with the blue attachement was used to break up the pellet.
6	Brisket	IV-IM	318C	26/01/2023	NaOH	0	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. 20ml of 10% CPC was used. The end pellet was hard and rubber-like. A 1000ml pipette with the blue attachement was used to break up the pellet.
6	Rump	IV-IM	318D	13/02/2023	CPC 1%	0	?	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. 20ml of 10% CPC was used. The end pellet was hard and rubber-like. A 1000ml pipette with the blue attachement was used to break up the pellet.
6	Rump	IV-IM	318D	13/02/2023	CPC 1%	0	?	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. 20ml of 10% CPC was used. The end pellet was hard and rubber-like. A 1000ml pipette with the blue attachement was used to break up the pellet.
6	Rump	IV-IM	318D	26/01/2023	NaOH	0	0	0	0	0	0	0	0	1	2/c	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. 20ml of 10% CPC was used. The end pellet was hard and rubber-like. A 1000ml pipette with the blue attachement was used to break up the pellet.
6	Rump	IV-IM	318D	26/01/2023	NaOH	0	0	0	0	0	0	0	0	0	1?	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. 20ml of 10% CPC was used. The end pellet was hard and rubber-like. A 1000ml pipette with the blue attachement was used to break up the pellet.

Buffalo_Number	Sample_Type	Vaccine_Treatment	Sample_(Lab)_Number	Date_Processed	Treatment	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9	Week 10	Final_Growth	PCR_Final_Result	Comments
7	Fillet	IV-Oral	319A	13/02/2023	CPC 1%	0	0	0	0	1?	1?	1?	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. 20ml of 10% CPC was used. The end pellet was hard and rubber-like. A 1000ml pipette with the blue attachement was used to break up the pellet.
7	Fillet	IV-Oral	319A	13/02/2023	CPC 1%	0	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. 20ml of 10% CPC was used. The end pellet was hard and rubber-like. A 1000ml pipette with the blue attachement was used to break up the pellet.
7	Fillet	IV-Oral	319A	26/01/2023	NaOH	0	0	1*-Egg Yellow	?	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. 20ml of 10% CPC was used. The end pellet was hard and rubber-like. A 1000ml pipette with the blue attachement was used to break up the pellet.
7	Fillet	IV-Oral	319A	26/01/2023	NaOH	0	0	0	0	0	0	0	0	0	1?	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. 20ml of 10% CPC was used. The end pellet was hard and rubber-like. A 1000ml pipette with the blue attachement was used to break up the pellet.
7	Silverside	IV-Oral	319B	13/02/2023	CPC 1%	0	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. 20ml of 10% CPC was used. The end pellet was hard and rubber-like. A 1000ml pipette with the blue attachement was used to break up the pellet.
7	Silverside	IV-Oral	319B	13/02/2023	CPC 1%	0	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. 20ml of 10% CPC was used. The end pellet was hard and rubber-like. A 1000ml pipette with the blue attachement was used to break up the pellet.
7	Silverside	IV-Oral	319B	27/01/2023	NaOH	0	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. 20ml of 10% CPC was used. The end pellet was hard and rubber-like. A 1000ml pipette with the blue attachement was used to break up the pellet.
7	Silverside	IV-Oral	319B	27/01/2023	NaOH	0	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. 20ml of 10% CPC was used. The end pellet was hard and rubber-like. A 1000ml pipette with the blue attachement was used to break up the pellet.
7	Brisket	IV-Oral	319C	13/02/2023	CPC 1%	0	?	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	A single small ball-like lesion was found (0.4x0.4x0.4cm). It was incorporated into the homogenate for culture. The sample decontaminated with CPC formed a hard pellet after centrifugation.
7	Brisket	IV-Oral	319C	13/02/2023	CPC 1%	0	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	A single small ball-like lesion was found (0.4x0.4x0.4cm). It was incorporated into the homogenate for culture. The sample decontaminated with CPC formed a hard pellet after centrifugation.
7	Brisket	IV-Oral	319C	27/01/2023	NaOH	0	0	?	0	0	0	0	0	1?	1?	Yes	Culture_Suspect_PCR_Negative	A single small ball-like lesion was found (0.4x0.4x0.4cm). It was incorporated into the homogenate for culture. The sample decontaminated with CPC formed a hard pellet after centrifugation. The colony formed was opaque white.
7	Brisket	IV-Oral	319C	27/01/2023	NaOH	0	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	A single small ball-like lesion was found (0.4x0.4x0.4cm). It was incorporated into the homogenate for culture. The sample decontaminated with CPC formed a hard pellet after centrifugation.
7	Rump	IV-Oral	319D	13/02/2023	CPC 1%	0	?	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. CPC was used to decontaminate the homogenate. The pellet formed after centriguing was hard
7	Rump	IV-Oral	319D	13/02/2023	CPC 1%	0	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. CPC was used to decontaminate the homogenate. The pellet formed after centriguing was hard
7	Rump	IV-Oral	319D	27/01/2023	NaOH	0	0	0	?	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. CPC was used to decontaminate the homogenate. The pellet formed after centriguing was hard
7	Rump	IV-Oral	319D	27/01/2023	NaOH	0	0	0	?	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. CPC was used to decontaminate the homogenate. The pellet formed after centriguing was hard

Buffalo_Number	Sample_Type	Vaccine_Treatment	Sample_(Lab)_Number	Date_Processed	Treatment	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9	Week 10	Final_Growth	PCR_Final_Result	Comments
8	Fillet	IV-IM	320A	14/02/2023	CPC 1%	0	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. CPC was used. It didn't turn the homogenate to jelly but the end pellet was harder than the other treatments. (20ml of 10% CPC was used). 2ml of distilled water was added to the pellet after the supernatant was poured off and left for 2 minutes. After the 2 minutes, the pellet had softened.
8	Fillet	IV-IM	320A	14/02/2023	CPC 1%	0	?	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. CPC was used. It didn't turn the homogenate to jelly but the end pellet was harder than the other treatments. (20ml of 10% CPC was used). 2ml of distilled water was added to the pellet after the supernatant was poured off and left for 2 minutes. After the 2 minutes, the pellet had softened.
8	Fillet	IV-IM	320A	30/01/2023	NaOH	0	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. CPC was used. It didn't turn the homogenate to jelly but the end pellet was harder than the other treatments. (20ml of 10% CPC was used). 2ml of distilled water was added to the pellet after the supernatant was poured off and left for 2 minutes. After the 2 minutes, the pellet had softened.
8	Fillet	IV-IM	320A	30/01/2023	NaOH	0	0	1/c	1/c	1/c	1/c	1/c	1/c	1/c	1/c	Yes	Culture_Suspect_PCR_Negative	No abnormalities were found on first inspection. CPC was used. It didn't turn the homogenate to jelly but the end pellet was harder than the other treatments. (20ml of 10% CPC was used). 2ml of distilled water was added to the pellet after the supernatant was poured off and left for 2 minutes. After the 2 minutes, the pellet had softened. The colony formed was clear.
8	Silverside	IV-IM	320B	14/02/2023	CPC 1%	0	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. CPC was used. It didn't turn the homogenate to jelly but the end pellet was harder than the other treatments. (20ml of 10% CPC was used). 2ml of distilled water was added to the pellet after the supernatant was poured off and left for 2 minutes. After the 2 minutes, the pellet had softened.
8	Silverside	IV-IM	320B	14/02/2023	CPC 1%	0	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. CPC was used. It didn't turn the homogenate to jelly but the end pellet was harder than the other treatments. (20ml of 10% CPC was used). 2ml of distilled water was added to the pellet after the supernatant was poured off and left for 2 minutes. After the 2 minutes, the pellet had softened.
8	Silverside	IV-IM	320B	30/01/2023	NaOH	0	0	?	?	0	0	0	0	1	1	Yes	Culture_Suspect_PCR_Negative	No abnormalities were found on first inspection. CPC was used. It didn't turn the homogenate to jelly but the end pellet was harder than the other treatments. (20ml of 10% CPC was used). 2ml of distilled water was added to the pellet after the supernatant was poured off and left for 2 minutes. After the 2 minutes, the pellet had softened. The colony formed was opaque white
8	Silverside	IV-IM	320B	30/01/2023	NaOH	0	0	?	?	0	0	0	0	1	1/c	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. CPC was used. It didn't turn the homogenate to jelly but the end pellet was harder than the other treatments. (20ml of 10% CPC was used). 2ml of distilled water was added to the pellet after the supernatant was poured off and left for 2 minutes. After the 2 minutes, the pellet had softened. The sample was not used in PCR as the growth was non-specific and not thought to be M. bovis

8	Brisket	IV-IM	320C	14/02/2023	CPC 1%	0	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. CPC was used. It didn't turn the homogenate to jelly but the end pellet was harder than the other treatments. (20ml of 10% CPC was used). 2ml of distilled water was added to the pellet after the supernatant was poured off and left for 2 minutes. After the 2 minutes, the pellet had softened.
8	Brisket	IV-IM	320C	14/02/2023	CPC 1%	0	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. CPC was used. It didn't turn the homogenate to jelly but the end pellet was harder than the other treatments. (20ml of 10% CPC was used). 2ml of distilled water was added to the pellet after the supernatant was poured off and left for 2 minutes. After the 2 minutes, the pellet had softened.
8	Brisket	IV-IM	320C	30/01/2023	NaOH	0	0	?	1*-Clear	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. CPC was used. It didn't turn the homogenate to jelly but the end pellet was harder than the other treatments. (20ml of 10% CPC was used). 2ml of distilled water was added to the pellet after the supernatant was poured off and left for 2 minutes. After the 2 minutes, the pellet had softened.
8	Brisket	IV-IM	320C	30/01/2023	NaOH	0	0	0	3*-Clear	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. CPC was used. It didn't turn the homogenate to jelly but the end pellet was harder than the other treatments. (20ml of 10% CPC was used). 2ml of distilled water was added to the pellet after the supernatant was poured off and left for 2 minutes. After the 2 minutes, the pellet had softened.
8	Rump	IV-IM	320D	14/02/2023	CPC 1%	0	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. CPC was used. It didn't turn the homogenate to jelly but the end pellet was harder than the other treatments. (20ml of 10% CPC was used). 2ml of distilled water was added to the pellet after the supernatant was poured off and left for 2 minutes. After the 2 minutes, the pellet had softened.
8	Rump	IV-IM	320D	14/02/2023	CPC 1%	0	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. CPC was used. It didn't turn the homogenate to jelly but the end pellet was harder than the other treatments. (20ml of 10% CPC was used). 2ml of distilled water was added to the pellet after the supernatant was poured off and left for 2 minutes. After the 2 minutes, the pellet had softened.
8	Rump	IV-IM	320D	30/01/2023	NaOH	0	0	1*-Yellow	1*-Yellow	1*-Yellow	1*-Yellow	1*-Yellow	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. CPC was used. It didn't turn the homogenate to jelly but the end pellet was harder than the other treatments. (20ml of 10% CPC was used). 2ml of distilled water was added to the pellet after the supernatant was poured off and left for 2 minutes. After the 2 minutes, the pellet had softened.
8	Rump	IV-IM	320D	30/01/2023	NaOH	0	0	?	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. CPC was used. It didn't turn the homogenate to jelly but the end pellet was harder than the other treatments. (20ml of 10% CPC was used). 2ml of distilled water was added to the pellet after the supernatant was poured off and left for 2 minutes. After the 2 minutes, the pellet had softened.

