PREVALENCE OF BOVINE CYSTICERCOSIS IN SLAUGHTERED CATTLE AND KNOWLEDGE, ATTITUDES AND PRACTICES OF CATTLE FARMERS IN THE MATABELELAND REGION OF ZIMBABWE

BY

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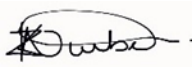
SIGNATURE OF STUDENT: 

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Abstract

Bovine cysticercosis is a zoonotic foodborne disease of socio-economic importance. The prevalence of bovine cysticercosis in Zimbabwe is underestimated, as it is currently only detected by the less sensitive meat inspection method. The objectives of the study were to determine the prevalence of bovine cysticercosis in slaughtered cattle using traditional meat inspection and B158/B60 monoclonal antibody-based ELISA for the detection of circulating antigens of *Taenia saginata*, confirming *T. saginata* cysticerci collected during meat inspection using PCR and to establish communal cattle farmers' knowledge, attitudes and practices on the risk factors of bovine cysticercosis in the Matabeleland Region of Zimbabwe. Serum was harvested from blood samples collected from 381 slaughtered cattle at three abattoirs in Matabeleland Region and subjected to Ag-ELISA for the detection of *Taenia saginata* antigens. *Taenia saginata* was characterized by cloning and sequencing of the 12s rDNA amplicons extracted from three cysticerci recovered on meat inspection. To determine farmers' knowledge, attitudes and practices on the risk factors of bovine cysticercosis, a total of 377 participants were interviewed in the Bulilima district of Matabeleland South Province. Results showed that the prevalence of cysticercosis detected by Ag-ELISA (140/369; 37.94%) was significantly higher ($p < 0.05$) than that detected by meat inspection (7/381; 1. 83%). Matabeleland South (92.13%) had the highest estimate of true prevalence amongst all the provinces in Matabeleland region. Seven out of 381(1.83%) cattle were positive for bovine cysticercosis on meat inspection, with the majority of the cysticerci identified being viable and mostly located on the masseter. All the metacestodes identified during meat inspection were confirmed as those of *T. saginata* by PCR with homology ranging between 99.75% and 99.87% *T. saginata* 12s rDNA sequence in the GenBank obtained from bovine cysticercosis cysts recovered from a slaughtered bovine in South Africa (MZ357054). Eighty-four percent (316/377) of the participants practised home slaughter and (263/377; 69.8%) performed meat inspection on their own which is deemed inadequate. About (366/377; 97%) of the participants lacked knowledge on bovine cysticercosis. Two-fifths of the participants had no access to toilets at home and (358/377; 95%) of the participants sometimes practised open defecation. Home slaughter, inadequate meat inspection, consumption of infected meat, lack of toilets and open defecation were identified risk factors for bovine cysticercosis in Matabeleland. Further studies are required to determine the prevalence of taeniasis cases in cattle farming communities at risk residing in Matabeleland region of Zimbabwe.

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Dedication

To family, the greatest backbone to success

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Chapter 1: Introduction

1.1 Background

Bovine cysticercosis is a zoonotic foodborne disease of socio-economic importance (Braae et al., 2018) caused by the larval stage of the human tapeworm causing taeniasis in humans. Taeniasis is classified by World Health Organisation (WHO) as a neglected tropical disease (Tegegne et al., 2018). Cattle become infected by ingesting feed and water contaminated with *T. saginata* eggs originating from human faecal matter (McBrien and Courcier, 2013). When cattle become infected, the eggs (oncosphere) develop into *T. saginata* cysticerci in striated muscles and become infective to humans approximately after ten weeks when eaten in raw/undercooked beef (McFadden et al., 2011a). Humans are the definitive hosts, harbouring the intestinal adult worm. They become infected when they consume raw or undercooked beef infected with *T. saginata* cysticerci (Qekwana et al., 2016). The hermaphrodite adult tapeworm in human intestines develops and matures to produces eggs that are excreted in faeces (free or within intact proglottids) (Saratsis et al., 2019). An average of 150,000 eggs per day can be shed by an infected person (Gholami et al., 2020) and they can survive in an appropriate environment for almost seven months (Abuseir et al., 2006).

Cysticerci can remain viable and infective to humans for at least two to three years before they start to degenerate (Scandrett et al., 2012). Bovine cysticercosis is usually an asymptomatic condition; hence, diagnosis is established post -mortem or during meat inspection (Gholami et al., 2020). Meat inspection is currently the only practical method used to detect bovine cysticercosis (Wanzala et al., 2002). However, it has a low sensitivity, especially in mildly infected carcasses (Da Silva et al., 2015). In a study carried out in Belgium, meat inspection was found to have a sensitivity of only 0.54% (viable and degenerate cysticerci) (Jansen et al., 2018).

Predilection sites for establishment of cysticerci are masseter muscles, the pericardial surface of the heart, triceps muscles of both sides of the shoulders, diaphragm, oesophagus, and tongue (Sungirai et al., 2014). In Zimbabwe, if twenty-one or more live or dead cysticerci are identified during meat inspection, the carcass and offals are condemned, if less than or equal to twenty cysticerci are identified, the carcass is frozen at -12^{o}C for not less than 12 days (Statutory instrument 50 of 1995). Condemnation of the head is executed if five or more cysticerci are

found in its musculature whilst the heart, the tongue and other organs are condemned if five or more cysticerci are identified in the organs. The carcass is condemned if eleven or more cysticerci are found in its musculature (Statutory instrument 50 of 1995).

The presence of viable cysticerci in the carcass can be detected in live cattle using a monoclonal antibody-based enzyme-linked immunosorbent assay (ELISA) (B158/B60 Ag-ELISA). Monoclonal antibodies were developed against excretory-secretory antigens of the metacestode larval stage of *T. saginata*, aiming at detecting viable cysticerci (Jansen et al., 2016). According to a study done in Belgium; B158/B60 Ag-ELISA has a sensitivity of 40% and a specificity of 100% (Jansen et al., 2017). A polymerase chain reaction (PCR) is used to detect the deoxyribonucleic acid (DNA) of suspected cysticerci sampled during meat inspection (Cuttell et al., 2013). PCR can be done by amplifying the 12s ribosomal DNA (rDNA) gene of the *T. saginata* cysticercus (Geysen et al., 2007).

A systematic review found that the reported prevalence of taeniasis in humans in Southern and Eastern Africa ranged between 0.02 and 8.1 % (microscopy) and 0.12-19.7% (coproAg- ELISA), whilst the prevalence of bovine cysticercosis in that region ranged between 0.02-26.3% (meat inspection) and 6.1-34.9% (Ag-ELISA) (Dermauw et al., 2018). Bovine cysticercosis causes economic losses as a result of condemnation of heavily infected carcasses, freezing costs of lowly infected meat and taeniasis treatment in humans (Tegegne et al., 2018).

As of 2019, Zimbabwe had a cattle population of 5443770 with Matabeleland North having 670,363 and Matabeleland South having 612,924 heads (MoLAWCRR, 2020). Eighty-nine percent of the cattle population is found in communal areas (Tavirimirwa et al., 2013). Analysis of meat inspection results (January 2006 to December 2007) of cattle slaughtered at a Cold Storage Company (Bulawayo, Zimbabwe) revealed an average prevalence of 1.6% (n=1364) of bovine cysticercosis; with Matabeleland North (Mat-North) having a prevalence of 2.8% (n=629) and Matabeleland South (Mat-South) a prevalence of 1.2% (n=735) (Sungirai et al., 2014).

1.2 Problem statement

Meat inspection is currently the only method used for the identification of bovine cysticercosis in Zimbabwe and it identifies mainly heavily infested carcasses (Wanzala et al., 2002). Due to the inadequate detection of lightly infected carcasses (Da Silva et al., 2015), the currently recorded low bovine cysticercosis prevalence in Matabeleland region, Zimbabwe might be underestimated. It is suggested by the author that the prevalence stated by Sungirai et al. (2014), obtained through meat inspection might be lower than expected (Matabeleland North 2.8% (n=629), Matabeleland South 1.2% (n=735) and Bulawayo 1.6 %(n=1364)). To the best knowledge of the author, serological detection of bovine cysticercosis using monoclonal antibody-based detecting ELISA (B158/B60 Ag-ELISA), has not been done in Zimbabwe. Moreover, molecular (PCR) confirmation of the cysticerci detected at meat inspection has not been done in Zimbabwe. Risk factors of bovine cysticercosis are unknown in Zimbabwe. The study was investigating risk factors of bovine cysticercosis in Matabeleland region, as studies from other countries have realised that transmission of bovine cysticercosis is mostly facilitated by poor personal hygiene, open defecation by infected humans, inappropriate cattle management practices and consumption of raw or undercooked beef.

1.3 Justification

Most Zimbabweans live in rural areas and rely mostly on agriculture for their livelihoods. Seventeen percent of the gross domestic product (GDP) is from the agricultural sector, with 60-70% of the population dependent on agriculture as the main source of income (FAO, 2022.). Twenty-five percent of the GDP is from the livestock sector (Sungirai et al.,2014), with cattle contributing 35-38% of the GDP linked with the agricultural sector (FAO, 2022.). As of 2019, 235,018 cattle were slaughtered in official abattoirs accounting for 4.31% of the total national herd (MoLAWCRR, 2020). Bovine cysticercosis is a disease of economic importance, with estimated losses in Africa of approximately $1.8 billion, as reviewed by Abusier et al. (2006). Economic losses are due to condemnation and treatment of infected carcasses (Tegegne et al.,2018). A monoclonal antibody-based detecting ELISA (B158/B60 Ag-ELISA) was used to compliment the current meat inspection method to determine the prevalence of bovine cysticercosis to best advise the relevant stakeholders on the country's situation regarding *T. saginata* cysticercosis. Bovine cysticercosis can be misdiagnosed during meat inspection with

other diseases such as sarcocystosis and actinobacillosis (Hassan El-Sayad et al., 2021), hence the need to confirm the cysticerci using Polymerase Chain Reaction (PCR). This study will help providing reliable prevalence rate of *T.saginata* cysticercosis in live animals and be able to take necessary prevention measures to reduce economic losses due to the disease. A study carried out by Sungirai et al. (2014) on the meat-inspection-based prevalence of bovine cysticercosis in Matabeleland province does state that there is a need for further studies aimed at reducing zoonotic consequences as a result of unobserved cases in the rural areas. Studies previously done in Matabeleland region of Zimbabwe determined the prevalence of bovine cysticercosis based on meat inspection (Pugh and Chambers., 1989; Sungirai et al., 2014) whilst no studies have been done to obtain information on farmers' knowledge, attitudes and practices on the risk factors of bovine cysticercosis in communal areas of Matabeleland.

1.4 Aim

To determine the prevalence of bovine cysticercosis and its associated risk factors for in Matabeleland province, Zimbabwe.

1.5 Objectives and hypothesis

- To determine the prevalence of bovine cysticercosis in cattle from communal areas of Matabeleland, using monoclonal antibody-based detecting ELISA (B158/B60 Ag-ELISA) and meat inspection.
- H_1: Monoclonal antibody-based antigen-ELISA has a higher sensitivity to detect bovine cysticercosis prevalence than meat inspection.
- To perform molecular confirmation on cysticerci identified during meat inspection
- H_1: PCR method is ideal for confirmation of suspected *T. saginata* cysticerci collected during meat inspection.
- To establish the cattle farmers' knowledge, attitudes and practices on the risk factors of bovine cysticercosis in communal areas of Matabeleland.
- H_1: Cattle farmers in Matabeleland region are aware of the risk factors of bovine cysticercosis.

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Chapter 2: Literature review

2.1 Introduction

Bovine cysticercosis (BCC) is a foodborne disease of cattle caused by the metacestode larval stage of the zoonotic *Taenia saginata* (*T. saginata*) (Dupuy et al., 2014). The current prevalence and associated risk factors of bovine cysticercosis in cattle in the communal areas of Matabeleland region are poorly understood despite the availability of more sensitive serological and molecular diagnostic tests, and thus warrant investigation. The current chapter details the *T. saginata* life cycle, risk factors and symptoms in taeniasis and bovine cysticercosis cases, economic importance of bovine cysticercosis, different diagnostic methods of bovine cysticercosis, different livestock farming systems practised in Zimbabwe, breeds and socio-economic importance of cattle in Zimbabwe and bovine cysticercosis in Zimbabwe.

2.2 Life cycle

Taenia saginata is a two-host parasite, with humans being the definitive host and cattle being the intermediate host (McFadden et al., 2011a). Humans get infected when they consume raw or undercooked beef (Fig 1.1) containing viable cysticerci (Kiermeier et al., 2019). Cattle get infected once they consume feed or drinking water containing *T. saginata* gravid proglottids or eggs (Laranjo-González et al., 2016; Fig 1.1).

After ingestion of mature cysts and digestion in humans, the cysticercus evaginates in the small intestines, and the scolex attaches to the mucosa of the intestines (Tagesu, 2017) and develops into an adult tapeworm. The adult tapeworm becomes sexually mature approximately three months after ingestion of the infected beef (McFadden et al., 2011a). The tapeworm can grow to ten meters in the small intestines (King and Fairley, 2014) and can be made up of approximately 1000 to 2000 proglottids (Wittner et al., 2011). Approximately three to seven proglottids containing between 30 000 to 50 000 eggs each are shed per day and are excreted with faecal matter or proglottids independently crawling outside the anus (Hassan El-Sayad et al., 2021).

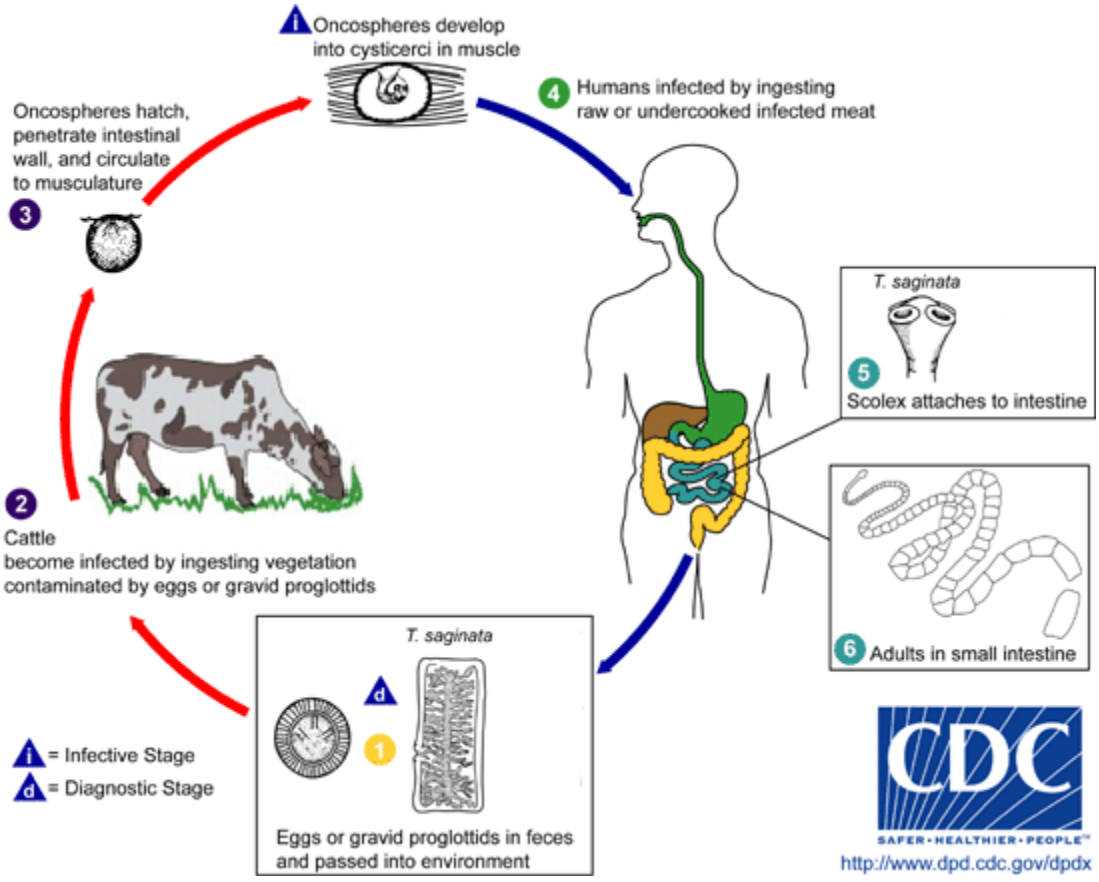

Figure 1.1: *Taenia saginata* life cycle: diagramme adapted from CDC cited by Tagesu. (2017)

Eggs excreted from human faecal matter can survive for several months in the environment (Bucur et al., 2019). In cattle, ingested eggs hatch into oncospheres in the gastrointestinal system and they penetrate the intestinal mucosa to reach the circulation system (Bruschi and Gómez-Morales, 2017). The hatching of eggs into oncospheres is facilitated by gastrointestinal juices (Murrell et al., 2005). Once in the circulation system, the oncospheres migrate to the skeletal and cardiac muscles (Alemneh et al., 2017). Despite the cysts being found anywhere in the striated muscles, the predilection sites are the tongue, heart, intercostal muscles and the masseter (Alemneh et al., 2017). Oncospheres rarely migrate to fat and visceral organs (Murrell et al., 2005).

In the muscles, the larval stage of *T. saginata* forms a fluid-filled cyst which is surrounded by a fibrous capsule (Tagesu, 2017). The cysticerci become infective for humans approximately after

ten weeks (McFadden et al., 2011b). After a few months, the cysticerci begin to degenerate and by the time they reach nine months, most of them will be dead (Murrell et al., 2005).

2.3 Risk factors

Consumption of raw or undercooked beef contributes massively to the presence of taeniasis cases (Jansen et al., 2018a). In some countries raw beef is eaten as a local tradition, for example in Ethiopia they consume raw beef called *kurt* in their local language (Amistu and Asrat, 2017) and in Bali where they eat raw beef in the form of *lawar* (Dharmawan et al., 2020). Home slaughter in the absence of inspection, inadequate meat inspection and poor meat inspection management control policies can contribute to a high number of taeniasis cases (Alemneh et al., 2017). A study carried out in a Kashmiri population in India, found that *T. saginata* infection and prevalence were due to food eating habits, age, sex and living conditions. Prevalence was 27.4% and more cases were detected in males than in females, especially in those above 60 years (Lateef et al., 2020).

Poor hygiene and inappropriate livestock husbandry practices are some of the risk factors for bovine cysticercosis (Alemneh et al., 2017). Access of cattle to water and pasture contaminated with human faecal matter, infected humans defecating near grazing areas and persistence of *T. saginata* eggs in treated water and irrigation of pastures with contaminated water can result in a high number of bovine cysticercosis cases12 (Laranjo-González et al., 2016). Due to the more intense contact between cattle and humans, the prevalence of bovine cysticercosis may be high in rural areas (Sadra Dharmawan et al., 2020).

2.4 Clinical symptoms

Natural infections of bovine cysticercosis are usually asymptomatic and are identified during routine meat inspection (Laranjo-González et al., 2016). Taeniasis can cause mild symptoms in humans such as loss of appetite, nausea and diarrhoea (Wittner et al., 2011). Movement of proglottids through the anus can result in irritation and their site on the stool or migrating to the patient’s clothes or body can result in anxiety and psychological distress in most patients (King and Fairley, 2014).

Complications can include vomiting with presence of proglottids in the vomit at times (O'Dempsey, 2010) and abdominal pain (Diemert, 2017). Eosinophilia may be present in mild form and immunoglobulin E levels are usually increased (Wittner et al., 2011). Taeniasis can rarely result in life-threatening situations due to intestinal, pancreatic or biliary obstruction (Wittner et al., 2011) (O'Dempsey, 2010).

2.5 Treatment

Taeniasis is treated using oral deworming medication such as praziquantel, niclosamide (King and Fairley, 2014; Bennet *et al.*, 2019; Bennett and Dolin, 2020) and albendazole (Okello and Thomas, 2017). There is currently no treatment against bovine cysticercosis in live cattle (*Canadian Food* Inspection *Agency*, 2022)

2.6 Global prevalence

Taenia saginata occurs worldwide, both in developing countries such as Zimbabwe, Kenya and South Africa but also in more industrialized countries such as Australia and New Zealand and Europe (Okello and Thomas, 2017).

Bovine cysticercosis cases can be classified into three separate groups depending on the level of infection, with low infection rates being less than 0.1%, highly endemic areas having a prevalence above 10% and medium infection rate being in between the low and high infection rates (Alemneh et al., 2017). In about 95% of European countries, bovine cysticercosis prevalence is below 6.2% when using meat inspection and it is between 0.41% and 14% when using serology and detailed meat inspection (Laranjo-González et al., 2016). Bovine cysticercosis in Egypt has been recorded in buffaloes and prevalence was estimated at 9% in buffaloes and 6, 09% in cattle (Hassan El-Sayad et al., 2021).

Using different diagnostic methods, the prevalence of taeniasis was found highly variable in endemic areas ranging between 0% to 13, 9% in Africa, 0, 24% to 17, 25% in Latin America and 0% to 3.02% in Asia.

Central and Eastern African countries (Kenya, Ethiopia and Zaire) and Mediterranean countries (Yugoslavia, Lebanon, and Syria) are said to be highly endemic to taeniasis. Information collected from 1990 up to 2017 in Southern and Northern Africa, showed that the prevalence

ranged between 0.2% -8.1% based on microscopy and 0.12%-19,7% based on coproAg-ELISA. In Central and Western Africa, unspecified human taeniasis cases were recorded from different countries, whilst *T. saginata*-specific data was reported from Cameroon (Hendrickx et al., 2019). Prevalence estimates in Nigeria ranged between 0-11%, with 0% recorded in suburban school children and 11% in community residents (Hendrickx et al., 2019).

Prevalence of taeniasis in Southern and Eastern Africa is between 0.02-8.1% (microscopy) and 0.12-19.7% (coproAg-ELISA), whilst prevalence of bovine cysticercosis in that region ranges between 0.02 - 26.3% (meat inspection) and 6.1-34.9% (Ag-ELISA) (Dermauw et al., 2018). More cases of bovine cysticercosis in South Africa are recorded from feedlots because of possible contaminated water used for irrigation and drinking water (Verwoerd, 2017).