Buffalo_Number	Sample_Type	Vaccine_Treatment	Sample_(Lab)_Number	Date_Processed	Treatment	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9	Week 10	Final_Growth	PCR_Final_Result	Comments
10	Fillet	IV-IM	321A	14/02/2023	CPC 1%	0	?	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. CPC was used. It didn't turn the homogenate to jelly but the end pellet was harder than the other treatments. (20ml of 10% CPC was used). 2ml of distilled water was added to the pellet after the supernatant was poured off and left for 2 minutes. After the 2 minutes, the pellet had softened.
10	Fillet	IV-IM	321A	14/02/2023	CPC 1%	0	?	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. CPC was used. It didn't turn the homogenate to jelly but the end pellet was harder than the other treatments. (20ml of 10% CPC was used). 2ml of distilled water was added to the pellet after the supernatant was poured off and left for 2 minutes. After the 2 minutes, the pellet had softened.
10	Fillet	IV-IM	321A	31/01/2023	NaOH	0	0	?	?	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. CPC was used. It didn't turn the homogenate to jelly but the end pellet was harder than the other treatments. (20ml of 10% CPC was used). 2ml of distilled water was added to the pellet after the supernatant was poured off and left for 2 minutes. After the 2 minutes, the pellet had softened.
10	Fillet	IV-IM	321A	31/01/2023	NaOH	0	0	?	?	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. CPC was used. It didn't turn the homogenate to jelly but the end pellet was harder than the other treatments. (20ml of 10% CPC was used). 2ml of distilled water was added to the pellet after the supernatant was poured off and left for 2 minutes. After the 2 minutes, the pellet had softened.
10	Silverside	IV-IM	321B	14/02/2023	CPC 1%	0	?	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. CPC was used. It didn't turn the homogenate to jelly but the end pellet was harder than the other treatments. (20ml of 10% CPC was used). 2ml of distilled water was added to the pellet after the supernatant was poured off and left for 2 minutes. After the 2 minutes, the pellet had softened.
10	Silverside	IV-IM	321B	14/02/2023	CPC 1%	0	?	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. CPC was used. It didn't turn the homogenate to jelly but the end pellet was harder than the other treatments. (20ml of 10% CPC was used). 2ml of distilled water was added to the pellet after the supernatant was poured off and left for 2 minutes. After the 2 minutes, the pellet had softened.
10	Silverside	IV-IM	321B	31/01/2023	NaOH	0	0	1*-Egg Yellow	3	0	0	0	0	1?	1/c	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. CPC was used. It didn't turn the homogenate to jelly but the end pellet was harder than the other treatments. (20ml of 10% CPC was used). 2ml of distilled water was added to the pellet after the supernatant was poured off and left for 2 minutes. After the 2 minutes, the pellet had softened. The final growth was 2x egg yellow spots which are not typical of M. bovis
10	Silverside	IV-IM	321B	31/01/2023	NaOH	0	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. CPC was used. It didn't turn the homogenate to jelly but the end pellet was harder than the other treatments. (20ml of 10% CPC was used). 2ml of distilled water was added to the pellet after the supernatant was poured off and left for 2 minutes. After the 2 minutes, the pellet had softened.

10	Brisket	IV-IM	321C	14/02/2023	CPC 1%	0	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. CPC was used. It didn't turn the homogenate to jelly but the end pellet was harder than the other treatments. (20ml of 10% CPC was used). 2ml of distilled water was added to the pellet after the supernatant was poured off and left for 2 minutes. After the 2 minutes, the pellet had softened.
10	Brisket	IV-IM	321C	14/02/2023	CPC 1%	0	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. CPC was used. It didn't turn the homogenate to jelly but the end pellet was harder than the other treatments. (20ml of 10% CPC was used). 2ml of distilled water was added to the pellet after the supernatant was poured off and left for 2 minutes. After the 2 minutes, the pellet had softened.
10	Brisket	IV-IM	321C	31/01/2023	NaOH	0	0	0	?	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. CPC was used. It didn't turn the homogenate to jelly but the end pellet was harder than the other treatments. (20ml of 10% CPC was used). 2ml of distilled water was added to the pellet after the supernatant was poured off and left for 2 minutes. After the 2 minutes, the pellet had softened.
10	Brisket	IV-IM	321C	31/01/2023	NaOH	0	0	0	?	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. CPC was used. It didn't turn the homogenate to jelly but the end pellet was harder than the other treatments. (20ml of 10% CPC was used). 2ml of distilled water was added to the pellet after the supernatant was poured off and left for 2 minutes. After the 2 minutes, the pellet had softened.
10	Rump	IV-IM	321D	14/02/2023	CPC 1%	0	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	A small lesion (0.1cm x 0.1cm x 0.1cm) was found in the middle of the muscle tissue. The lesion was cut out and added as part of the representative sample for culturing. The pellet formed after the CPC treatment was hard following the removal of the supernatant. 2ml of distilled water was added to the pellet and left for 2 minutes. After the 2 minutes the pellet had softened.
10	Rump	IV-IM	321D	14/02/2023	CPC 1%	0	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	A small lesion (0.1cm x 0.1cm x 0.1cm) was found in the middle of the muscle tissue. The lesion was cut out and added as part of the representative sample for culturing. The pellet formed after the CPC treatment was hard following the removal of the supernatant. 2ml of distilled water was added to the pellet and left for 2 minutes. After the 2 minutes the pellet had softened.
10	Rump	IV-IM	321D	31/01/2023	NaOH	0	0	1*-Clear	?	0	0	0	0	0	1/c	No	Not_Applicable_Not_Done	A small lesion (0.1cm x 0.1cm x 0.1cm) was found in the middle of the muscle tissue. The lesion was cut out and added as part of the representative sample for culturing. The pellet formed after the CPC treatment was hard following the removal of the supernatant. 2ml of distilled water was added to the pellet and left for 2 minutes. After the 2 minutes the pellet had softened.
10	Rump	IV-IM	321D	31/01/2023	NaOH	0	0	1*-Clear	?	0	0	0	0	0	0	No	Not_Applicable_Not_Done	A small lesion (0.1cm x 0.1cm x 0.1cm) was found in the middle of the muscle tissue. The lesion was cut out and added as part of the representative sample for culturing. The pellet formed after the CPC treatment was hard following the removal of the supernatant. 2ml of distilled water was added to the pellet and left for 2 minutes. After the 2 minutes the pellet had softened.

Buffalo_Number	Sample_Type	Vaccine_Treatment	Sample_(Lab)_Number	Date_Processed	Treatment	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9	Week 10	Final_Growth	PCR_Final_Result	Comments
14	Fillet	IV-IM	322A	02/02/2023	CPC 1%	0	1	2*-White	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. 20 ml of 10 % CPC was used to decontaminate the homogenate. The pellet formed after centriguing was hard and rubber-like. After a few minutes the pellet softened enough to be broken up more easily.
14	Fillet	IV-IM	322A	02/02/2023	CPC 1%	0	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. 20 ml of 10 % CPC was used to decontaminate the homogenate. The pellet formed after centriguing was hard and rubber-like. After a few minutes the pellet softened enough to be broken up more easily.
14	Fillet	IV-IM	322A	31/01/2023	NaOH	0	?	?		0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. 20 ml of 10 % CPC was used to decontaminate the homogenate. The pellet formed after centriguing was hard and rubber-like. After a few minutes the pellet softened enough to be broken up more easily.
14	Fillet	IV-IM	322A	31/01/2023	NaOH	0	0	?	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. 20 ml of 10 % CPC was used to decontaminate the homogenate. The pellet formed after centriguing was hard and rubber-like. After a few minutes the pellet softened enough to be broken up more easily.
14	Silverside	IV-IM	322B	02/02/2023	CPC 1%	0	*+-Egg Yellow	*+-Egg Yellow	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. 20 ml of 10 % CPC was used to decontaminate the homogenate. The pellet formed after centriguing was hard and rubber-like. After a few minutes the pellet softened enough to be broken up more easily.
14	Silverside	IV-IM	322B	02/02/2023	CPC 1%	0	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. 20 ml of 10 % CPC was used to decontaminate the homogenate. The pellet formed after centriguing was hard and rubber-like. After a few minutes the pellet softened enough to be broken up more easily.
14	Silverside	IV-IM	322B	31/01/2023	NaOH	0	?	?	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. 20 ml of 10 % CPC was used to decontaminate the homogenate. The pellet formed after centriguing was hard and rubber-like. After a few minutes the pellet softened enough to be broken up more easily.
14	Silverside	IV-IM	322B	31/01/2023	NaOH	0	0	?	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. 20 ml of 10 % CPC was used to decontaminate the homogenate. The pellet formed after centriguing was hard and rubber-like. After a few minutes the pellet softened enough to be broken up more easily.

14	Brisket	IV-IM	322C	01/02/2023	CPC 1%	0	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. 20 ml of 1% CPC was used to decontaminate the homogenate. The pellet formed after centriguing was hard and rubber-like. After a few minutes the pellet softened enough to be broken up more easily because 2ml of distilled water was added.
14	Brisket	IV-IM	322C	01/02/2023	NaOH	0	1*-Egg Yellow	1*-Clear	1	1	1	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. 20 ml of 1% CPC was used to decontaminate the homogenate. The pellet formed after centriguing was hard and rubber-like. After a few minutes the pellet softened enough to be broken up more easily because 2ml of distilled water was added.
14	Brisket	IV-IM	322C	01/02/2023	NaOH	0	0	1*-Clear	1	1	1	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. 20 ml of 1% CPC was used to decontaminate the homogenate. The pellet formed after centriguing was hard and rubber-like. After a few minutes the pellet softened enough to be broken up more easily because 2ml of distilled water was added.
14	Rump	IV-IM	322D	01/02/2023	CPC 1%	0	?	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. 20 ml of 1% CPC was used to decontaminate the homogenate. The pellet formed after centriguing was hard and rubber-like. After a few minutes the pellet softened enough to be broken up more easily because 2ml of distilled water was added.
14	Rump	IV-IM	322D	01/02/2023	CPC 1%	0	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. 20 ml of 1% CPC was used to decontaminate the homogenate. The pellet formed after centriguing was hard and rubber-like. After a few minutes the pellet softened enough to be broken up more easily because 2ml of distilled water was added.
14	Rump	IV-IM	322D	01/02/2023	NaOH	0	1*-Clear	1*-Clear	1*-Clear	1*-Clear	1*-Clear	1*-Clear	1*-Clear	1*-Clear	1*-Clear	Yes	Culture_Suspect_PCR_Negative	No abnormalities were found on first inspection. 20 ml of 1% CPC was used to decontaminate the homogenate. The pellet formed after centriguing was hard and rubber-like. After a few minutes the pellet softened enough to be broken up more easily because 2ml of distilled water was added. The colony formed was clear.
14	Rump	IV-IM	322D	01/02/2023	NaOH	0	?	1*-Clear	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. 20 ml of 1% CPC was used to decontaminate the homogenate. The pellet formed after centriguing was hard and rubber-like. After a few minutes the pellet softened enough to be broken up more easily because 2ml of distilled water was added.

Buffalo_Number	Sample_Type	Vaccine_Treatment	Sample_(Lab)_Number	Date_Processed	Treatment	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9	Week 10	Final_Growth	PCR_Final_Result	Comments
15	Fillet	Control - No Vaccine	323A	01/02/2023	CPC 1%	0	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. 20 ml of 1% CPC was used to decontaminate the homogenate. The pellet formed after centriguing was hard and rubber-like. After a few minutes the pellet softened enough to be broken up more easily because 2ml of distilled water was added.
15	Fillet	Control - No Vaccine	323A	01/02/2024	CPC 1%	0	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. 20 ml of 1% CPC was used to decontaminate the homogenate. The pellet formed after centriguing was hard and rubber-like. After a few minutes the pellet softened enough to be broken up more easily because 2ml of distilled water was added.
15	Fillet	Control - No Vaccine	323A	01/03/2023	NaOH	0	2*-Egg Yellow	?	?	?	?	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. 20 ml of 1% CPC was used to decontaminate the homogenate. The pellet formed after centriguing was hard and rubber-like. After a few minutes the pellet softened enough to be broken up more easily because 2ml of distilled water was added.
15	Fillet	Control - No Vaccine	323A	01/02/2025	NaOH	0	0	?	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. 20 ml of 1% CPC was used to decontaminate the homogenate. The pellet formed after centriguing was hard and rubber-like. After a few minutes the pellet softened enough to be broken up more easily because 2ml of distilled water was added.
15	Silverside	Control - No Vaccine	323B	01/04/2023	CPC 1%	0	2*-Egg Yellow	2*-Egg Yellow	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. 20 ml of 1% CPC was used to decontaminate the homogenate. The pellet formed after centriguing was hard and rubber-like. After a few minutes the pellet softened enough to be broken up more easily because 2ml of distilled water was added.
15	Silverside	Control - No Vaccine	323B	01/02/2026	CPC 1%	0	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. 20 ml of 1% CPC was used to decontaminate the homogenate. The pellet formed after centriguing was hard and rubber-like. After a few minutes the pellet softened enough to be broken up more easily because 2ml of distilled water was added.
15	Silverside	Control - No Vaccine	323B	01/05/2023	NaOH	0	0	?	?	?	?	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. 20 ml of 1% CPC was used to decontaminate the homogenate. The pellet formed after centriguing was hard and rubber-like. After a few minutes the pellet softened enough to be broken up more easily because 2ml of distilled water was added.
15	Silverside	Control - No Vaccine	323B	01/02/2027	NaOH	0	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. 20 ml of 1% CPC was used to decontaminate the homogenate. The pellet formed after centriguing was hard and rubber-like. After a few minutes the pellet softened enough to be broken up more easily because 2ml of distilled water was added.

15	Brisket	Control - No Vaccine	323C	01/02/2028	CPC 1%	0	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. 20 ml of 1% CPC was used to decontaminate the homogenate. The pellet formed after centriguing was hard and rubber-like. After a few minutes the pellet softened enough to be broken up more easily because 2ml of distilled water was added.
15	Brisket	Control - No Vaccine	323C	01/07/2023	NaOH	0	?	?	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. 20 ml of 1% CPC was used to decontaminate the homogenate. The pellet formed after centriguing was hard and rubber-like. After a few minutes the pellet softened enough to be broken up more easily because 2ml of distilled water was added.
15	Brisket	Control - No Vaccine	323C	01/02/2029	NaOH	0	0	c/?	c/0	c/0	c/0	c/0	c/0	c/0	c/0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. 20 ml of 1% CPC was used to decontaminate the homogenate. The pellet formed after centriguing was hard and rubber-like. After a few minutes the pellet softened enough to be broken up more easily because 2ml of distilled water was added. The media shrank but did not dry out.
15	Rump	Control - No Vaccine	323D	01/08/2023	CPC 1%	0	0	?	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. 20 ml of 1% CPC was used to decontaminate the homogenate. The pellet formed after centriguing was hard and rubber-like. After a few minutes the pellet softened enough to be broken up more easily because 2ml of distilled water was added.
15	Rump	Control - No Vaccine	323D	01/02/2030	CPC 1%	0	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. 20 ml of 1% CPC was used to decontaminate the homogenate. The pellet formed after centriguing was hard and rubber-like. After a few minutes the pellet softened enough to be broken up more easily because 2ml of distilled water was added.
15	Rump	Control - No Vaccine	323D	01/09/2023	NaOH	0	C	C	C	C	C	C	C	C	C	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. 20 ml of 1% CPC was used to decontaminate the homogenate. The pellet formed after centriguing was hard and rubber-like. After a few minutes the pellet softened enough to be broken up more easily because 2ml of distilled water was added. The media changed colour to a peach yellow.
15	Rump	Control - No Vaccine	323D	01/02/2031	NaOH	0	C	C	C	C	C	C	C	C	C	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. 20 ml of 1% CPC was used to decontaminate the homogenate. The pellet formed after centriguing was hard and rubber-like. After a few minutes the pellet softened enough to be broken up more easily because 2ml of distilled water was added. The media changed colour to a yellow.

Buffalo_Number	Sample_Type	Vaccine_Treatment	Sample_(Lab)_Number	Date_Processed	Treatment	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9	Week 10	Final_Growth	PCR_Final_Result	Comments
16	Fillet	BCG	324A	02/02/2023	CPC 1%	0	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. 20 ml of 1% CPC was used to decontaminate the homogenate. The pellet formed after centriguing was hard and rubber-like. After a few minutes the pellet softened enough to be broken up more easily because 2ml of distilled water was added.
16	Fillet	BCG	324A	02/02/2023	CPC 1%	0	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. 20 ml of 1% CPC was used to decontaminate the homogenate. The pellet formed after centriguing was hard and rubber-like. After a few minutes the pellet softened enough to be broken up more easily because 2ml of distilled water was added.
16	Fillet	BCG	324A	02/02/2023	NaOH	0	?	0	0	0	0	0	0	?	0/c	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. 20 ml of 1% CPC was used to decontaminate the homogenate. The pellet formed after centriguing was hard and rubber-like. After a few minutes the pellet softened enough to be broken up more easily because 2ml of distilled water was added.
16	Fillet	BCG	324A	02/02/2023	NaOH	0	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. 20 ml of 1% CPC was used to decontaminate the homogenate. The pellet formed after centriguing was hard and rubber-like. After a few minutes the pellet softened enough to be broken up more easily because 2ml of distilled water was added.
16	Silverside	BCG	324B	02/02/2023	CPC 1%	0	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. 20 ml of 1% CPC was used to decontaminate the homogenate. The pellet formed after centriguing was hard and rubber-like. After a few minutes the pellet softened enough to be broken up more easily because 2ml of distilled water was added.
16	Silverside	BCG	324B	02/02/2023	CPC 1%	0	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. 20 ml of 1% CPC was used to decontaminate the homogenate. The pellet formed after centriguing was hard and rubber-like. After a few minutes the pellet softened enough to be broken up more easily because 2ml of distilled water was added.
16	Silverside	BCG	324B	02/02/2023	NaOH	0	0	?	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. 20 ml of 1% CPC was used to decontaminate the homogenate. The pellet formed after centriguing was hard and rubber-like. After a few minutes the pellet softened enough to be broken up more easily because 2ml of distilled water was added.
16	Silverside	BCG	324B	02/02/2023	NaOH	0	?	?	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. 20 ml of 1% CPC was used to decontaminate the homogenate. The pellet formed after centriguing was hard and rubber-like. After a few minutes the pellet softened enough to be broken up more easily because 2ml of distilled water was added.

16	Brisket	BCG	324C	02/02/2023	CPC 1%	?	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. 20 ml of 1% CPC was used to decontaminate the homogenate. The pellet formed after centriguing was hard and rubber-like. After a few minutes the pellet softened enough to be broken up more easily because 2ml of distilled water was added.
16	Brisket	BCG	324C	02/02/2023	CPC 1%	0	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. 20 ml of 1% CPC was used to decontaminate the homogenate. The pellet formed after centriguing was hard and rubber-like. After a few minutes the pellet softened enough to be broken up more easily because 2ml of distilled water was added.
16	Brisket	BCG	324C	02/02/2023	NaOH	0	?	0	0	0	0	0	0	1	1/c	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. 20 ml of 1% CPC was used to decontaminate the homogenate. The pellet formed after centriguing was hard and rubber-like. After a few minutes the pellet softened enough to be broken up more easily because 2ml of distilled water was added.
16	Brisket	BCG	324C	02/02/2023	NaOH	0	0	0	0	0	0	0	0	1	1/c	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. 20 ml of 1% CPC was used to decontaminate the homogenate. The pellet formed after centriguing was hard and rubber-like. After a few minutes the pellet softened enough to be broken up more easily because 2ml of distilled water was added.
16	Rump	BCG	324D	02/02/2023	CPC 1%	0	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. 20 ml of 1% CPC was used to decontaminate the homogenate. The pellet formed after centriguing was hard and rubber-like. After a few minutes the pellet softened enough to be broken up more easily because 2ml of distilled water was added.
16	Rump	BCG	324D	02/02/2023	CPC 1%	0	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. 20 ml of 1% CPC was used to decontaminate the homogenate. The pellet formed after centriguing was hard and rubber-like. After a few minutes the pellet softened enough to be broken up more easily because 2ml of distilled water was added.
16	Rump	BCG	324D	02/02/2023	NaOH	0	0	C	C	C	C	C	C	C	C	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. 20 ml of 1% CPC was used to decontaminate the homogenate. The pellet formed after centriguing was hard and rubber-like. After a few minutes the pellet softened enough to be broken up more easily because 2ml of distilled water was added.
16	Rump	BCG	324D	02/02/2023	NaOH	0	0	?	?	?	?	0	0	0	1/c	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. 20 ml of 1% CPC was used to decontaminate the homogenate. The pellet formed after centriguing was hard and rubber-like. After a few minutes the pellet softened enough to be broken up more easily because 2ml of distilled water was added.

Buffalo_Number	Sample_Type	Vaccine_Treatment	Sample_(Lab)_Number	Date_Processed	Treatment	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9	Week 10	Final_Growth	PCR_Final_Result	Comments
17	Fillet	IV-IM	325A	03/02/2023	CPC 1%	0	0	?	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. 20 ml of 1% CPC was used to decontaminate the homogenate. The pellet formed after centriguing was hard and rubber-like. After a few minutes the pellet softened enough to be broken up more easily because 2ml of distilled water was added.
17	Fillet	IV-IM	325A	03/02/2023	CPC 1%	0	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. 20 ml of 1% CPC was used to decontaminate the homogenate. The pellet formed after centriguing was hard and rubber-like. After a few minutes the pellet softened enough to be broken up more easily because 2ml of distilled water was added.
17	Fillet	IV-IM	325A	03/02/2023	NaOH	0	0	?	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. 20 ml of 1% CPC was used to decontaminate the homogenate. The pellet formed after centriguing was hard and rubber-like. After a few minutes the pellet softened enough to be broken up more easily because 2ml of distilled water was added.
17	Fillet	IV-IM	325A	03/02/2023	NaOH	0	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. 20 ml of 1% CPC was used to decontaminate the homogenate. The pellet formed after centriguing was hard and rubber-like. After a few minutes the pellet softened enough to be broken up more easily because 2ml of distilled water was added.
17	Silverside	IV-IM	325B	03/02/2023	CPC 1%	0	0	1*-Clear	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. 20 ml of 1% CPC was used to decontaminate the homogenate. The pellet formed after centriguing was hard and rubber-like. After a few minutes the pellet softened enough to be broken up more easily because 2ml of distilled water was added.
17	Silverside	IV-IM	325B	03/02/2023	CPC 1%	0	0	1*-Clear	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. 20 ml of 1% CPC was used to decontaminate the homogenate. The pellet formed after centriguing was hard and rubber-like. After a few minutes the pellet softened enough to be broken up more easily because 2ml of distilled water was added.
17	Silverside	IV-IM	325B	03/02/2023	NaOH	0	c/0	c/2*	0	0	0	0	0	0	0	Yes	Culture_Suspect_PCR_Negative	No abnormalities were found on first inspection. 20 ml of 1% CPC was used to decontaminate the homogenate. The pellet formed after centriguing was hard and rubber-like. After a few minutes the pellet softened enough to be broken up more easily because 2ml of distilled water was added. The colony formed was clear.
17	Silverside	IV-IM	325B	03/02/2023	NaOH	0	0	c/2*	c/2*	c/2*	c/2*?	c/2	c/2	c/2	c/2	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. 20 ml of 1% CPC was used to decontaminate the homogenate. The pellet formed after centriguing was hard and rubber-like. After a few minutes the pellet softened enough to be broken up more easily because 2ml of distilled water was added.