2.7 Economic importance of bovine cysticercosis

Taenia saginata cysticercosis causes high economic losses due to carcass quality loss, freezing costs of infected meat, condemnation of heavily infected carcasses and taeniasis treatment in humans (Tegegne et al., 2018). Economic losses in Brazil due to bovine cysticercosis have been estimated to range between US$ 167 868.53 and US$ 11 313 816.67 (Comin et al., 2021).

In a study done in Belgium from 2012 to 2016 it was calculated that economic losses associated with bovine cysticercosis were €3 408 455/ year (€ 2954061/ year due to BCC insurance, €453 024/ year due to value losses of beef from uninsured carcasses and € 1370/ year being due to destruction costs of uninsured carcasses (Jansen et al., 2018c)). A study carried out in Catalonia north-eastern Spain from 2008-2015 estimated the annual mean economic importance of *T. saginata* to be €154 903/ year (Laranjo-González et al., 2018).

It is estimated that Africa experiences about $1.8 billion losses to BCC, as reviewed by Abusier et al. (2006). Economic losses associated with BCC and *T. saginata* in Ethiopia were estimated at US$ 212 202.76 (Hiko and Seifu, 2019). An average of US$ 2407.2 was spent in Wolaita Soddo town in Ethiopia on taeniasis treatment (Tesfaye et al., 2012).

2.8 Diagnosis

Meat inspection (Dupuy *et al.*, 2014;Nzeyimana *et al.*, 2015;Jansen *et al.*, 2017) and serological tests (Allepuz *et al.*, 2012;Jansen *et al.*, 2018), are the two major methods used in determining the prevalence.

Meat inspection is of public health importance, as it helps detect infected carcasses and viscera, and its application is often regulated by law (Henckel et al., 2020). Meat inspection entails visual inspection, palpation and incision on different predilection sites (Henckel et al., 2020). Bovine cysticercosis is mostly observed from various predilection sites, which are palpated and incised during meat inspection, namely the masseter muscle, pterygoid muscle, oesophagus, heart, tongue and diaphragm (El-Sayad et al., 2021).

Viable *T. saginata* cysticerci are identified as fluid-filled cysts with a scolex often visible in viable cysticerci (Ogunremi et al., 2004). Degenerated or calcified cysts are identified as firm nodular lesions having yellowish material and at times a calcareous aspect (Costa et al., 2012). Viable cysticerci are often more difficult to detect as compared to degenerated or calcified cysts. The sensitivity of meat inspection is estimated at <16 % (Jansen et al., 2018b) and the specificity is also suboptimal, as metacestodes can be confused with possible actinobacillosis and *Sarcocystis* lesions or other local alterations (El-Sayad et al., 2021).

It is difficult to differentiate adult *T. saginata* from *T. solium* using morphological features because they have indistinguishable eggs (Mayta *et al.*, 2000). Different molecular tests such as PCR have been successfully developed to differentiate *Taenia spp*. PCR-restriction fragment length polymorphism (PCR-RFLP) has been used by amplifying the 3’ region of the 18S and the 5’ region of the 28S ribosomal gene and using three restriction enzymes (AluI, DdeI or MboI) for analysis of the PCR amplicons (Mayta *et al.*, 2000), whilst Rodriguez-Hialgo et al differentiated *Taenia spp*, by using PCR-RFLP using 12S rDNA (Mwape and Gabriël, 2014).

PCR can be used to confirm the results obtained during visual inspection of meat (Geysen *et al.*, 2007); however, at the moment it is mostly used in the context of research projects and more rarely for routinely detected cysticerci. PCR detects the deoxyribonucleic acid (DNA) of bovine cysticerci sampled during meat inspection (Cuttell et al., 2013). The sensitivity of PCR decreases as cysticerci degenerate (Cuttell et al., 2013). Conventional PCR can be done by amplifying the 12s rDNA gene of *Taenia saginata* cysticercus followed by restriction fragment length

polymorphism (RFLP) (Geysen et al., 2007). Real-time PCR can also be used to detect *T. saginata* DNA, by using a cytochrome c oxidase subunit gene 1 (Cuttell et al., 2013)

The sensitivity of meat inspection is thought to be considerably lower than serological tests (Allepuz et al., 2012). ELISA is an immune assay, mostly used in veterinary medicine and labelled- antigen ELISA is frequently used to detect antibodies, whilst antibody-based sandwich ELISA is used to detect and measure specific antigens (Tizard, 2013). Monoclonal antibody-based antigen-ELISA (HP10 Ag-ELISA) (Wanzala et al., 2002) and monoclonal antibody-based circulating antigen-detecting ELISA (B158/B60 Ag ELISA) (Jansen et al., 2016), are the two different types of ELISAs used to detect viable *T. saginata* cysticerci in cattle using serum samples.

The monoclonal antibody-based circulating antigen detecting ELISA (B158/B60 Ag ELISA) was first described by Brandt et al. (1992) and later modified by Dorny et al. (2004). In this test, monoclonal antibodies were developed against excretory-secretory antigens of *T. saginata* cysticerci to only detect viable cysticerci. According to a study done in Belgium B158/B60 Ag-ELISA has a sensitivity of 40% and a specificity of 100% (Jansen et al., 2017).

Another method of detecting live *T. saginata* cysticerci is a double sandwich ELISA based on a mouse monoclonal antibody (McAb), coded as HP10 (Wanzala et al., 2002). This monoclonal antibody is an immunoglobulin M (IgM) isotope that can detect antigens of only viable metacestodes and has a sensitivity that ranges between 60-80% (Wanzala et al., 2002).

2.9 Cattle farming and bovine cysticercosis in Zimbabwe

Zimbabwe is a landlocked country in Southern Africa and shares borders with South Africa, Mozambique, Zambia and Botswana (Schneider and Ferguson, 2020). The country has different agroecological regions and farming sectors (Tawonezvi et al., 2004). Zimbabwe was divided into agroecological zones in 1960 by Vincent and Thomas based on mean annual rainfall (Mugandani et al 2012);Table 2.1. Controlled grazing in commercial farms and uncontrolled grazing in communal farms are the two main types of grazing systems in Zimbabwe (Tavirimirwa *et al.*, 2013). Production systems in the agroecological regions IV, V and parts of region III, are mostly extensive grazing on natural rangeland (Tawonezvi et al., 2004). Fully commercial, partially

communal/commercial and fully communal, are the three farming systems practised in Zimbabwe (Bennet *et al.*, 2019) (Table 2.2).

Table 2.1: Description of agro-ecological zones of Zimbabwe (Mugandani et al.,2012)

Agroecological zone	Mean annual rainfall	Mean annual temperature	Farming activities
I	>1000mm	15-18ºC	Coffee, tea, potatoes
II	1000-700mm	16-19ºC	Maize, tobacco, cotton, wheat and intensive livestock production
III	700-550mm	18-22ºC	Maize, tobacco, cotton, wheat and cattle ranching
IV	600-450mm	18-24ºC	Extensive livestock production and crops such as; sorghum, millet
V	<500mm	21-25ºC	Extensive cattle ranching and game protection

Table 2.2: Description of beef farming systems practised in Zimbabwe (Bennet et al.,2019)

Farming System	Description of the farming system
Fully commercial	Semi-intensive production system on enclosed land with supplement feeding. Animals are mostly used for sale. Highly commercialized
Fully communal	Subsistence mixed farming system, with usage of animal-drawn implements. Animals are used for substance provision of milk, savings and status. Shared communal grazing area.
Partially communal/commercial	Relocated farmers who use communal grazing land and

have limited usage of tractor-drawn implements. Animals are used for both communal and commercial purposes and supplies to the market.

Cattle are the most predominant livestock species in Zimbabwe (Chinembiri, 1999) (Tawonezvi et al., 2004). The total herd size of cattle in Zimbabwe is approximated at 5.4 million (MoLAWCRR, 2020). Most of the exotic breeds are owned in the commercial sector with approximately 88% of households owning indigenous breeds or crossbreeds (Tavirimirwa et al., 2013). In Zimbabwe, cattle have numerous functions such as food security, drought power provision, hides, manure and socio-cultural functions such as lobola payments (Maburutse et al., 2012). Cattle are a sign of wealth status for most communal farmers and a source of income for most households, through cattle sales and their products (Tavirimirwa et al., 2013). Brahman, Simmental, Hereford, Sussex, Aberdeen, Angus, Beef master, Limousine, and Charolais are the different exotic breeds found in Zimbabwe with Jersey, Holstein-Friesian, the Red Dane and dual-purpose Simmental being common in dairy farming (Tavirimirwa *et al.*, 2013). Mashona, Tuli and Nkone are the three local sanga cattle (Gororo et al., 2018) characterised as indigenous cattle breeds of Zimbabwe (Consultancy, 2002).

Few studies have investigated the presence of bovine cysticercosis in Zimbabwe. In a study carried out between January 2006 to December 2007 using meat inspection of cattle slaughtered at a cold storage company (CSC), Bulawayo, Zimbabwe, an average prevalence of 1.6% (n=1364) of bovine cysticercosis was determined; with Matabeleland North (Mat-North) having a prevalence of 2.8% (n=629) and Matabeleland South (Mat-South) a prevalence of 1.2% (n=735) (Sungirai et al., 2014). Most bovine cysticercosis cases were observed in communal farming systems (Sungirai et al.,2014). An in-depth investigation of the local risk factors for bovine cysticercosis is lacking up to now for Zimbabwe.

In Zimbabwe, predilection sites that are palpated and incised during routine meat inspection are the masseter muscles, the pericardial surface of the heart, triceps muscles of both sides of the shoulders, diaphragm, oesophagus, and tongue (Sungirai et al., 2014). Local legislation dictates that if more than or equal to twenty-one live or dead cysticerci are identified during meat inspection, the carcass and offals are condemned, if less than or equal to twenty cysticerci are

identified, the carcass is frozen at -12ºC for not less than 12 days. Condemnation of the head is executed if five or more cysticerci are found in its musculature whilst the heart, the tongue and other organs are condemned if five or more cysticerci are identified in the organ. The carcass is condemned if eleven or more cysticerci are found in its musculature (Statutory instrument 50 of 1995). To assure public safety, measled meat is treated for a period of >12 days through freezing in a cold storage room not used for other purposes or frozen in a blast-freezing tunnel for a period laid by the director or boiled at a temperature not less than 95ºC for 150 minutes (Statutory instrument 50 of 1995).

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Chapter 3: Risk factors associated with bovine cysticercosis in the Matabeleland region of Zimbabwe

Abstract

Bovine cysticercosis, caused by the zoonotic tapeworm *Taenia saginata* (*T. saginata*), is one of the most important zoonotic neglected tropical diseases of livestock, especially in low-income countries. Communal farmers' knowledge, attitudes and perceptions of the risk factors for bovine cysticercosis in Matabeleland region of Zimbabwe are not fully known. The current study investigated communal farmers' knowledge, attitudes and practices on the risk factors of bovine cysticercosis in the Matabeleland region of Zimbabwe. A total of 377 participants were interviewed in the Bulilima district of Matabeleland South Province in October 2021 for seven days. Eighty-six point two percent (325/377; 86.2%) of the respondents were livestock owners, with the majority (223/377; 59.2%) indicating cattle as their livestock species of choice and (300/377; 79.6%) relying on livestock as their source of income. Majority of the participants (316/377; 83.8%) practised home slaughter with inadequate meat inspection and (263/377; 69.8%) of the slaughters were performed by the farmer. Most of the participants (366/377; 97.1%) did not know and had never heard about bovine cysticercosis. Two-fifths of the participants had no access to toilets at home and (358/377; 95%) of the participants sometimes practiced open defecation. This study identified home slaughter, inadequate meat inspection, consumption of meat with visible cysts, lack of toilets or inadequate toilets and open defecation as risk factors for bovine cysticercosis in Matabeleland South.