17	Brisket	IV-IM	325C	03/02/2023	CPC 1%	0	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	What looked to be freezer burn was visible on the outside of the meat. It did not penetrate the meat very deep. No lesions were found during the initial inspection. 20ml of 1% CPC was used to decontaminate the homogenate. The pellet formed after centrifuging was rubber-like.
17	Brisket	IV-IM	325C	03/02/2023	CPC 1%	0	0	c/0	c/0	c/0	c/0	c/0	c/0	c/0	c/0	No	Not_Applicable_Not_Done	What looked to be freezer burn was visible on the outside of the meat. It did not penetrate the meat very deep. No lesions were found during the initial inspection. 20ml of 1% CPC was used to decontaminate the homogenate. The pellet formed after centrifuging was rubber-like.
17	Brisket	IV-IM	325C	03/02/2023	NaOH	0	0	3*-Opaque	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	What looked to be freezer burn was visible on the outside of the meat. It did not penetrate the meat very deep. No lesions were found during the initial inspection. 20ml of 1% CPC was used to decontaminate the homogenate. The pellet formed after centrifuging was rubber-like.
17	Brisket	IV-IM	325C	03/02/2023	NaOH	0	0	?	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	What looked to be freezer burn was visible on the outside of the meat. It did not penetrate the meat very deep. No lesions were found during the initial inspection. 20ml of 1% CPC was used to decontaminate the homogenate. The pellet formed after centrifuging was rubber-like.
17	Rump	IV-IM	325D	03/02/2023	CPC 1%	0	0	1*-Opaque White	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abornmalities were found on first inspection. 20ml of 1% CPC was used to decontaminate the homogenate. The pellet formed after centrifuging was rubber-like. After adding 2ml of water and waiting a minute the pellet softened.
17	Rump	IV-IM	325D	03/02/2023	CPC 1%	0	0	1*-Opaque White	?	?	?	0	0	0	0	No	Not_Applicable_Not_Done	No abornmalities were found on first inspection. 20ml of 1% CPC was used to decontaminate the homogenate. The pellet formed after centrifuging was rubber-like. After adding 2ml of water and waiting a minute the pellet softened.
17	Rump	IV-IM	325D	03/02/2023	NaOH	0	0	?	?	?	?	0	0	0	0	Yes	Culture_Suspect_PCR_Negative	No abornmalities were found on first inspection. 20ml of 1% CPC was used to decontaminate the homogenate. The pellet formed after centrifuging was rubber-like. After adding 2ml of water and waiting a minute the pellet softened. The colony formed was opaque white
17	Rump	IV-IM	325D	03/02/2023	NaOH	0	0	?	?	?	?	0	0	0	0	No	Not_Applicable_Not_Done	No abornmalities were found on first inspection. 20ml of 1% CPC was used to decontaminate the homogenate. The pellet formed after centrifuging was rubber-like. After adding 2ml of water and waiting a minute the pellet softened.

Buffalo_Number	Sample_Type	Vaccine_Treatment	Sample_(Lab)_Number	Date_Processed	Treatment	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9	Week 10	Final_Growth	PCR_Final_Result	Comments
18	Fillet	IV-Oral	326A	09/02/2023	CPC 1%	0	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. The CPC 1% decontamination method caused the pellet to become hard and rubber-like. 2ml of distilled water was added to help soften the pellet.
18	Fillet	IV-Oral	326A	09/02/2023	CPC 1%	0	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. The CPC 1% decontamination method caused the pellet to become hard and rubber-like. 2ml of distilled water was added to help soften the pellet.
18	Fillet	IV-Oral	326A	09/02/2023	NaOH	0	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. The CPC 1% decontamination method caused the pellet to become hard and rubber-like. 2ml of distilled water was added to help soften the pellet.
18	Fillet	IV-Oral	326A	09/02/2023	NaOH	0	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. The CPC 1% decontamination method caused the pellet to become hard and rubber-like. 2ml of distilled water was added to help soften the pellet.
18	Silverside	IV-Oral	326B	09/02/2023	CPC 1%	0	0	0	0	0	0	0	0	1	1	Yes	Culture_Suspect_PCR_Negative	No abnormalities were found on first inspection. The CPC 1% decontamination method caused the pellet to become hard and rubber-like. 2ml of distilled water was added to help soften the pellet. The colony formed was opaque white
18	Silverside	IV-Oral	326B	09/02/2023	CPC 1%	?	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. The CPC 1% decontamination method caused the pellet to become hard and rubber-like. 2ml of distilled water was added to help soften the pellet.
18	Silverside	IV-Oral	326B	09/02/2023	NaOH	0	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. The CPC 1% decontamination method caused the pellet to become hard and rubber-like. 2ml of distilled water was added to help soften the pellet.
18	Silverside	IV-Oral	326B	09/02/2023	NaOH	0	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. The CPC 1% decontamination method caused the pellet to become hard and rubber-like. 2ml of distilled water was added to help soften the pellet.
18	Brisket	IV-Oral	326C	09/02/2023	CPC 1%	?	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. The CPC 1% decontamination method caused the pellet to become hard and rubber-like. 2ml of distilled water was added to help soften the pellet.
18	Brisket	IV-Oral	326C	09/02/2023	CPC 1%	0	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. The CPC 1% decontamination method caused the pellet to become hard and rubber-like. 2ml of distilled water was added to help soften the pellet.
18	Brisket	IV-Oral	326C	09/02/2023	NaOH	?	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. The CPC 1% decontamination method caused the pellet to become hard and rubber-like. 2ml of distilled water was added to help soften the pellet.
18	Brisket	IV-Oral	326C	09/02/2023	NaOH	0	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. The CPC 1% decontamination method caused the pellet to become hard and rubber-like. 2ml of distilled water was added to help soften the pellet.
18	Rump	IV-Oral	326D	09/02/2023	CPC 1%	0	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. The CPC 1% decontamination method caused the pellet to become hard and rubber-like. 2ml of distilled water was added to help soften the pellet.
18	Rump	IV-Oral	326D	09/02/2023	CPC 1%	0	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. The CPC 1% decontamination method caused the pellet to become hard and rubber-like. 2ml of distilled water was added to help soften the pellet.
18	Rump	IV-Oral	326D	09/02/2023	NaOH	1*?-Clear	1*?-Clear	1*?-Clear	1*?-Clear	1*?-Clear	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. The CPC 1% decontamination method caused the pellet to become hard and rubber-like. 2ml of distilled water was added to help soften the pellet.
18	Rump	IV-Oral	326D	09/02/2023	NaOH	0	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. The CPC 1% decontamination method caused the pellet to become hard and rubber-like. 2ml of distilled water was added to help soften the pellet.

Buffalo_Number	Sample_Type	Vaccine_Treatment	Sample_(Lab)_Number	Date_Processed	Treatment	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9	Week 10	Final_Growth	PCR_Final_Result	Comments
20	Fillet	IV-Oral	327A	10/02/2023	CPC 1%	0	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. The CPC 1% decontamination method caused the pellet to become hard and rubber-like. 2ml of distilled water was added to help soften the pellet.
20	Fillet	IV-Oral	327A	10/02/2023	CPC 1%	0	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. The CPC 1% decontamination method caused the pellet to become hard and rubber-like. 2ml of distilled water was added to help soften the pellet.
20	Fillet	IV-Oral	327A	10/02/2023	NaOH	0	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. The CPC 1% decontamination method caused the pellet to become hard and rubber-like. 2ml of distilled water was added to help soften the pellet.
20	Fillet	IV-Oral	327A	10/02/2023	NaOH	0	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. The CPC 1% decontamination method caused the pellet to become hard and rubber-like. 2ml of distilled water was added to help soften the pellet.
20	Silverside	IV-Oral	327B	10/02/2023	CPC 1%	0	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. The CPC 1% decontamination method caused the pellet to become hard and rubber-like. 2ml of distilled water was added to help soften the pellet.
20	Silverside	IV-Oral	327B	10/02/2023	CPC 1%	0	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. The CPC 1% decontamination method caused the pellet to become hard and rubber-like. 2ml of distilled water was added to help soften the pellet.
20	Silverside	IV-Oral	327B	10/02/2023	NaOH	0	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. The CPC 1% decontamination method caused the pellet to become hard and rubber-like. 2ml of distilled water was added to help soften the pellet.
20	Silverside	IV-Oral	327B	10/02/2023	NaOH	?	0	0	0	0	0	0	0	1*-Clear	1/c	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. The CPC 1% decontamination method caused the pellet to become hard and rubber-like. 2ml of distilled water was added to help soften the pellet.
20	Brisket	IV-Oral	327C	10/02/2023	CPC 1%	0	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. The CPC 1% decontamination method caused the pellet to become hard and rubber-like. 2ml of distilled water was added to help soften the pellet.
20	Brisket	IV-Oral	327C	10/02/2023	CPC 1%	0	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. The CPC 1% decontamination method caused the pellet to become hard and rubber-like. 2ml of distilled water was added to help soften the pellet.
20	Brisket	IV-Oral	327C	10/02/2023	NaOH	0	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. The CPC 1% decontamination method caused the pellet to become hard and rubber-like. 2ml of distilled water was added to help soften the pellet.
20	Brisket	IV-Oral	327C	10/02/2023	NaOH	0	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. The CPC 1% decontamination method caused the pellet to become hard and rubber-like. 2ml of distilled water was added to help soften the pellet.
20	Rump	IV-Oral	327D	10/02/2023	CPC 1%	0	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. The CPC 1% decontamination method caused the pellet to become hard and rubber-like. 2ml of distilled water was added to help soften the pellet.
20	Rump	IV-Oral	327D	10/02/2023	CPC 1%	0	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. The CPC 1% decontamination method caused the pellet to become hard and rubber-like. 2ml of distilled water was added to help soften the pellet.
20	Rump	IV-Oral	327D	10/02/2023	NaOH	?	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. The CPC 1% decontamination method caused the pellet to become hard and rubber-like. 2ml of distilled water was added to help soften the pellet.
20	Rump	IV-Oral	327D	10/02/2023	NaOH	0	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. The CPC 1% decontamination method caused the pellet to become hard and rubber-like. 2ml of distilled water was added to help soften the pellet.

Buffalo_Number	Sample_Type	Vaccine_Treatment	Sample_(Lab)_Number	Date_Processed	Treatment	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9	Week 10	Final_Growth	PCR_Final_Result	Comments
21	Fillet	Control - No Vaccine	328A	13/02/2023	CPC 1%	1	1	1	1	1	1	1	1	1	1/c	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. The CPC 1% decontamination method caused the pellet to become hard and rubber-like. 2ml of distilled water was added to help soften the pellet. The final growth was clear.
21	Fillet	Control - No Vaccine	328A	13/02/2023	CPC 1%	1	1	1	1	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. The CPC 1% decontamination method caused the pellet to become hard and rubber-like. 2ml of distilled water was added to help soften the pellet.
21	Fillet	Control - No Vaccine	328A	13/02/2023	NaOH	0	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. The CPC 1% decontamination method caused the pellet to become hard and rubber-like. 2ml of distilled water was added to help soften the pellet.
21	Fillet	Control - No Vaccine	328A	13/02/2023	NaOH	0	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. The CPC 1% decontamination method caused the pellet to become hard and rubber-like. 2ml of distilled water was added to help soften the pellet.
21	Silverside	Control - No Vaccine	328B	13/02/2023	CPC 1%	?	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. The CPC 1% decontamination method caused the pellet to become hard and rubber-like. 2ml of distilled water was added to help soften the pellet.
21	Silverside	Control - No Vaccine	328B	13/02/2023	CPC 1%	0	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. The CPC 1% decontamination method caused the pellet to become hard and rubber-like. 2ml of distilled water was added to help soften the pellet.
21	Silverside	Control - No Vaccine	328B	13/02/2023	NaOH	0	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. The CPC 1% decontamination method caused the pellet to become hard and rubber-like. 2ml of distilled water was added to help soften the pellet.
21	Silverside	Control - No Vaccine	328B	13/02/2023	NaOH	0	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. The CPC 1% decontamination method caused the pellet to become hard and rubber-like. 2ml of distilled water was added to help soften the pellet.
21	Brisket	Control - No Vaccine	328C	13/02/2023	CPC 1%	0	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. The CPC 1% decontamination method caused the pellet to become hard and rubber-like. 2ml of distilled water was added to help soften the pellet.
21	Brisket	Control - No Vaccine	328C	13/02/2023	CPC 1%	0	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. The CPC 1% decontamination method caused the pellet to become hard and rubber-like. 2ml of distilled water was added to help soften the pellet.
21	Brisket	Control - No Vaccine	328C	13/02/2023	NaOH	0	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. The CPC 1% decontamination method caused the pellet to become hard and rubber-like. 2ml of distilled water was added to help soften the pellet.
21	Brisket	Control - No Vaccine	328C	13/02/2023	NaOH	0	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. The CPC 1% decontamination method caused the pellet to become hard and rubber-like. 2ml of distilled water was added to help soften the pellet.
21	Rump	Control - No Vaccine	328D	13/02/2023	CPC 1%	0	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. The CPC 1% decontamination method caused the pellet to become hard and rubber-like. 2ml of distilled water was added to help soften the pellet.
21	Rump	Control - No Vaccine	328D	13/02/2023	CPC 1%	0	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. The CPC 1% decontamination method caused the pellet to become hard and rubber-like. 2ml of distilled water was added to help soften the pellet.
21	Rump	Control - No Vaccine	328D	13/02/2023	NaOH	?	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. The CPC 1% decontamination method caused the pellet to become hard and rubber-like. 2ml of distilled water was added to help soften the pellet.
21	Rump	Control - No Vaccine	328D	13/02/2023	NaOH	?	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. The CPC 1% decontamination method caused the pellet to become hard and rubber-like. 2ml of distilled water was added to help soften the pellet.

Buffalo_Number	Sample_Type	Vaccine_Treatment	Sample_(Lab)_Number	Date_Processed	Treatment	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9	Week 10	Final_Growth	PCR_Final_Result	Comments
22	Fillet	BCG	329A	13/02/2023	CPC 1%	0	0	0	0	0	0	0	0	0	0?	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. The CPC 1% decontamination method caused the pellet to become hard and rubber-like. 2ml of distilled water was added to help soften the pellet.
22	Fillet	BCG	329A	13/02/2023	CPC 1%	0	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. The CPC 1% decontamination method caused the pellet to become hard and rubber-like. 2ml of distilled water was added to help soften the pellet.
22	Fillet	BCG	329A	13/02/2023	NaOH	0	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. The CPC 1% decontamination method caused the pellet to become hard and rubber-like. 2ml of distilled water was added to help soften the pellet.
22	Fillet	BCG	329A	13/02/2023	NaOH	0	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. The CPC 1% decontamination method caused the pellet to become hard and rubber-like. 2ml of distilled water was added to help soften the pellet.
22	Silverside	BCG	329B	13/02/2023	CPC 1%	0	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. The CPC 1% decontamination method caused the pellet to become hard and rubber-like. 2ml of distilled water was added to help soften the pellet.
22	Silverside	BCG	329B	13/02/2023	CPC 1%	0	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. The CPC 1% decontamination method caused the pellet to become hard and rubber-like. 2ml of distilled water was added to help soften the pellet.
22	Silverside	BCG	329B	13/02/2023	NaOH	?	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. The CPC 1% decontamination method caused the pellet to become hard and rubber-like. 2ml of distilled water was added to help soften the pellet.
22	Silverside	BCG	329B	13/02/2023	NaOH	0	0	0	0	0	0	0	0	0	1/c	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. The CPC 1% decontamination method caused the pellet to become hard and rubber-like. 2ml of distilled water was added to help soften the pellet.
22	Brisket	BCG	329C	14/02/2023	CPC 1%	?	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. The CPC 1% decontamination method caused the pellet to become hard and rubber-like. 2ml of distilled water was added to help soften the pellet.
22	Brisket	BCG	329C	14/02/2023	CPC 1%	0	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. The CPC 1% decontamination method caused the pellet to become hard and rubber-like. 2ml of distilled water was added to help soften the pellet.
22	Brisket	BCG	329C	14/02/2023	NaOH	0	0	0	0	0	0	0	0	0	1/c	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. The CPC 1% decontamination method caused the pellet to become hard and rubber-like. 2ml of distilled water was added to help soften the pellet.
22	Brisket	BCG	329C	14/02/2023	NaOH	0	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. The CPC 1% decontamination method caused the pellet to become hard and rubber-like. 2ml of distilled water was added to help soften the pellet.
22	Rump	BCG	329D	14/02/2023	CPC 1%	0	0	0	0	0	0	0	0	0	?	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. The CPC 1% decontamination method caused the pellet to become hard and rubber-like. 2ml of distilled water was added to help soften the pellet.
22	Rump	BCG	329D	14/02/2023	CPC 1%	0	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. The CPC 1% decontamination method caused the pellet to become hard and rubber-like. 2ml of distilled water was added to help soften the pellet.
22	Rump	BCG	329D	14/02/2023	NaOH	0	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. The CPC 1% decontamination method caused the pellet to become hard and rubber-like. 2ml of distilled water was added to help soften the pellet.
22	Rump	BCG	329D	14/02/2023	NaOH	0	0	0	0	0	0	0	0	?	?	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. The CPC 1% decontamination method caused the pellet to become hard and rubber-like. 2ml of distilled water was added to help soften the pellet.

Buffalo_Number	Sample_Type	Vaccine_Treatment	Sample_(Lab)_Number	Date_Processed	Treatment	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9	Week 10	Final_Growth	PCR_Final_Result	Comments
23	Fillet	BCG	330A	14/02/2023	CPC 1%	0	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. The CPC 1% decontamination method caused the pellet to become hard and rubber-like. 2ml of distilled water was added to help soften the pellet.
23	Fillet	BCG	330A	14/02/2023	CPC 1%	0	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. The CPC 1% decontamination method caused the pellet to become hard and rubber-like. 2ml of distilled water was added to help soften the pellet.
23	Fillet	BCG	330A	14/02/2023	NaOH	?	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. The CPC 1% decontamination method caused the pellet to become hard and rubber-like. 2ml of distilled water was added to help soften the pellet.
23	Fillet	BCG	330A	14/02/2023	NaOH	?	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. The CPC 1% decontamination method caused the pellet to become hard and rubber-like. 2ml of distilled water was added to help soften the pellet.
23	Silverside	BCG	330B	14/02/2023	CPC 1%	0	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. The CPC 1% decontamination method caused the pellet to become hard and rubber-like. 2ml of distilled water was added to help soften the pellet.
23	Silverside	BCG	330B	14/02/2023	CPC 1%	0	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. The CPC 1% decontamination method caused the pellet to become hard and rubber-like. 2ml of distilled water was added to help soften the pellet.
23	Silverside	BCG	330B	14/02/2023	NaOH	?	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. The CPC 1% decontamination method caused the pellet to become hard and rubber-like. 2ml of distilled water was added to help soften the pellet.
23	Silverside	BCG	330B	14/02/2023	NaOH	0	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. The CPC 1% decontamination method caused the pellet to become hard and rubber-like. 2ml of distilled water was added to help soften the pellet.
23	Brisket	BCG	330C	14/02/2023	CPC 1%	0	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. The CPC 1% decontamination method caused the pellet to become hard and rubber-like. 2ml of distilled water was added to help soften the pellet.
23	Brisket	BCG	330C	14/02/2023	CPC 1%	0	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. The CPC 1% decontamination method caused the pellet to become hard and rubber-like. 2ml of distilled water was added to help soften the pellet.
23	Brisket	BCG	330C	14/02/2023	NaOH	0	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. The CPC 1% decontamination method caused the pellet to become hard and rubber-like. 2ml of distilled water was added to help soften the pellet.
23	Brisket	BCG	330C	14/02/2023	NaOH	0	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. The CPC 1% decontamination method caused the pellet to become hard and rubber-like. 2ml of distilled water was added to help soften the pellet.
23	Rump	BCG	330D	14/02/2023	CPC 1%	0	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. The CPC 1% decontamination method caused the pellet to become hard and rubber-like. 2ml of distilled water was added to help soften the pellet.
23	Rump	BCG	330D	14/02/2023	CPC 1%	0	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. The CPC 1% decontamination method caused the pellet to become hard and rubber-like. 2ml of distilled water was added to help soften the pellet.
23	Rump	BCG	330D	14/02/2023	NaOH	0	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. The CPC 1% decontamination method caused the pellet to become hard and rubber-like. 2ml of distilled water was added to help soften the pellet.
23	Rump	BCG	330D	14/02/2023	NaOH	0	0	0	0	0	0	0	0	1	1	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. The CPC 1% decontamination method caused the pellet to become hard and rubber-like. 2ml of distilled water was added to help soften the pellet. The final growth was clear.

Buffalo_Number	Sample_Type	Vaccine_Treatment	Sample_(Lab)_Number	Date_Processed	Treatment	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9	Week 10	Final_Growth	PCR_Final_Result	Comments
24	Fillet	IV-Oral	331A	15/02/2023	CPC 1%	0	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. The CPC 1% decontamination method caused the pellet to become hard and rubber-like. 2ml of distilled water was added to help soften the pellet.
24	Fillet	IV-Oral	331A	15/02/2023	CPC 1%	0	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. The CPC 1% decontamination method caused the pellet to become hard and rubber-like. 2ml of distilled water was added to help soften the pellet.
24	Fillet	IV-Oral	331A	15/02/2023	NaOH	1?	1*-Clear	1*-Clear	1*-Clear	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. The CPC 1% decontamination method caused the pellet to become hard and rubber-like. 2ml of distilled water was added to help soften the pellet.
24	Fillet	IV-Oral	331A	15/02/2023	NaOH	0	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. The CPC 1% decontamination method caused the pellet to become hard and rubber-like. 2ml of distilled water was added to help soften the pellet.
24	Silverside	IV-Oral	331B	15/02/2023	CPC 1%	0	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. The CPC 1% decontamination method caused the pellet to become hard and rubber-like. 2ml of distilled water was added to help soften the pellet.
24	Silverside	IV-Oral	331B	15/02/2023	CPC 1%	0	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. The CPC 1% decontamination method caused the pellet to become hard and rubber-like. 2ml of distilled water was added to help soften the pellet.
24	Silverside	IV-Oral	331B	15/02/2023	NaOH	c/0	c/0	c/0	c/0	c/0	c/0	c/0	c/0	c/0	c/0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. The CPC 1% decontamination method caused the pellet to become hard and rubber-like. 2ml of distilled water was added to help soften the pellet.
24	Silverside	IV-Oral	331B	15/02/2023	NaOH	c/0	c/0	c/0	c/0	c/0	c/0	c/0	c/0	c/0	c/0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. The CPC 1% decontamination method caused the pellet to become hard and rubber-like. 2ml of distilled water was added to help soften the pellet.
24	Brisket	IV-Oral	331C	15/02/2023	CPC 1%	?	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. The CPC 1% decontamination method caused the pellet to become hard and rubber-like. 2ml of distilled water was added to help soften the pellet.
24	Brisket	IV-Oral	331C	15/02/2023	CPC 1%	0	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. The CPC 1% decontamination method caused the pellet to become hard and rubber-like. 2ml of distilled water was added to help soften the pellet.
24	Brisket	IV-Oral	331C	15/02/2023	NaOH	0	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. The CPC 1% decontamination method caused the pellet to become hard and rubber-like. 2ml of distilled water was added to help soften the pellet.
24	Brisket	IV-Oral	331C	15/02/2023	NaOH	?	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. The CPC 1% decontamination method caused the pellet to become hard and rubber-like. 2ml of distilled water was added to help soften the pellet.
24	Rump	IV-Oral	331D	15/02/2023	CPC 1%	0	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection except that there appeared to be some freezer burn. The affected area was approximately 0.5cm deep along the one whole edge of the meat. The CPC 1% decontamination method caused the pellet to become hard and rubber-like.
24	Rump	IV-Oral	331D	15/02/2023	CPC 1%	0	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection except that there appeared to be some freezer burn. The affected area was approximately 0.5cm deep along the one whole edge of the meat. The CPC 1% decontamination method caused the pellet to become hard and rubber-like.
24	Rump	IV-Oral	331D	15/02/2023	NaOH	1*-Clear	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection except that there appeared to be some freezer burn. The affected area was approximately 0.5cm deep along the one whole edge of the meat. The CPC 1% decontamination method caused the pellet to become hard and rubber-like.
24	Rump	IV-Oral	331D	15/02/2023	NaOH	0	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection except that there appeared to be some freezer burn. The affected area was approximately 0.5cm deep along the one whole edge of the meat. The CPC 1% decontamination method caused the pellet to become hard and rubber-like.