Keywords: Bovine cysticercosis, communal cattle, Matabeleland South, risk factors, *Taenia saginata*

3.1 Introduction

Taenia saginata is a zoonotic tapeworm (Torgerson *et al.*, 2019) that causes bovine cysticercosis and is one of the zoonotic neglected tropical diseases of livestock (Tegegne et al.,2018). Humans act as the definitive host for this parasite, while bovines are the intermediate hosts (Dermauw *et al.*, 2018). Humans get infected when they consume raw or undercooked beef containing *T. saginata* cysticerci (Diemert, 2017), whilst cattle get infected when they consume feed or water that is contaminated with eggs or proglottids from human faecal matter (Held and Cappello, 2004). Bovine cysticercosis is usually asymptomatic (Braae *et al.*, 2018) causing losses due to carcass condemnations or treatment of infected carcass to destroy cysts. Taeniasis can be asymptomatic, yet it can also cause symptoms such as perianal itchiness due to the active passage of proglottid segments through the anus, loss of appetite, nausea and vague abdominal pain in infected people (O'Dempsey, 2010). Controlling this disease is vital in breaking its economic and public health significance.

Bovine cysticercosis is highly prevalent in low-income countries where hygiene and sanitation standards are poor, routine meat inspection is not enforced (Braae *et al.*, 2018) and poor husbandry methods are practiced (Alemneh et al., 2017). Open defecation, especially on grazing land is a risk factor for bovine cysticercosis (Laranjo-González *et al.*, 2016). The presence of bovine cysticercosis is associated with numerous environmental factors linked to water sources, such as cattle having access to surface water, flooding pastures with wastewater or proximity to wastewater sources (Braae *et al.*, 2018) and persistence of *T. saginata* eggs in treated water (Laranjo-González *et al.*, 2016).

Zimbabwe is a landlocked country in Southern Africa, with an area of approximately 39 million hectares, of which 33.3% of the land area is used for agricultural purposes (FAO, 2022). Agriculture is a means of livelihood for approximately 60-70% of the population (FAO, 2022), with most of the people practising subsistence crop and livestock production (Chigonda, 2018). Livestock production is of great importance because it contributes 25% towards the gross domestic product (GDP) of the country (Sungirai, 2014), with cattle contributing 35-38% of that GDP (FAO, 2022). In Zimbabwe, only two studies have so far investigated the prevalence of *T. saginata*. Based on abattoir records, prevalence estimates for bovine cysticercosis ranged between 1.6% and 2.2% (Sungirai et al., 2014; Pugh & Chambers, 1989). Several factors seem conducive for the transmission of *T. saginata* in Zimbabwe; according to a demographic survey,

35% of households use flush toilets, while 43% use pit latrines and 22 % lack access to toilets (ZIMSTAT, 2017). Bovine cysticercosis is associated with poor personal hygiene, consumption of raw or undercooked beef and inappropriate cattle management practices, which are thought to prevail in the country.

Increasing the awareness of risk factors amongst cattle-producing communities is essential to fight the transmission of *T. saginata*. Up to now, however, there have been few attempts to determine communal farmers' perceptions of the risk factors of bovine cysticercosis in cattle farmers in Zimbabwe. It is hypothesised that communal cattle farmers Matabeleland South province have limited knowledge on the risk factors of bovine cysticercosis. Therefore, the current study aimed to assess knowledge, attitudes and practices on the risk factors of bovine cysticercosis in the Matabeleland region of Zimbabwe.

3.2 Materials and Methodology

3.2.1 Ethical clearance

Ethical clearance for the study was obtained from the University of Pretoria's Faculty of Veterinary Science Research Ethics Committee (Appendix 1), Faculty of Humanities Ethics Committee (Appendix 2) and National Animal Research Committee of Zimbabwe (Appendix 3).

3.2.2 Study design and study area

A cross-sectional study was conducted in 10 wards of the Bulilima district located in Matabeleland South Province of Zimbabwe. The Matabeleland region carries about a fifth of the national cattle herd, and most of these are reared extensively by resource-limited communal farmers (Tavirimirwa *et al.*, 2013). The Bulilima district is in Natural Region 5 with an annual rainfall of approximately 400 mm and is known to be a cattle and game ranching area (Matsa and Mini, 2014). The district has 22 wards, with a population of 90,757 and approximately 19,761 households (Zimstat, 2013). Approval from the rural district council, district administration office and the district police services was obtained before conducting the survey. The study was conducted in 10 out of 22 wards: Nyele, Masendu, Gala centre, Huwana, Ngwala, Ndolwane, Bambadzi, Madlambuzi, Dombodema, Gwambe and Butshe (Figure 3.1). The wards in the study were selected due to their easy accessibility. The study area was selected based on earlier reports

about bovine cysticercosis in the Matabeleland province (Sungirai et al., 2014; Pugh and Chambers, 1989), retrospective data obtained from the Department of Livestock and Veterinary Services of Zimbabwe, the willingness of farmers to participate in the study and the presence of cattle communal farmers.

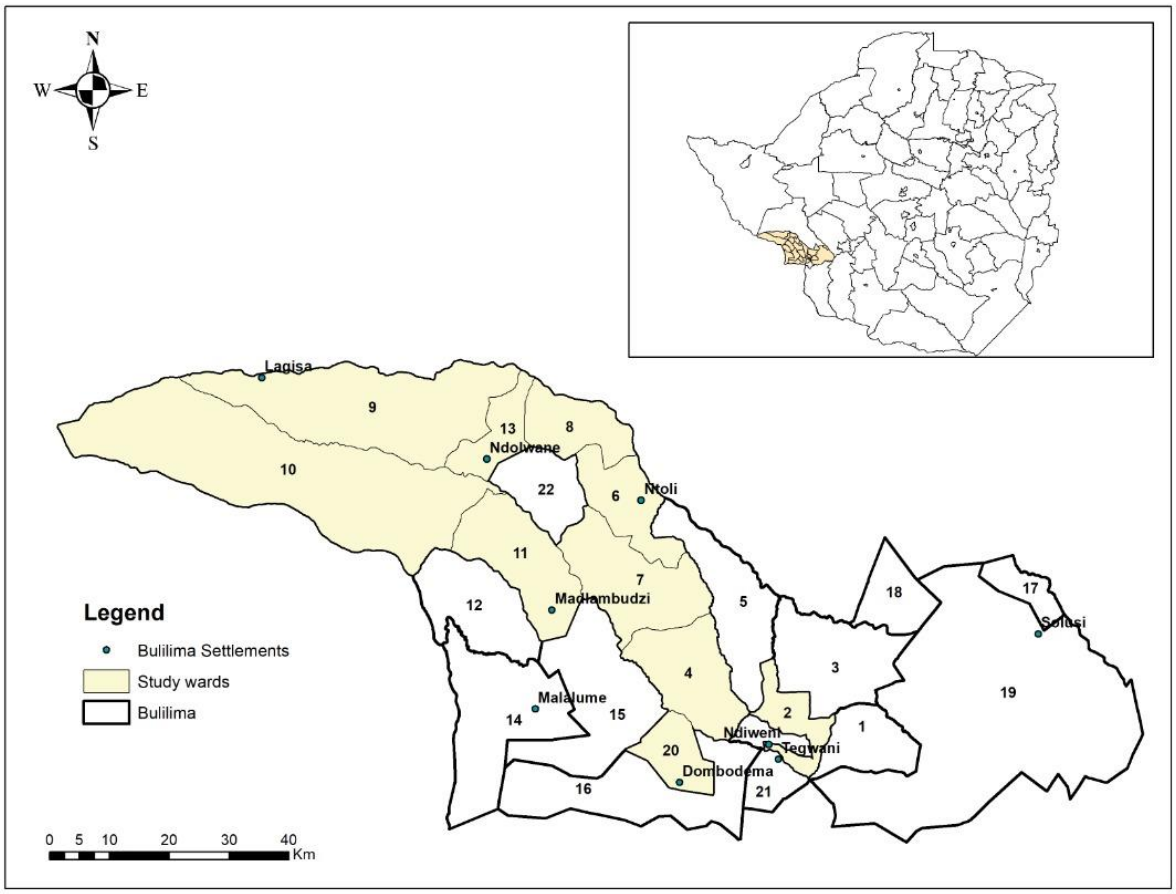

Figure 3.1: Map of Zimbabwe (on the top) with Bulilima district (in colour). Bulilima district map highlighting the study wards; ward 2 (Gwambe), ward 4 (Nyele), ward 6 (Gala centre), ward 7 (Masendu), ward 8 (Huwana), ward 8 (Ngwala), ward 9 (Butshe), ward 10 (Bambadzi), ward 11 (Madlambuzi), ward 13 (Ndolwane), ward 20 (Dombodema)

3.2.3 Data collection

Smallholder farmers were identified using a snowball sampling technique (Qokweni et al., 2020). All individuals were at least 18 years of age and willingly participated by signing consent forms before participating. All participants were informed of their right to quit the study at any point if they felt uncomfortable with the questions. Confidentiality was assured for all

participants and all participants had to sign a consent form that stated the guarantee of confidentiality, no particular names and/or identity numbers were collected. Using the KoboCollect v2021.3.4 software, an electronic questionnaire was administered to a total of 377 farmers in 10 wards in the Bulilima district. The questionnaire (Appendix 10) sought to collect information regarding farmers' and cattle herd demographics; farm infrastructure; and farmers' knowledge, attitudes and practices on the risk factors of bovine cysticercosis. Knowledge on bovine cysticercosis and zoonotic transmission of *T. saginata* was determined using the responses of the participants to questions about previous knowledge on bovine cysticercosis, how cattle acquire bovine cysticercosis, whether bovine cysticercosis is dangerous to humans, on whether humans get infected after consumption of raw or undercooked beef or offal. Information about respondent gender, age, level of education and experience in livestock keeping was also collected.

3.2.4 Statistical analysis

The questionnaire data were exported to Microsoft Excel. First, descriptive statistics were used to analyse data on farmer and herd demography, farm characteristics and management practices. The association between individuals diagnosed with verminosis and demographic data and the association between demographic data and individuals dewormed was calculated using the Pearson Chi-Square test with the null hypothesis being rejected when the *P*-value was < 0.05. Strength of association was calculated using Phi and Cramer's V, with values; >0.25 signifying very strong association, >0.15 strong association, >0.10 moderate association, >0.05 weak association and 0 no association or very weak association.

Knowledge scores were calculated, with a score of one indicating knowledgeable and zero indicating lack of knowledge. Binary logistic regression was used to calculate the association between demographic data and knowledge scores. All statistical analyses were run using IBM SPSS Statistics Version 28.0 (International Business Machines Corp., Armonk, NY, USA software).