Buffalo_Number	Sample_Type	Vaccine_Treatment	Sample_(Lab)_Number	Date_Processed	Treatment	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9	Week 10	Final_Growth	PCR_Final_Result	Comments
25	Fillet	BCG	332A	15/02/2023	CPC 1%	1*-Egg Yellow	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. The CPC 1% decontamination method caused the pellet to become hard and rubber-like. 2ml of distilled water was added to help soften the pellet.
25	Fillet	BCG	332A	15/02/2023	CPC 1%	0	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. The CPC 1% decontamination method caused the pellet to become hard and rubber-like. 2ml of distilled water was added to help soften the pellet.
25	Fillet	BCG	332A	15/02/2023	NaOH	?	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. The CPC 1% decontamination method caused the pellet to become hard and rubber-like. 2ml of distilled water was added to help soften the pellet.
25	Fillet	BCG	332A	15/02/2023	NaOH	0	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. The CPC 1% decontamination method caused the pellet to become hard and rubber-like. 2ml of distilled water was added to help soften the pellet.
25	Silverside	BCG	332B	15/02/2023	CPC 1%	?	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. The CPC 1% decontamination method caused the pellet to become hard and rubber-like. 2ml of distilled water was added to help soften the pellet.
25	Silverside	BCG	332B	15/02/2023	CPC 1%	?	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. The CPC 1% decontamination method caused the pellet to become hard and rubber-like. 2ml of distilled water was added to help soften the pellet.
25	Silverside	BCG	332B	15/02/2023	NaOH	0	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. The CPC 1% decontamination method caused the pellet to become hard and rubber-like. 2ml of distilled water was added to help soften the pellet.
25	Silverside	BCG	332B	15/02/2023	NaOH	c?	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. The CPC 1% decontamination method caused the pellet to become hard and rubber-like. 2ml of distilled water was added to help soften the pellet.
25	Brisket	BCG	332C	16/02/2023	CPC 1%	0	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. The CPC 1% decontamination method caused the pellet to become hard and rubber-like. 2ml of distilled water was added to help soften the pellet.
25	Brisket	BCG	332C	16/02/2023	CPC 1%	0	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. The CPC 1% decontamination method caused the pellet to become hard and rubber-like. 2ml of distilled water was added to help soften the pellet.
25	Brisket	BCG	332C	16/02/2023	NaOH	0	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. The CPC 1% decontamination method caused the pellet to become hard and rubber-like. 2ml of distilled water was added to help soften the pellet.
25	Brisket	BCG	332C	16/02/2023	NaOH	0	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. The CPC 1% decontamination method caused the pellet to become hard and rubber-like. 2ml of distilled water was added to help soften the pellet.
25	Rump	BCG	332D	16/02/2023	CPC 1%	0	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection but the meat appeared to have some freezer burn. The discolouration did not penetrate deeply into the meat. The CPC 1% decontamination method caused the pellet to become hard and rubber-like. 2ml of distilled water was added to help soften the pellet.
25	Rump	BCG	332D	16/02/2023	CPC 1%	0	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection but the meat appeared to have some freezer burn. The discolouration did not penetrate deeply into the meat. The CPC 1% decontamination method caused the pellet to become hard and rubber-like. 2ml of distilled water was added to help soften the pellet.
25	Rump	BCG	332D	16/02/2023	NaOH	?	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection but the meat appeared to have some freezer burn. The discolouration did not penetrate deeply into the meat. The CPC 1% decontamination method caused the pellet to become hard and rubber-like. 2ml of distilled water was added to help soften the pellet.
25	Rump	BCG	332D	16/02/2023	NaOH	?	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection but the meat appeared to have some freezer burn. The discolouration did not penetrate deeply into the meat. The CPC 1% decontamination method caused the pellet to become hard and rubber-like. 2ml of distilled water was added to help soften the pellet.

Buffalo_Number	Sample_Type	Vaccine_Treatment	Sample_(Lab)_Number	Date_Processed	Treatment	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9	Week 10	Final_Growth	PCR_Final_Result	Comments
26	Fillet	Control - No Vaccine	333A	16/02/2023	CPC 1%	0	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. The CPC 1% decontamination method caused the pellet to become hard and rubber-like.
26	Fillet	Control - No Vaccine	333A	16/02/2023	CPC 1%	0	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. The CPC 1% decontamination method caused the pellet to become hard and rubber-like.
26	Fillet	Control - No Vaccine	333A	16/02/2023	NaOH	0	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. The CPC 1% decontamination method caused the pellet to become hard and rubber-like.
26	Fillet	Control - No Vaccine	333A	16/02/2023	NaOH	0	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. The CPC 1% decontamination method caused the pellet to become hard and rubber-like.
26	Silverside	Control - No Vaccine	333B	16/02/2023	CPC 1%	1*-Egg Yellow	1*-Egg Yellow	1*-Egg Yellow	1*-Egg Yellow	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection but there was slight discolouration on the meat tht did not penetrate deeply into the meat. The appearance was similar to freezer burn. The CPC 1% decontamination method caused the pellet to become hard and rubber-like.
26	Silverside	Control - No Vaccine	333B	16/02/2023	CPC 1%	0	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection but there was slight discolouration on the meat tht did not penetrate deeply into the meat. The appearance was similar to freezer burn. The CPC 1% decontamination method caused the pellet to become hard and rubber-like.
26	Silverside	Control - No Vaccine	333B	16/02/2023	NaOH	0	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection but there was slight discolouration on the meat tht did not penetrate deeply into the meat. The appearance was similar to freezer burn. The CPC 1% decontamination method caused the pellet to become hard and rubber-like.
26	Silverside	Control - No Vaccine	333B	16/02/2023	NaOH	0	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection but there was slight discolouration on the meat tht did not penetrate deeply into the meat. The appearance was similar to freezer burn. The CPC 1% decontamination method caused the pellet to become hard and rubber-like.
26	Brisket	Control - No Vaccine	333C	17/02/2023	CPC 1%	0	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. The CPC 1% decontamination method caused the pellet to become hard and rubber-like. 2ml of distilled water was added to help soften the pellet.
26	Brisket	Control - No Vaccine	333C	17/02/2023	CPC 1%	0	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. The CPC 1% decontamination method caused the pellet to become hard and rubber-like. 2ml of distilled water was added to help soften the pellet.
26	Brisket	Control - No Vaccine	333C	17/02/2023	NaOH	0	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. The CPC 1% decontamination method caused the pellet to become hard and rubber-like. 2ml of distilled water was added to help soften the pellet.
26	Brisket	Control - No Vaccine	333C	17/02/2023	NaOH	0	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. The CPC 1% decontamination method caused the pellet to become hard and rubber-like. 2ml of distilled water was added to help soften the pellet.
26	Rump	Control - No Vaccine	333D	17/02/2023	CPC 1%	?	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. The CPC 1% decontamination method caused the pellet to become hard and rubber-like. 2ml of distilled water was added to help soften the pellet.
26	Rump	Control - No Vaccine	333D	17/02/2023	CPC 1%	0	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. The CPC 1% decontamination method caused the pellet to become hard and rubber-like. 2ml of distilled water was added to help soften the pellet.
26	Rump	Control - No Vaccine	333D	17/02/2023	NaOH	0	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. The CPC 1% decontamination method caused the pellet to become hard and rubber-like. 2ml of distilled water was added to help soften the pellet.
26	Rump	Control - No Vaccine	333D	17/02/2023	NaOH	0	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. The CPC 1% decontamination method caused the pellet to become hard and rubber-like. 2ml of distilled water was added to help soften the pellet.

Buffalo_Number	Sample_Type	Vaccine_Treatment	Sample_(Lab)_Number	Date_Processed	Treatment	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9	Week 10	Final_Growth	PCR_Final_Result	Comments
Negative_Control_#1	Sub_iliac_lymph_node_from_Buffalo_#20	IV-Oral	Neg_Control_#1	19/01/2023	HCl	0	0	0	0	0	0	0	0	0	0	No	Negative_Control_Water	No abnormalities were found. The sample had slices in it from the inspection process.
Negative_Control_#1	Sub_iliac_lymph_node_from_Buffalo_#20	IV-Oral	Neg_Control_#1	19/01/2023	HCl	0	0	0	0	0	0	0	0	0	0	No	Negative_Control_Water	No abnormalities were found. The sample had slices in it from the inspection process.
Negative_Control_#1	Sub_iliac_lymph_node_from_Buffalo_#20	IV-Oral	Neg_Control_#1	19/01/2023	NaOH	0	0	0	0	0	0	0	0	0	0	No	Negative_Control_Water	No abnormalities were found. The sample had slices in it from the inspection process.
Negative_Control_#1	Sub_iliac_lymph_node_from_Buffalo_#20	IV-Oral	Neg_Control_#1	19/01/2023	NaOH	0	0	0	0	0	0	0	0	0	0	No	Negative_Control_Water	No abnormalities were found. The sample had slices in it from the inspection process.
Negative_Control_#2	Parotid_Lymph_Node_Buffalo_21	Control - No Vaccine	Neg_Control_#2	27/01/2023	NaOH	0	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. The sample had slices in it from the initial inspection process.
Negative_Control_#2	Parotid_Lymph_Node_Buffalo_21	Control - No Vaccine	Neg_Control_#2	27/01/2023	NaOH	0	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. The sample had slices in it from the initial inspection process.
Negative_Control_#3	Beef_Rump	N/A	Neg_Control_#3	03/02/2023	CPC 1%	0	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abornmalities were found on first inspection. 20ml of 1% CPC was used to decontaminate the homogenate. The pellet formed after centrifuging was softer than the buffalo samples.
Negative_Control_#3	Beef_Rump	N/A	Neg_Control_#3	03/02/2023	CPC 1%	0	0	?	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abornmalities were found on first inspection. 20ml of 1% CPC was used to decontaminate the homogenate. The pellet formed after centrifuging was softer than the buffalo samples.
Negative_Control_#3	Beef_Rump	N/A	Neg_Control_#3	03/02/2023	NaOH	C	C	C	C	C	C	C	C	C	C	No	Not_Applicable_Not_Done	No abornmalities were found on first inspection. 20ml of 1% CPC was used to decontaminate the homogenate. The pellet formed after centrifuging was softer than the buffalo samples. The medium turned a bright turqoise and with lots of liquid.
Negative_Control_#3	Beef_Rump	N/A	Neg_Control_#3	03/02/2023	NaOH	0	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abornmalities were found on first inspection. 20ml of 1% CPC was used to decontaminate the homogenate. The pellet formed after centrifuging was softer than the buffalo samples.
Negative_Control_#4	Beef_Rump	N/A	Neg_Control_#4	10/02/2023	CPC 1%	?	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. The CPC 1% decontamination method caused the pellet to become hard and rubber-like. 2ml of distilled water was added to help soften the pellet.
Negative_Control_#4	Beef_Rump	N/A	Neg_Control_#4	10/02/2023	CPC 1%	?	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. The CPC 1% decontamination method caused the pellet to become hard and rubber-like. 2ml of distilled water was added to help soften the pellet.
Negative_Control_#4	Beef_Rump	N/A	Neg_Control_#4	10/02/2023	NaOH	0	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. The CPC 1% decontamination method caused the pellet to become hard and rubber-like. 2ml of distilled water was added to help soften the pellet.
Negative_Control_#4	Beef_Rump	N/A	Neg_Control_#4	10/02/2023	NaOH	0	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. The CPC 1% decontamination method caused the pellet to become hard and rubber-like. 2ml of distilled water was added to help soften the pellet.
Negative_Control_#5	Beef_Rump	N/A	Neg_Control_#5	17/02/2023	CPC 1%	2*-Sunshine Yellow	1*?	1*?	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. The pellet formed from the 1% CPC decontamination was a lot softer than the buffalo samples and did not require time to soften with the 2ml of distilled water,
Negative_Control_#5	Beef_Rump	N/A	Neg_Control_#5	17/02/2023	CPC 1%	0	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. The pellet formed from the 1% CPC decontamination was a lot softer than the buffalo samples and did not require time to soften with the 2ml of distilled water,
Negative_Control_#5	Beef_Rump	N/A	Neg_Control_#5	17/02/2023	NaOH	0	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. The pellet formed from the 1% CPC decontamination was a lot softer than the buffalo samples and did not require time to soften with the 2ml of distilled water,
Negative_Control_#5	Beef_Rump	N/A	Neg_Control_#5	17/02/2023	NaOH	0	0	0	0	0	0	0	0	0	0	No	Not_Applicable_Not_Done	No abnormalities were found on first inspection. The pellet formed from the 1% CPC decontamination was a lot softer than the buffalo samples and did not require time to soften with the 2ml of distilled water,