3.3 Results

3.3.1 Farmer demographics

Out of the 377 participants, the majority were livestock owners, accounting for 86.2% and 12.7% were animal attendants (Table 3.1). Most participants were females (72.4%) while males were the minority (27.6%). Respondents' age ranged from 18 to 92 years, with 52.8% having gone through primary education, 36.1% having high school education, 1.1% having tertiary education, and 6.9% having no formal education (Table 3.1). Experience in livestock keeping ranged from less than 10 years to 67 years, with the majority (56.50%) having less than 10 years of experience (Table 3.1)

Table 3.1: Farmer demographics in Bulilima district, Zimbabwe (n=377)

Variable		Count	Percentage
Ownership	Owner	325	86.2
	Animal attendant	48	12.7
	No response	4	1.10
Gender	Male	104	27.6
	Female	273	72.4
Age (years)	18-28	39	10.3
	29-39	53	14.1
	40-50	84	22.3
	51-60	95	25.2
	61-70	62	16.4
	71-80	33	8.8
	81-92	10	2.7
	No response	1	0.30
Level of education	Primary	199	52.8
	High school	136	36.1
	Tertiary level	4	1.10
	No formal education	26	6.9
	No response	12	3.2
Experience in livestock keeping (years)	≤10	157	41.6

	11-20	87	23.1
	21-30	49	13.0
	31-40	37	9.8
	41-50	29	7.7
	51-67	11	2.9
	No response	7	1.90

3.3.2 Farm characteristics

Livestock species kept were cattle (64.2%), sheep (4.2%), goats (81.4%), poultry (84.4%) and other species (24.9) %. About 59.20% of participants chose cattle as their livestock of preference (Table 3.2). About 58.62% relied on both livestock and crops as their source of income. The majority of the participants (97.10%) had no calving pens, with 99.70% having no restraining equipment and 99.70% having no weight estimation equipment (Table 3.2). Extensive livestock production was the most popular livestock production system used, with 81.4% of the participants practising it and only 0.30% practising semi-intensive. About half of the respondents (48.8%) reported using streams or rivers, 14.6% used unprotected wells and a tenth of the respondents (9.5%) used boreholes as a source of drinking water for livestock (Table 3.2).

Table 3.2: Farm characteristics according to questionnaire respondents in Bulilima district, Zimbabwe (n=377)

Variable		Count	Percentages
Livestock species kept and poultry	Cattle	242	64.2%
	Sheep	16	4.2%
	Goats	307	81.4%
	Chickens	318	84.4%
	Other	94	24.9%
Cattle being livestock of choice	1st choice	223	59.2%
	2nd choice	11	2.9%
	3rd choice	8	2.1%
Source of income	Livestock	300	79.6%
	Crops	270	71.6%

	Pension	21	5.6%
	salary/ wages	93	24.7%
	Other	40	10.6%
Production type	Semi-intensive	1	0.30%
	Extensive	307	81.4%
	No response	69	18.3%
Source of water	Borehole	36	9.5%
	Unprotected well	55	14.6%
	Stream/river	184	48.8%
	No response	102	27.1%
Feeding system	Pasture grazing	184	48.8%
	Grazing & supplement feeding	91	24.1%
	No response	102	27.1%

3.3.3 Slaughter and meat inspection

Only 0.5% of participants slaughtered their cattle at the abattoir, whilst 1.1% slaughtered from designated slaughter points and the majority (83.8%) slaughtered at home (Table 3.3). Most (69.8%) of the cattle slaughtered at home had the inspection done by farmers. Farmers mostly looked for wounds (7/377; 1.9%), unspecified diseases and infections (50/377; 13.30%), abscesses (3/377; 0.8%), organs and intestinal abnormalities (66/377; 17.5%), colour and other abnormalities (8/377; 2.1%), wounds, plastics and body condition (4/377; 1.1%), ticks and abscesses (3/377; 0.8%), intestinal abnormalities and wounds (2/377; 0.5%). About 39.8% of participants consumed the meat even though abnormalities are observed during inspection, 36.9% discarded the part with abnormalities and the minority 0.5% sold the meat with abnormalities (Table 3.3).

Table 3.3: Descriptive statistics on slaughter and meat inspection practices in Bulilima district, Zimbabwe

Meat inspection practices		Count	Percentage
Slaughtering places	Abattoir	2	0.50%
	Designated slaughter	4	1.10%

	points		
	Home	316	83.8%
	No response	55	14.6%
Is meat inspected	Yes	23	6.1%
	No	298	79.0%
	No response	56	14.9%
Who inspects the meat	Veterinarian	13	3.4%
	Animal health technician	6	1.60%
	Farmer	263	69.8%
	No response	95	25.2%
If abnormalities are observed what do you do	Consume	150	39.8%
	Discard	139	36.9%
	Sell	2	0.50%
	No response	86	22.8%

3.3.4 Personal hygiene

Sixty percent of the participants had access to toilets at home while 39.5% had no access to toilets at home (Table 3.4). Most (52.8%) of the respondents with toilets at home, always used the toilet, whilst 2.4% often used the toilet and 4.77% sometimes used toilets. Majority of the respondents (69.5%) had access to toilets at gathering points with 34.7% of those with access to toilets always using the toilets, 16.4% often using the toilet, 17% using the toilet sometimes and the minority at 1.3% never using the toilet. Almost a third (30.5%) of the respondents had no access to toilets at gathering points. The majority of the participants representing 76.1% had no access to toilets at grazing lands whilst the minority at 23.9% had access to toilets and 23.6% of those with access to toilets always used the toilets and 0.3% often used the toilets. Most of the participants (95%), used the bush when they had no access to toilets (Table 3.4).

Table 3.4: Personal hygiene practices in farmers in Bulilima district, Zimbabwe

Sanitation practices		Count	Percentages
Access to toilets at home	Yes	227	60.2%

	No	149	39.5%
	No response	1	0.30%
Access to toilets at gathering points	Yes	262	69.5%
	No	115	30.5%
Access to toilets at grazing lands	Yes	90	23.9%
	No	287	76.1%

3.3.5 Taeniasis

Amongst the participants, 26.5% had been previously diagnosed with verminosis and 26% of the participants had been previously dewormed. There was no association between demographic data and diagnosis of verminosis ($P>0.05$). There was no association between demographic data and individuals dewormed ($P>0.05$).

3.3.6 Knowledge scores

Hosmer and Lemeshow test indicated that; the model adequately fits the data as $P > 0.05$, therefore there was no difference between the observed and predicted model. The model correctly classified 66.8% of the respondents who were knowledgeable about bovine cysticercosis and zoonotic transmission. Gender, Age and Level of education were generally not significant ($P>0.05$), whilst experience in livestock keeping was significant ($P<0.05$) and a positive beta coefficient (0.93 in individuals with less than ten years of experience meaning that for every one-unit increase inexperience in livestock keeping, level of knowledge increases by 0.93.

3.4 Discussion

Demographic data observed on gender and age were in accordance with data from the Zimbabwe National Statistical Agency (ZIMSTAT) findings (ZIMSTAT, 2013.; ZIMSTAT, 2017). The majority of the interviewees had primary-level education and few had up to tertiary education with some having no formal education, agreeing with literature. which states that in Matabeleland South 8.8% (3-24 years) and 6% (25 years and above) had never attended school, whilst 37.6% were at school and 53.6% had dropped out of school (ZIMSTAT, 2021). Common

livestock farmed in the Bulilima were similar to livestock species found among farmers in Zimbabwe (Tawonezvi *et al.*, 2004). Majority (223/377; 59.2%) of the participants indicated that cattle were their preferred livestock. Cattle are a sign of wealth and a source of income for most communal households (Tavirimirwa *et al.*, 2013). Despite many years of experience among farmers, low management level in communal production systems is still of concern as it results in high disease and parasite challenges (Tavirimirwa *et al.*, 2013)

Uncontrolled grazing is practised by most communal farmers, agreeing with a study done by Tavirimirwa et al. 2013, with approximately (275/377; 72.9%) of the cattle in Bulilima exposed to pasture grazing at some point. Majority of the cattle in Bulilima drank water from unprotected wells and the river, increasing the likelihood of exposure to bovine cysticercosis together with pasture grazing due to the possibility of contamination of grazing land and water by human faecal matter (Laranjo-González et al., 2018).

T. saginata cases can be increased by the absence of meat inspection and inadequate meat inspection (Alemneh et al., 2017), as observed in Bulilima where (316;37783.8%) of meat inspection is done at home, with (263/377;69.8%) of the inspection done by farmers who have very little to no knowledge of the disease. Moreover, (366/377; 97.08%) did not know bovine cysticercosis and what they looked for during inspection had nothing to do with the disease. Even when abnormalities are observed during meat inspection, (150/377; 39.8%) of the participants will still consume the meat and (2/377; 0.5%) will sell it, increasing the likelihood of taeniasis cases. A study carried out in Gauteng province in South Africa, demonstrated that home slaughter in the absence of meat inspection contributes to risk factors of bovine cysticercosis (Tsotetsi-Khambule *et al.*, 2018).

Gender, Age, and level of education had no significant effect on knowledge about bovine cysticercosis and its zoonotic transmission, whilst years of experience in livestock keeping had a significant effect on the bovine cysticercosis and zoonotic transmission. Level of knowledge increased with the level of experience. This study partly agrees with a study done in South Africa which realised that there was no significant association between level of education and years in farming and outcome knowledge in porcine cysticercosis (Shongwe *et al.*, 2020).

Poor sanitation practices increase the risk of bovine cysticercosis as cattle get infected by ingesting feed or drinking water contaminated with *T. saginata* eggs shed in human faecal matter

(McBrien and Courcier, 2013); (Bruschi and Gómez-Morales, 2017). Eggs excreted from human faecal matter can survive in the environment for several months. Bucur et al. 2019 and Abuseir et al. 2006 stated that in an appropriate environment the eggs can survive for almost seven months. In Bulilima, (149/377; 39.5%) of the participants had no access to toilets and (358/377; 95%) of the participants sometimes defecated in the bush when in places with no toilets. Despite the presence of toilets, some participants had a habit of using the bush, thereby increasing the risk of bovine cysticercosis (Tsotetsi-Khambule *et al.*, 2018). Open defecation increases the chance of cattle getting infected with bovine cysticercosis, as the majority of the cattle owned by the participants were at some point on pasture grazing and drank from unprotected water sources (Laranjo-González *et al.*, 2018).

Diagnosis of taeniasis in humans is of importance in determining the risk factors of bovine cysticercosis, as humans are the definitive host of *T. saginata* (McFadden *et al.*, 2011). Though there was no association between demographic data and diagnosis of worms in individuals in the present study, a previous study in India reported that *T. saginata* infection and prevalence was associated with age, sex, living conditions and eating habits, with more cases recorded in males than females especially those above 60 years (Lateef *et al.*, 2020). Differences in study location and cultural practices may account for the discrepancies with the current study. There is, therefore, a need to further investigate the prevalence of taeniasis cases and risk factors in humans in the Matabeleland region. Previous diagnosis of taeniasis in individuals needs to be investigated. There is need to understand traditional practices on meat eating habits such as; existence of traditional ceremonies were meat is eaten raw and if all gender and age groups are allowed to partake in those ceremonies.