Buffalo_Number	Sample_Type	Vaccine_Treatment	Sample_(Lab)_Number	Date_Processed	Treatment	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9	Week 10	Final_Growth	PCR_Final_Result	Comments
Positive_Control_#1	Lung_sample_from_a_Male_Buffalo	N/A	Pos_Control_#1	19/01/2023	HCl	0	0	0	0	1	2	2	2	2	2	No	Not_Applicable_Not_Done	Consolidated caseous lung sample. No lung structure. No calcification. The lesion was approximately 1x1x1cm.
Positive_Control_#1	Lung_sample_from_a_Male_Buffalo	N/A	Pos_Control_#1	19/01/2023	HCl	0	0	0	0	1	1	1	1	1	1	No	Not_Applicable_Not_Done	Consolidated caseous lung sample. No lung structure. No calcification. The lesion was approximately 1x1x1cm.
Positive_Control_#1	Lung_sample_from_a_Male_Buffalo	N/A	Pos_Control_#1	19/01/2023	NaOH	0	0	0	1	1	1	1	1	1	1	No	Not_Applicable_Not_Done	Consolidated caseous lung sample. No lung structure. No calcification. The lesion was approximately 1x1x1cm.
Positive_Control_#1	Lung_sample_from_a_Male_Buffalo	N/A	Pos_Control_#1	19/01/2023	NaOH	0	0	0	0	1	1	1	1	1	1	No	Not_Applicable_Not_Done	Consolidated caseous lung sample. No lung structure. No calcification. The lesion was approximately 1x1x1cm.
Positive_Control_#2	R_Cranial_Lobe_Mixed_Lesions	IV-Oral	Pos_Control_#2	27/01/2023	NaOH	0	?	4	4?	4?	4?	4?	4?	4	4	No	Not_Applicable_Not_Done	The sample was taken from Buffalo #5 from the vaccine trial. The sample contained many lesions of varying sizes. The whole sample was used to create the homogenate. Once the homogenizing was complete and the homogenate placed into the 50ml tubes. Some of the homogenate floated at the top and after centrifuging, there was still homogenate floating. There was enough of the homogenate that formed a pellet. This occured for both the NaOH and CPC decontamination methods.
Positive_Control_#2	R_Cranial_Lobe_Mixed_Lesions	IV-Oral	Pos_Control_#2	27/01/2023	NaOH	0	?	4	4?	4?	4?	4?	4?	4	4	No	Not_Applicable_Not_Done	The sample was taken from Buffalo #5 from the vaccine trial. The sample contained many lesions of varying sizes. The whole sample was used to create the homogenate. Once the homogenizing was complete and the homogenate placed into the 50ml tubes. Some of the homogenate floated at the top and after centrifuging, there was still homogenate floating. There was enough of the homogenate that formed a pellet. This occured for both the NaOH and CPC decontamination methods.
Positive_Control_#3	Right_Cranial_Lung_Lesion	BCG	Pos_Control_#3	03/02/2023	CPC 1%	0	?	1	1	1	1	1	1	1	1	No	Not_Applicable_Not_Done	Lesions 2, 3, and 4 of the right cranial lobe were used from number 23. There were many lesions of varying sizes with the largest being approximately 0.5cm x 0.5cm x 0.5cm. A hard rubber-like pellet formed after decontamination with 1% CPC.
Positive_Control_#3	Right_Cranial_Lung_Lesion	BCG	Pos_Control_#3	03/02/2023	CPC 1%	0	?	1	1	1	1	1	1	1	1	No	Not_Applicable_Not_Done	Lesions 2, 3, and 4 of the right cranial lobe were used from number 23. There were many lesions of varying sizes with the largest being approximately 0.5cm x 0.5cm x 0.5cm. A hard rubber-like pellet formed after decontamination with 1% CPC.
Positive_Control_#3	Right_Cranial_Lung_Lesion	BCG	Pos_Control_#3	03/02/2023	NaOH	Big Growth	1/c	1/c	(+)/c	(+)/c	(+)/c	(+)/c	(+)/c	(+)/c	(+)/c	No	Not_Applicable_Not_Done	Lesions 2, 3, and 4 of the right cranial lobe were used from number 23. There were many lesions of varying sizes with the largest being approximately 0.5cm x 0.5cm x 0.5cm. A hard rubber-like pellet formed after decontamination with 1% CPC. There was a large cream white fungal growth that grew during the first week of incubation.
Positive_Control_#3	Right_Cranial_Lung_Lesion	BCG	Pos_Control_#3	03/02/2023	NaOH	0	1	1	1	1	1	1	1	1/c	1/c	No	Not_Applicable_Not_Done	Lesions 2, 3, and 4 of the right cranial lobe were used from number 23. There were many lesions of varying sizes with the largest being approximately 0.5cm x 0.5cm x 0.5cm. A hard rubber-like pellet formed after decontamination with 1% CPC.

Positive_Control_M4	Left_Caudal_Lung	Control - No Vaccine	Pos_Control_M4	10/02/2023	CPC 1%	0	0	0	0	0	0	0	0	0?	0?	No	Not_Applicable_Not_Done	Mixed granular lesions from buffalo 26. The lesions were of varying small sizes. The largest being about 0.2cm x 0.2cm x 0.3 cm.
Positive_Control_M4	Left_Caudal_Lung	Control - No Vaccine	Pos_Control_M4	10/02/2023	CPC 1%	0	0	0	0	1	1	1	1	1	1	No	Not_Applicable_Not_Done	Mixed granular lesions from buffalo 26. The lesions were of varying small sizes. The largest being about 0.2cm x 0.2cm x 0.3 cm.
Positive_Control_M4	Left_Caudal_Lung	Control - No Vaccine	Pos_Control_M4	10/02/2023	NaOH	1	1	1	1	1	1	1	1	1	1	Yes	PCR_and_Culture_Positive	Mixed granular lesions from buffalo 26. The lesions were of varying small sizes. The largest being about 0.2cm x 0.2cm x 0.3 cm.
Positive_Control_M4	Left_Caudal_Lung	Control - No Vaccine	Pos_Control_M4	10/02/2023	NaOH	c/?	1	1	1	1	1	1	1	1	1	Yes	Not_Applicable_Not_Done	Mixed granular lesions from buffalo 26. The lesions were of varying small sizes. The largest being about 0.2cm x 0.2cm x 0.3 cm.
Positive_Control_M4	Left_Caudal_Lung	Control - No Vaccine	Pos_Control_M4	10/02/2023	NaOH	3-Opaque White; 1-Dull Yellow	1	1	1	1	1	1	1	1	1	Yes	Not_Applicable_Not_Done	Mixed granular lesions from buffalo 26. The lesions were of varying small sizes. The largest being about 0.2cm x 0.2cm x 0.3 cm.
Positive_Control_M5	Left_Caudal_Lung	Control - No Vaccine	Pos_Control_M5	17/02/2023	CPC 1%	0	0	0	0	0	0	0	0	1	1	Yes	Not_Applicable_Not_Done	Mixed lung lesions from buffalo 26. The lesions were of varying sizes. The largest lesion was approximately 0.7cm x 0.7cm x 0.7cm. The larger lesions were all encased. Some of the homogenated material floated to the top in both decontamination methods
Positive_Control_M5	Left_Caudal_Lung	Control - No Vaccine	Pos_Control_M5	17/02/2023	CPC 1%	0	0	0	0	0	0	0	0	1	1	Yes	Not_Applicable_Not_Done	Mixed lung lesions from buffalo 26. The lesions were of varying sizes. The largest lesion was approximately 0.7cm x 0.7cm x 0.7cm. The larger lesions were all encased. Some of the homogenated material floated to the top in both decontamination methods
Positive_Control_M5	Left_Caudal_Lung	Control - No Vaccine	Pos_Control_M5	17/02/2023	NaOH	?	0	0	0	0	0	0	0	1	1	Yes	Not_Applicable_Not_Done	Mixed lung lesions from buffalo 26. The lesions were of varying sizes. The largest lesion was approximately 0.7cm x 0.7cm x 0.7cm. The larger lesions were all encased. Some of the homogenated material floated to the top in both decontamination methods
Positive_Control_M5	Left_Caudal_Lung	Control - No Vaccine	Pos_Control_M5	17/02/2024	NaOH	?	0	0	0	0	0	0	0	1	1	Yes	Not_Applicable_Not_Done	Mixed lung lesions from buffalo 26. The lesions were of varying sizes. The largest lesion was approximately 0.7cm x 0.7cm x 0.7cm. The larger lesions were all encased. Some of the homogenated material floated to the top in both decontamination methods
Positive_Control_M5	Left_Caudal_Lung	Control - No Vaccine	Pos_Control_M5	17/02/2023	NaOH	?	0	0	0	0	0	0	0	1	1	Yes	Not_Applicable_Not_Done	Mixed lung lesions from buffalo 26. The lesions were of varying sizes. The largest lesion was approximately 0.7cm x 0.7cm x 0.7cm. The larger lesions were all encased. Some of the homogenated material floated to the top in both decontamination methods

Appendix C: Animal Ethics Committee Ethical Approval REC163-22

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Faculty of Veterinary Science
Animal Ethics Committee

4 April 2023

Approval Certificate
New Application

AEC Reference No.:	REC163-22
Title:	Investigation of the Meat Safety of BCG-Vaccinated African Buffalos (Synerus caffer) Experimentally Infected with Mycobacterium bovis that are typically used in human consumption
Researcher:	Miss ME Antrobus
Student's Supervisor:	Prof AL Michel

Dear Miss ME Antrobus,

The **New Application** as supported by documents received between 2022-11-17 and 2023-03-27 for your research, was approved by the Animal Ethics Committee on its quorate meeting of 2023-03-27.