3.5 Conclusion.

Home slaughter, inadequate or absent meat inspection, consumption of infected meat or abnormalities, lack of toilets or inadequate toilets and open defecation were factors identified to increase the risk for bovine cysticercosis and taeniasis in communal farming areas of Matabeleland. Retrospective bovine cysticercosis prevalence of 1.08% in the Matabeleland region based on meat inspection data may be an underestimate and a more sensitive test should be used in future studies.

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Chapter 4: Determination of the prevalence of bovine cysticercosis in Matabeleland region of Zimbabwe based on the application of B158/B60 Ag-ELISA and meat inspection done in abattoirs.

Abstract

Taenia saginata is a zoonotic tapeworm of economic importance, with humans being the definitive hosts and cattle the intermediate hosts. The current study aimed to determine the prevalence of bovine cysticercosis using antigen enzyme-linked immunosorbent assay (Ag-ELISA) and confirm the cysticerci detected on meat inspection using polymerase chain reaction (PCR) and sequencing in cattle slaughtered in three abattoirs in the Matabeleland region of Zimbabwe. Cysticerci identified during meat inspection were collected from 381 slaughtered cattle, serum samples were successfully extracted from 369 blood samples collected in the abattoir and analysed by the monoclonal antibody-based B158/B60 Ag ELISA while deoxyribonucleic acid (DNA) was extracted from the cysticerci, amplified and sequenced. The Ag-ELISA determined an apparent prevalence of bovine cysticercosis of 37.9 %. Seven of the 381 cattle (1.90%) were positive to bovine cysticercosis on meat inspection. The majority of the cysticerci identified were viable and mostly located in the masseter muscle. All the cysticerci identified during meat inspection were confirmed to be *T. saginata* cysticerci on PCR and when the sequences were aligned to other sequences from the Gene bank, they showed 99.2 % to 99.8 % similarity to a sequence corresponding to 12s rDNA gene of *T. saginata* genome. Bayesian methods were used to determine true prevalence of bovine cysticercosis. Matabeleland South had the highest estimate for true prevalence (92.1%), followed by Matabeleland North (89.3%) and Bulawayo (83.2%). The present study confirmed that Ag ELISA gives a better estimate of bovine cysticercosis prevalence than meat inspection. The high prevalence of bovine cysticercosis and the presence of viable cysticerci observed in this study suggests that humans could be frequently exposed to *T. saginata* cysticerci warranting further investigation of taeniasis prevalence in the human population.

Keywords: antigen ELISA, bovine cysticercosis, Matabeleland, meat inspection, *Taenia saginata*,

4.1 Introduction

Taenia saginata is a zoonotic tapeworm of economic importance (Braae et al., 2018). Humans are the definitive host being infected when they consume raw or undercooked beef or offal containing *T. saginata* cysticerci (Dermauw et al., 2018). This results in the development of the adult worm in the small intestine or taeniasis in humans. Cattle, the intermediate hosts, get infected when they consume feed or drinking water contaminated with *T. saginata* eggs or proglottids containing eggs, shed from human faecal matter (Saratsis et al., 2019). This results in the development of larvae in striated muscle, a condition called bovine cysticercosis. Cysticercosis causes great economic losses to the cattle farmers, the cost of which have been calculated to be 154,943 euros per year in Spain (Laranjo-González et al., 2018), approximately 3,408,455 euros per year in Belgium (Jansen et al., 2018) and US$ 212 202.76 in Ethiopia combined for taeniasis and bovine cysticercosis (Hiko and Seifu, 2019). These losses were either related to meat inspection costs, carcass condemnation and freezing or insurance fees (Jansen et al., 2018). In Zimbabwe no attempts have been made to estimate the economic losses associated with bovine cysticercosis, however, the determination of the prevalence of bovine cysticercosis is a prerequisite for such studies.

Meat inspection is the only routinely used diagnostic tool in several countries to diagnose bovine cysticercosis (Wanzala et al., 2002). In Zimbabwe, it is conducted following the guidelines stated in statutory instrument 50 of 1995 (Public Health Regulations 50 of 1995). Previous studies based on the post-mortem identification of cysticerci during meat inspection have reported a low prevalence (1.65 % up to 2.16 %) of bovine cysticercosis in slaughtered cattle (Pugh and Chambers, 1989; Sungirai et al, 2014). However, meat inspection is believed to have a low sensitivity of <15% (Jansen et al., 2016), especially in mildly infected carcasses (Da Silva et al., 2015) and poor ability to differentiate viable from calcified cysts (Tsotetsi-Khambule et al., 2017) It has also been established that meat inspection is less specific as there can be misdiagnosis of bovine cysticercosis with other diseases such as actinobacillosis and sarcocystosis which cause lesions in striated muscle similar to *T. saginata* cysticerci (Hassan El-Sayad et al., 2021). Direct immunodiagnosis (detection of products of the infective agent in the host) utilizing monoclonal antigen enzyme-linked immunosorbent assays developed for *T. saginata* greatly improved the sensitivity of detection of cattle infections (Harrison et al., 1989)

These assays have the advantage of demonstrating active infection and are more sensitive than meat inspection (Rodriguez et al., 2012). According to Jansen et al. (2017), Ag-ELISA (B158/B60 Ag ELISA) has a sensitivity of 40 % and a specificity of 100 %. The use of the highly sensitive and specific polymerase chain reaction (PCR) with restricted fragment length polymorphism (RFLP) or sequencing has also been applied to confirm the identity of the cysts collected on meat inspection and thus improve specificity (Geysen et al., 2007).

At present, there have been no attempts to estimate the prevalence of bovine cysticercosis utilizing the more sensitive Ag ELISA nor to confirm the identity of the cysts detected at meat inspection using PCR and sequencing in Zimbabwe. Studies utilizing the Ag ELISA assays will be essential for the generation of reliable prevalence data on bovine cysticercosis and stimulate the prompt development of control strategies that will mitigate the risk of taeniasis in humans and serious economic losses to the cattle industry (Jansen et al., 2017). The Matabeleland region produces approximately a fifth of the total cattle population of the country (Tavirimirwa et al., 2013), and yet the prevalence data on bovine cysticercosis remains scanty in the region. The objectives of the current study were therefore to determine the prevalence of bovine cysticercosis in the Matabeleland region utilizing Ag ELISA and to confirm the identity of *T. saginata* cysticerci detected on meat inspection.

4.2 Materials and Methods

4.2.1 Ethical clearance

Ethical clearance for the study was obtained from the University of Pretoria's Faculty of Veterinary Science Research Ethics Committee (Appendix 1), National Animal Research Committee of Zimbabwe (Appendix 3), University of Pretoria's Faculty of Veterinary Science

Animal Ethics Committee (appendix 4), and Medical Research Council of Zimbabwe (Appendix 5). Permission to conduct research under Section 20 of the Animal Diseases Act (Act 35 of 1984) (Appendix 6) and its amendment (Appendix 7) was obtained from the Department of Agriculture, Land Reform and Rural Development, Republic of South Africa. An import permit (Appendix 8) to move serum and tissue cyst samples from Zimbabwe to South Africa was obtained from the Directorate of Animal Health Import-Export Policy Unit of the Department of

Agriculture, Land Reform and Rural Development, Republic of South Africa, while an export permit (Appendix 9) was obtained from the Department of Veterinary Services, Zimbabwe.

4.2.2 Study design and study area

In October 2021, a cross-sectional study was conducted in the Matabeleland region of Zimbabwe. Matabeleland region is located in South Western Zimbabwe and comprises three provinces, Matabeleland North, Matabeleland South and Bulawayo ("Matabeleland - Wikipedia," 2022.). In the region, there are fourteen grade B abattoirs, where more than five animals but not more than 100 animals per day are slaughtered Three grade B abattoirs were conveniently selected, based on easy accessibility.

4.2.3 Sampling procedures

In each of the selected abattoirs, all carcasses processed during five days were sampled. Some of the processed carcasses at slaughter were not from the chosen study area (Matabeleland region), therefore these were not included in the statistical analysis. Samples were collected from abattoir A (n = 183) located in Bulawayo metropolitan province and abattoir B (n = 175) located in Matobo district, Matabeleland South province, and abattoir C (n = 23) located in Umguza District, Matabeleland North province. The blood samples were collected from the jugular vein within the first 30 seconds after slaughter and stored in red-top vacutainer plain tubes. The blood tubes were labelled before being transported on ice to Bulawayo Provincial Veterinary Laboratory (BPVL). Serum was separated by centrifugation of blood tubes for 5 minutes at 3000 rpm and stored in 2 ml cryotubes. This was followed by preheating of the cryotubes at 56 ºC for 30 minutes to inactivate any potentially infectious material, storage at -20 ºC and then transportation to the Research and Training laboratories at the Department of Veterinary Tropical Diseases (DVTD), University of Pretoria, South Africa for further processing.

4.2.4 Meat Inspection

Meat inspection was conducted by food inspectors on each of the selected carcasses, according to the Zimbabwean statutory instrument 50 of 1995 (Public Health Regulations 50 of 1995). Predilection sites namely the masseter muscles, oesophagus, pericardial surface of the heart, diaphragm and triceps of both sides of the shoulders were palpated before being incised to

inspect for cysts/cysticerci (Sungirai et al., 2014) and the tongue was palpated. Cysts were collected from infected carcasses detected by meat inspection and stored on ice during transportation to Bulawayo Provincial Veterinary Laboratory (BPVL)for classification. Cysts were classified as viable if they were translucent and fluid filled with a visible scolex (Ogunremi et al., 2004), degenerate if they were soft, semi-translucent with nodular lesions having yellowish material and calcified if they were firm, opaque and calcareous (Costa et al., 2012). The cysts were placed in 70% ethanol-bearing 2 ml cryotubes, preheated at 56 ºC for 30 minutes to inactivate any potentially infectious material, stored at -20 ºC and then transported for further processing to the Research and Training laboratories at the DVTD.

4.2.5 DNA extraction

Cysts were cut into pieces and crushed, with each cyst cut using a new scalpel blade and cleaning the working desk with corox in between different samples. The QIAamp® DNA mini kit (Qiagen, Germany) was used for DNA extraction as per guidelines of the manufacturer (Quiagen, 2019). All centrifugation was done at room temperature (15-25ºC) The crushed cysts were put in 2ml microtubes and 180µl of buffer ATL was added, followed by 20µl proteinase K. Samples were incubated at 56ºC overnight in an accublock digital dry bath with occasional vortexing during the incubation. 4µl RNase was added to the sample followed by incubation at room temperature for 2 minutes. 200µl buffer AL was added to the sample, followed by incubation at 70ºC for ten minutes. 200µl of 100% ethanol was added. Pipet the mixture including the precipitate onto the QIAamp® mini spin column (in a 2ml collection tube) without wetting the rim, followed by centrifugation at 8000rmp for a minute. The QIAamp® mini spin column was placed in a clean 2ml collection tube and the tube containing the filtrate was discarded. 500µl Buffer AW1 was added without wetting the rim and centrifugation was performed for a minute at 8000rpm. The QIAamp® mini spin column was placed in a clean 2ml collection tube and 500µl buffer AW2 was added without wetting the rim, followed by centrifugation at 12600rpm for three minutes. The QIAamp® mini spin column was placed in a clean 1.5ml microcentrifuge tube and 50µl buffer AE was added and incubation at room temperature for a minute was done, followed by centrifugation at 8000rpm for a minute to elute the DNA.

4.2.6 Polymerase Chain Reaction (PCR)

DNA amplification and restriction fragment length polymorphism were performed following the technique reported by (Geysen et al., 2007). Two rounds of conventional PCR assay amplifying the12s rDNA gene were performed with primer set ITM TnR-TaenF used for the first round PCR assay and primer set ITM TnR- nTAE used for the second round PCR assay. Five µl of cyst DNA was used as a DNA template for the first round PCR and 0.5 µl of first round PCR assay product was used as template DNA for the second round PCR. Positive control was *T. saginata* DNA provided by the Institute of Tropical Medicine, Antwerp, Belgium and the negative control was nuclease-free water. Initial denaturation was at 98 ºC for 10 seconds, run in one cycle, denaturation at 98 ºC for 45 seconds, annealing at 57 ºC for 45 seconds and extension at 72 ºC for a minute were followed by final extension which was set at 72 ºC for ten minutes for one cycle. Denaturation, annealing and extension were run for 35 cycles during the first round PCR assay and 30 cycles for the second round PCR assay. Gel electrophoresis was prepared using 4 g of agarose molecular grade, 200 ml 1x tae buffer and 6 µl of ethidium bromide. Gel electrophoresis was set at 100 V for one hour, with the12s rDNA gene of *T. saginata* read at 900 bp for the first round and 798 bp for the second round.

Restriction fragment length polymorphism (RFLP) was conducted according to Geysen et al., 2007 description, using second round PCR (ITMTnR-nTae) product, with addition of 0.3µl of *DdeI*, 0.3µl *HinfI*, 0.6µl *HpaI* restriction enzymes. Gel electrophoresis was performed at 100 V for one hour and *T. saginata* was read at 471 bp, 165 bp, 128 bp, 34 bp, 27 bp and 21 bp. DNA cloning was done using the second round PCR product. Colonies randomly selected from DNA cloning were used for colony PCR. Colony PCR reagents used were, 10 µl Dream Taq, 0.4µl of PJET F (10µl), 0.4µl PJET R (10µl), and 9.2µl nuclease-free water. The 12S rDNA secondary products read at approximately 798bp of three *T. saginata* PCR-RFLP field samples (10, 14 and 270) were randomly selected and cloned. A total of six clones (2 clones from each sample) were sequenced at Inqaba biotechnical Industries (Pty) Ltd, Pretoria, South Africa using the colony PCR product, PJET F and PJET R. A basic local alignment search tool (BLAST) (https://blast.ncbi.nlm.nih.gov/Blast.cgi), was used to search for sequences identical to a sequence corresponding to 12s rDNA gene of the *T. saginata* genome with a query cover of 100%. *Taenia saginata* 12s rDNA sequence obtained from bovine cysts recovered from a

slaughtered bovine in an abattoir in Gauteng province, South Africa (MZ357054) (Dube, 2020) and *T. saginata* genome (accession number: AY684274), which was sequenced from a *T. saginata* adult stage collected from a Belgian patient (Jeon et al., 2007) were used to search for sequences.

4.2.7 Cysticercosis Antigen ELISA

Serum was analysed using the B158/B60 monoclonal antibody-based Ag ELISA (apDia cysticercosis Antigen (Ag) ELISA REF 650510) following the manufacturer's instruction (Jansen et al., 2017). Serum samples were thawed at room temperature. Indirect ELISA was performed using microtiter plates with 12 x 8 well strips coated with B158C11A10 monoclonal antibodies (ApDia bvba, n.d.), negative control, positive control, conjugate (60ml peroxidase-conjugated monoclonal B60H8A4 antibodies having a red dye and antimicrobial agents) and washing solution (125ml x 20x concentrate phosphate buffered washing solution which contains 0.05% Proclin 300). Samples with Antigen index (Ag) ≥ 1.3 were considered positive, samples with Ag ≤ 0.8 were considered negative and samples with 0.8 < Ag index< 1.3 were considered doubtful. The final reading was calculated by subtracting the 650nm reading from the 450nm reading. The mean was calculated by dividing the sum of the duplicates of each sample by two. The Ag index was calculated according to the equation below, with the cut-off value calculated, as being equal to the mean optical density of negative control multiplied by two.

$$\text{Ag index} = \frac{Mean\ optical\ density\ sample}{Cut\ off\ value}$$

The coefficient of variation (CV) was calculated to judge whether the replicates are too far apart, with all samples having a CV>20% being retested. The coefficient of variation was calculated as being equal to the standard deviation of the 450nm and 650nm reading divided by the mean value of 450nm and 650nm reading and the result was multiplied by 100.

4.2.8 Data analysis

All data were entered in Microsoft Excel sheets and checked for consistency. First, descriptive statistics were calculated (e.g. proportion of cysts found in the different predilection sites). Then,

based on the Ag-ELISA and meat inspection results, the apparent prevalence was calculated according to the formula below (Hunt and Kaloshin, 2022):

$$Apparent\ prevalence = \frac{Number\ of\ diseased}{Total\ sample\ size} x\ 100$$

Next, the true prevalence obtained from Ag ELISA was calculated using the Bayesian method (Speybroeck et al., 2012) using R 4.2.1. The Bayesian method was done using previously published sensitivity and specificity of the B158/B60 Ag ELISA (Jansen et al., 2017), sample size, total number of positive test results and a priori probability distribution for true prevalence (Speybroeck et al., 2012). A uniform distribution ranging from 0 to 100%, was used as prior probability distribution for true prevalence, as it expresses that true prevalence can take any possible value and each possible value is equally likely.

To determine which test better estimates the prevalence of bovine cysticercosis between meat inspection and B158/B60 Ag ELISA test, Pearson Chi-Square test was used with null hypothesis accepted when *P* value > 0.05 and the null hypothesis rejected when *P* value ≤ 0.05. Strength of association between the meat inspection test and Ag-ELISA test was calculated using Phi and Cramers' V, with values > 0.25 signifying very strong association, > 0.15 strong association, > 0.10 moderate association, > 0.05 weak association and 0 no association or very weak association (Akoglu, 2018). Unless stated otherwise, the statistical analysis was done using IBM SPSS Statistics Version 28.0 (International Business Machines Corp., Armonk, NY, USA software).

4.3 Results

Seven out of 381 (1.84 %) cattle were positive to bovine cysticercosis on meat inspection, with *T. saginata* cysticerci mostly observed from the masseter as compared to other predilection sites (Figure 4.1).

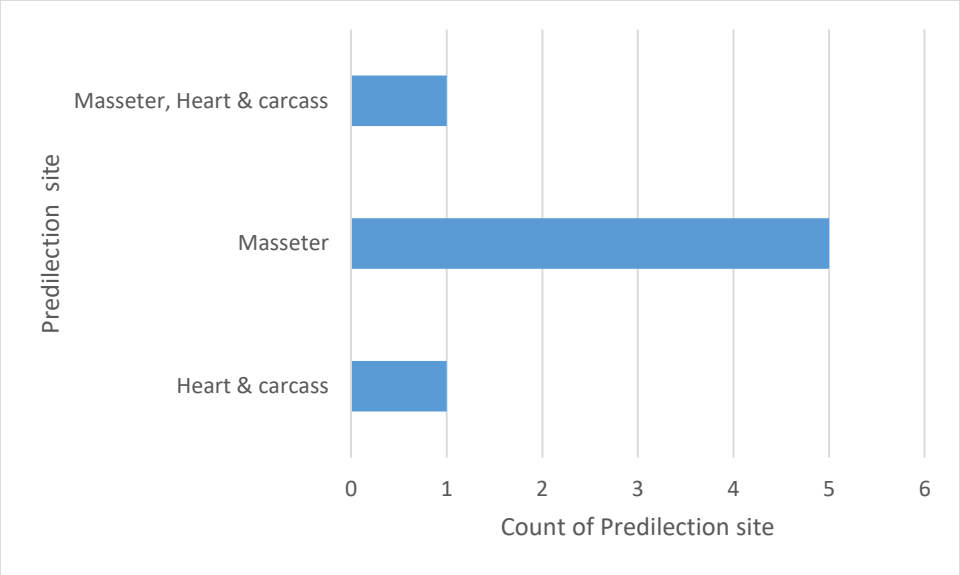

Figure 4.1: Number of *Taenia saginata* cysts recorded from various predilection sites during meat inspection

In some instances, viable cysts, degenerate cysts and calcified cysts were observed in the same animal. Majority (85.7%) of the positive cattle had viable cysts, whilst 28.7 % had degenerated cysts and 28.7% had calcified cysts (Table 4.1).

Table 4.1: Classification of cysts recovered from the study animals

Cattle identification number	Number of cysts observed	Viable	Degenerate	Calcified	Predilection site
10	1	1	0	0	Masseter
14	1	1	0	0	Masseter
41	12	11	0	1	Heart, carcass
95	1	1	0	0	Masseter
270	1	0	0	1	Masseter
329	8	4	4	0	Masseter
373	13	10	3	0	Masseter,

		heart, carcass

All cysticerci from the seven cattle that were positive on meat inspection were confirmed positive on PCR (Figure 4.2). The RFLP further confirmed the identity of the cysts recovered during meat inspection to be *T. saginata* cysticerci (Figure 4.3).

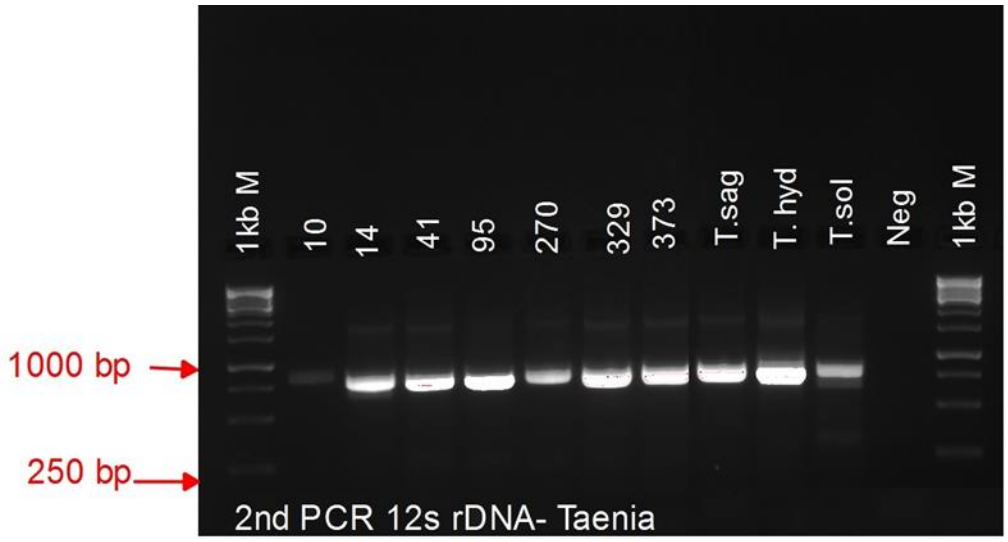

Figure: 4.2: Second round rDNA PCR product obtained from seven *Taenia saginata* positive cattle samples. Molecular weight marker (M) = 1kb DNA ladder, Tsag, T.hyd and T.sol= *Taenia saginata*, *T hydatigena*, *T solium* positive control; N=negative control (no DNA template; 10, 14, 41, 95, 270, 329, 373 = PCR products from cattle samples under investigations