Please note the following about your ethics approval:

1. The use of species is approved:

Samples	Number
African Buffalo - Muscle Tissue (Brisket) Stored- Historic/Retrospective	21
African Buffalo - Muscle Tissue (Fillet) Stored- Historic/Retrospective	21
African Buffalo - Muscle Tissue (Rump) Stored- Historic/Retrospective	21
African Buffalo - Muscle Tissue (Silverside) Stored- Historic/Retrospective	21

2. Ethics Approval is valid for 1 year and needs to be renewed annually by 2024-04-04.
3. Please remember to use your protocol number (REC163-22) on any documents or correspondence with the AEC regarding your research.
4. Please note that the AEC may ask further questions, seek additional information, require further modification, monitor the conduct of your research, or suspend or withdraw ethics approval.
5. **All incidents** must be reported by the PI by email to Ms Marleze Rheeder (AEC Coordinator) within 3 days, and must be subsequently submitted electronically on the application system within 14 days.
6. The committee also requests that you record major procedures undertaken during your study for own-archiving, using any available digital recording system that captures in adequate quality, as it may be required if the committee needs to evaluate a complaint. However, if the committee has monitored the procedure previously or if it is generally can be considered routine, such recording will not be required.

Ethics approval is subject to the following:

- The ethics approval is conditional on the research being conducted as stipulated by the details of all documents submitted to the Committee. In the event that a further need arises to change who the investigators are, the methods or any other aspect, such changes must be submitted as an Amendment for approval by the Committee.

Room 6-13, Arnold Theiler Building, Onderstepoort
Private Bag X04, Onderstepoort 0110, South Africa
Tel +27 12 529 8434
Fax +27 12 529 8321
Email: marleze.rheeder@up.ac.za

Fakulteit Veeartsenykunde
Lefapha la Diseanse tša Bongakadiruiwa

We wish you the best with your research.

Yours sincerely

Prof V Naidoo
CHAIRMAN: UP-Animal Ethics Committee

Room 6-13, Arnold Theiler Building, Onderstepoort
Private Bag X04, Onderstepoort 0110, South Africa
Tel +27 12 529 8434
Fax +27 12 529 8321
Email: marleze.rheeder@up.ac.za

Fakulteit Veeartsenykunde
Lefapha la Diseanse tša Bongakadiruiwa

Appendix D: AEC Approval for the Vaccination Trial (REC 148-19)

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Faculty of Veterinary Science
Animal Ethics Committee

7 September 2020

Approval Certificate
New Application

AEC Reference No.:	REC148-19
Title:	Vaccination of African buffalo against Bovine Tuberculosis
Researcher:	Dr JM Hewlett
Student's Supervisor:	Prof AL Michel

Dear Dr JM Hewlett,

The **New Application** as supported by documents received between 2020-01-29 and 2020-09-04 for your research, was approved by the Animal Ethics Committee on its quorate meeting of 2020-09-04.

Please note the following about your ethics approval:

1. The use of species is approved:

Species and Samples	**Number**
Buffalo (African buffalo)	24
Samples Blood EDTA samples	288 (9 ml each)
Blood Serum samples	288 (9 ml each)
Blood Heparin samples	288 (9 ml each)

2. Ethics Approval is valid for 1 year and needs to be renewed annually by 2021-09-07.
3. Please remember to use your protocol number (REC148-19) on any documents or correspondence with the AEC regarding your research.
4. Please note that the AEC may ask further questions, seek additional information, require further modification, monitor the conduct of your research, or suspend or withdraw ethics approval.
5. **All incidents** must be reported by the PI by email to Ms Marleze Rheeder (AEC Coordinator) within 3 days, and must be subsequently submitted electronically on the application system within 14 days.
6. As part of your approval, the committee requires that you record a **short video footage** of major animal procedures approved in your study. The committee may request them for monitoring purposes at any later point.

Ethics approval is subject to the following:

- The ethics approval is conditional on the research being conducted as stipulated by the details of all documents submitted to the Committee. In the event that a further need arises to change who the investigators are, the methods or any other aspect, such changes must be submitted as an Amendment for approval by the Committee.

Room 6-13, Arnold Theiler Building, Onderstepoort
Private Bag X04, Onderstepoort 0110, South Africa
Tel +27 12 529 8434
Fax +27 12 529 8021
Email: marleze.rheeder@up.ac.za

Fakulteit Veeartsenykunde
Lefapha la Diseanse tša Bongakadiruiwa

We wish you the best with your research.
Yours sincerely

Prof V Naidoo
CHAIRMAN: UP-Animal Ethics Committee

Faculty of Veterinary Science
Fakulteit Veeartsenykunde
Lefapha la Diseanse tša Bongakadiruiwa

Appendix E: Section 20 for the Vaccination Trial

agriculture,
forestry & fisheries

Department:
Agriculture, Forestry and Fisheries
REPUBLIC OF SOUTH AFRICA

Directorate Animal Health, Department of Agriculture, Forestry and Fisheries
Private Bag X138, Pretoria 0001

Enquiries: Mr Herry Gololo • Tel: +27 12 319 7532 • Fax: +27 12 319 7470 • E-mail: HerryG@daff.gov.za
Reference: 12/11/1/1/6

Dr Jennie Hewlett
Faculty of Veterinary Science
Onderstepoort
0110
Email: Jennie.hewlett@up.ac.za; Anita.michel@up.ac.za; LinmarieDK@daff.gov.za

Dear Dr Hewlett,

RE: PERMISSION TO DO RESEARCH IN TERMS OF SECTION 20 OF THE ANIMAL DISEASES ACT, 1984 (ACT NO. 35 OF 1984)

Your application sent per email, requesting permission under Section 20 of the Animal Disease Act, 1984 (Act No. 35 of 1984) to perform a research project or study, refers. I am pleased to inform you that permission is hereby granted to perform the following study, with the following conditions:

Conditions:

1. This permission does not relieve the researcher of any responsibility which may be placed on him by any other act of the Republic of South Africa;
2. The study is approved as per the application form dated 25 October 2018 and the correspondence thereafter. Written permission from the Director: Animal Health must be obtained prior to any deviation from the conditions approved for this study under this Section 20 permit. Please apply in writing to HerryG@daff.gov.za;
3. All potentially infectious material utilised, collected or generated during the study are to be destroyed at the completion of the study. Records must be kept for five years for auditing purposes;
4. Buffalo can only be obtained and moved to Skukuza subject to permission and movement permits issued by the responsible state veterinarian of the area;
5. Buffalo can only be kept at the animal holding facilities within the quarantine area of the Skukuza State Veterinary Services;

6. Testing of serum and whole blood, as well as bacterial culture of tissue lesions may only be performed at the Skukuza State Veterinary Services BSL2+ laboratory;
7. Tissue samples in formalin may be transported to the Department of Pathology at the Onderstepoort Campus of the University of Pretoria subject to the necessary State veterinary movement permits and the conditions contained therein;
8. The buffalo carcasses must be disposed of at Skukuza abattoir rendering plant;
9. The *Mycobacterium bovis* vaccine must be imported in compliance with a veterinary import permit issued in terms of the Animal Diseases Act, 1984 (Act 35 of 1984) prior to the start of the study. Records must be kept for five years for auditing purposes;
10. The vaccine must be imported and the study performed in compliance with the Medicines and Related Substances Control Act, 1965 (Act 101 of 1965) and the Fertilizers, Farm Feeds, Agricultural Remedies and Stock Remedies Act, 1947 (Act 36 of 1947);
11. The study must be conducted in compliance with the Veterinary and Para-Veterinary Professions Act 1982 (Act 19 of 1982);
12. The study may not commence until a valid ethics approval has been obtained, in writing, from the relevant ethics approval body;
13. If required, an application for an extension must be made by the responsible researcher at least one month prior to the expiry of this Section 20 approval.

Title of research/study: Vaccination of African buffalo against Bovine Tuberculosis

Researcher: Dr Jennie Hewlett

Institution: Faculty of Veterinary Science, University of Pretoria

Our ref Number: 12/11/1/1/6

Your ref: none provided

Expiry date: 2021-12

Kind regards,

DR. MPHO MAJA
DIRECTOR OF ANIMAL HEALTH
Date: 2019-10-22

SUBJECT: S20 PERMISSION FOR: VACCINATION OF AFRICAN BUFFALO AGAINST BOVINE TUBERCULOSIS - LJVR

Appendix F: Section 20 for the current Study

agriculture, land reform & rural development
Department:
Agriculture, Land Reform and Rural Development
REPUBLIC OF SOUTH AFRICA

2030 NDP

Directorate Animal Health, Department of Agriculture, Land Reform and Rural Development Private Bag X138, Pretoria 0001
Enquiries: Ms Marna Laing • Tel: +27 12 319 7532 • Fax: +27 12 319 7470 • E-mail: MarnaL@dalrrd.gov.za Website: www.dalrrd.gov.za
Reference: 12/11/1/1/8 (2868PM)

Ms Megan Elizabeth Antrobus
University of Pretoria, Faculty of Veterinary Sciences
Old Soutpan Road
Onderstepoort 0110
E-mail: antrobus.meg@gmail.com

Dear Ms Anthrobus

RE: PERMISSION TO DO RESEARCH IN TERMS OF SECTION 20 OF THE ANIMAL DISEASES ACT, 1984 (ACT NO 35 OF 1984)

Your application dated 19 January 2023 requesting permission under Section 20 of the Animal Disease Act, 1984 (Act No. 35 of 1984) to perform a research project or study, refers. I am pleased to inform you that permission is hereby granted to perform the following study, with the following conditions:

Conditions:

1. This permission does not relieve the researcher of any responsibility which may be placed on him by any other act of the Republic of South Africa.
2. The research project is approved as per the application form dated 19 January 2023 and the correspondence thereafter. Written permission from the Director: Animal Health must be obtained prior to any deviation from the conditions approved for this research project under this Section 20 permit. Please apply in writing to MarnaL@dalrrd.gov.za.
3. All potentially infectious material utilised or collected during the study is to be destroyed at the completion of the study using the specified waste contractor

Compass Medical Waste. Records must be kept for five years for audit purposes. A dispensation application may be made to the Director Animal Health in the event that any of the above is to be stored or distributed;

4. Permission in terms of the Fertilizers, Farm Feeds, Agricultural Remedies and Stock Remedies Act, 1947 (Act No 36 of 1947) and/or the Medicines and Related Substances Control Act, 1965 (Act No 101 of 1965) may be needed prior to the start of the study;
5. Ethics approval must be obtained prior to the start of the study
6. Appropriate personal protective equipment and precautions must be taken by all persons when handling meat samples from buffalo TB vaccine trial.
7. Only buffalo meat samples bio-banked at the Hans Hoheisen Wildlife Research Station laboratory may be used in this study. No samples may be obtained from another biobank or another species without written permission from the Director: Animal Health;
8. It is the responsibility of the researcher and laboratory/facility manager to ensure that the human health and safety aspects of this study are adequately addressed;
9. If required, an application for an extension must be made by the responsible researcher at least one month prior to the expiry of this Section 20 permit. Please apply in writing to MarnaL@dalrrd.gov.za;

Title of research/study: Investigation of the Meat Safety of BCG-Vaccinated African Buffalos (*Synerus caffer*) Experimentally Infected with *Mycobacterium bovis* that are typically used in human consumption.
Researchers: Ms Megan Elizabeth Antrobus
Institution:

- University of Pretoria, Faculty of Veterinary Sciences
- Old Soutpan Road, Onderstepoort 0110
- Hans Hoheisen Wildlife Research Station (HHWRS)

Permit Expiry Date: 31 December 2023
Our ref Number: 12/11/1/1/8 (2868PM)
Your ref: REC 163-22

Kind regards,

DR. MPHO MAJA
DIRECTOR: ANIMAL HEALTH
Date: 2023-03-03