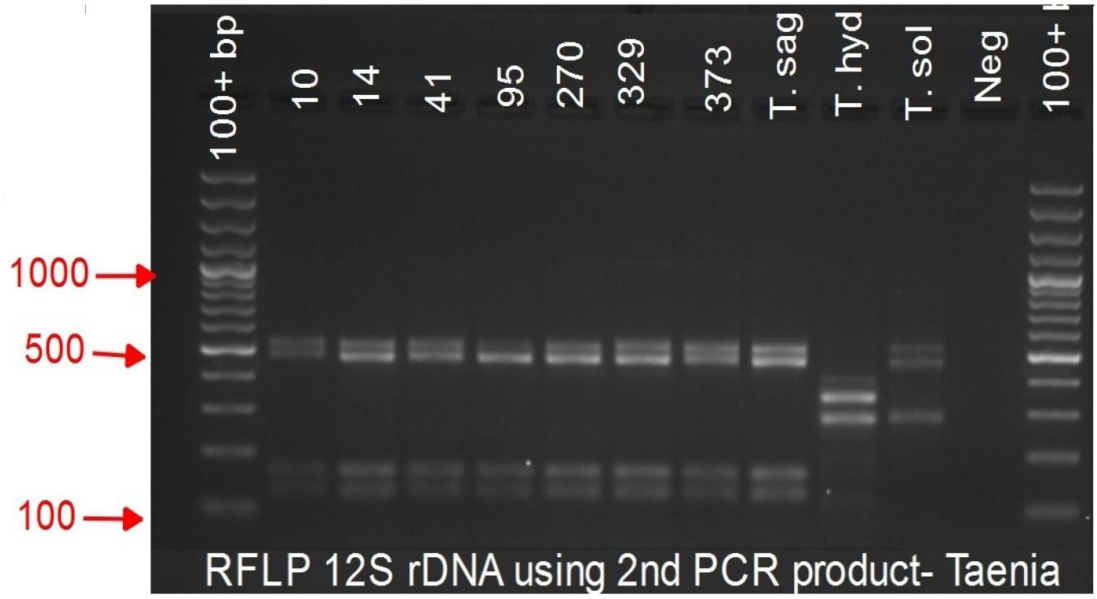

Figure 4.3: RFLP 12s rDNA second round PCR product. Molecular weight marker (M) = 100 bp DNA ladder; T. sag, T. hyd and T. sol = *Taenia saginata*, *Taenia hydatigena* and *Taenia solium* positive control; N = negative control (no DNA template); 10, 14, 41, 95, 270, 329, 373 = PCR products from cattle samples under investigation.

Search for sequences highlighted that they were 99.2 to 99.8 % identical (query cover 100%) to a sequence corresponding to the 12s rDNA gene of the *T. saginata* genome (accession number: AY684274), which was sequenced from a *T. saginata* adult stage collected from a Belgian patient (Jeon et al., 2007). The sequences were 99.8 % to 99.9 % identical (query cover 100%) to a *T. saginata* 12s rDNA sequence obtained from bovine cysticercosis cysts recovered from a slaughtered bovine in an abattoir in Gauteng province, South Africa (MZ357054) (Dube, 2020). Two sequences from two of the samples, 14 (clone ID 4) and 270 (cone ID 2) have been deposited in the GenBank under accession numbers ON692928 and ON692929 respectively.

A total of 369 out of 384 cattle inspected during meat inspection were from Matabeleland region. Therefore, out of the 369 cattle from the Matabeleland region, on Ag-ELISA, the majority (177; 48.0 %) were negative, 140 (37.9 %) were positive and 52 (14.1 %) had doubtful results (Figure 4.4).

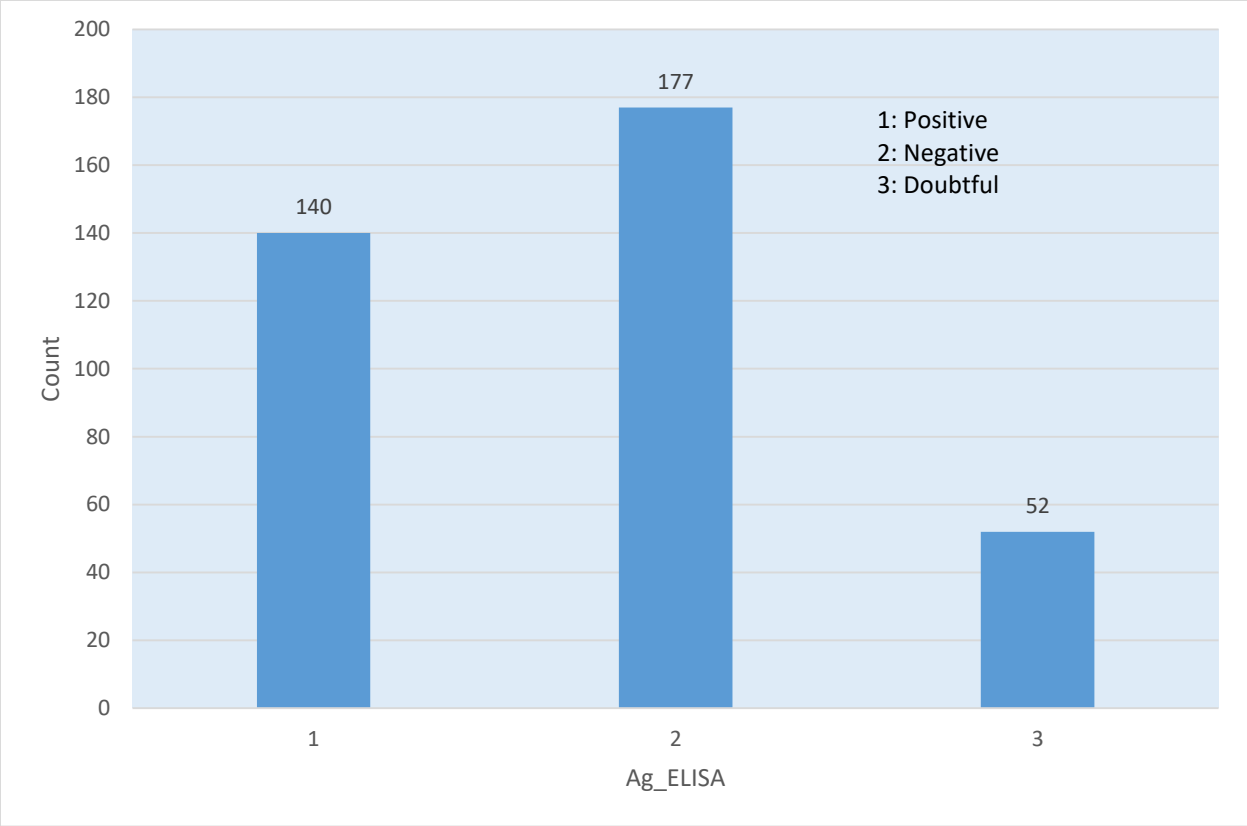

Figure 4.4: Bar chat illustrating the number of Ag-ELISA results of bovine cysticercosis samples collected in 2021 in Matabeleland region of Zimbabwe.

Apparent prevalence of meat inspection of cattle from Matabeleland region was twenty times less than that of Ag-ELISA (Table 4.2). Matabeleland North had the highest apparent prevalence on meat inspection whilst Matabeleland South had the highest apparent prevalence on Ag-ELISA (Table 4.3).

Table 4.2: Apparent prevalence of bovine cysticercosis in Matabeleland region

Test	N positive / N sampled	Apparent prevalence
Meat inspection	7/369	1.90%
Ag-ELISA	140/369	37.9%

Table 4.3: Apparent prevalence of bovine cysticercosis per province in Matabeleland region

Province	Meat Inspection	Ag-ELISA
Matabeleland South	1/1140.88%)	47 /114 (41.2%)
Matabeleland North	5/202 (2.48%)	74 / 202(36.6%)
Bulawayo	1/53 (1.89%)	19/53 (35.9%)

Matabeleland South had the highest 97.5 quantile considered as true prevalence on the Bayesian method, followed by Matabeleland North and lastly Bulawayo (Table 4.4).

Table 4.4: True prevalence estimation from the Bayesian model

Province	Mean	Standard deviation	2.5% quantile	97.5% quantile
Matabeleland South	92.1	6.17	77.2	99.7
Matabeleland North	89.3	6.56	75.2	99.1
Bulawayo	83.2	11.08	58.7	99.3

4.4 Discussion

The current study reports for the first time the prevalence of bovine cysticercosis in cattle slaughtered in Matabeleland region of Zimbabwe using meat inspection confirmed by PCR and sequencing of cysts and Ag ELISA as detection methods. Previous studies on the prevalence of bovine cysticercosis in this region used only meat inspection (Pugh and Chambers, 1989;Sungirai et al., 2014) and thus may have underestimated the true prevalence of the disease as this method is known to have low sensitivity, especially in lightly infected carcasses (Jansen et al 2015). The low prevalence of bovine cysticercosis (1.84 %) as detected by meat inspection in the present study was expected and is comparable to the 2.1 % that was reported by Pugh and Chambers (1989) and 1.6 % reported by Sungirai et al. (2014). These results indicate that most of the cattle slaughtered in this region may have light positive cases infections and could thus require more sensitive methods than meat inspection to detect these infections and prevent the introduction of infected carcasses into the human food chain.

Meat inspection can mistakenly detect lesions similar to *T. saginata* cysticerci such as actinobacillosis and *Sarcocystis* (Hassan El-Sayad et al., 2021; Laranjo-González et al., 2016),

therefore it is essential to confirm the metacestodes isolated during meat inspection with PCR (Cuttell et al., 2013; Geysen et al., 2007). The high specificity of meat inspection in detecting *T. saginata* cysticerci as confirmed by the PCR and sequencing in the present study could be attributed to the experience of the inspectors or the absence of other diseases which can cause lesions similar to *T. saginata* cysts. Though meat inspection has a low sensitivity, its accuracy is improved when performed by experienced personnel. Six of the seven cattle positive on meat inspection (85.71%) in the present study had viable cysticerci suggesting that these were easily recognized by meat inspection personnel. The experience of the inspectors may be responsible for the detection of the one calcified cyst which was confirmed to be a *T. saginata* cysticercus on RFLP PCR and sequencing. All these seven cattle were also confirmed to be positive for bovine cysticercosis by Ag ELISA which detects the viable cysticerci of *T. saginata* in cattle (Jansen et al., 2016). Viable cysticerci are the infective stage to humans when consumed in raw or undercooked beef (FAO, 1994). The present results confirm the assertion that, though meat inspection is a widely adopted tool to detect the presence of bovine cysticercosis in cattle, it may not be enough to prevent the inadvertent introduction of undetected infected carcasses into the human food chain.

In the present study, *T. saginata* cysticerci were observed in the masseter, heart and the carcass as per known predilection sites (Alemneh et al., 2017; Kebede et al., 2008). Majority of cysticerci were observed in the masseter muscle (>72%) whilst in the previous studies carried out in Zimbabwe approximately 58% of the metacestodes were observed inthe head only, 20% on the shoulder, 8% on the heart and the majority accounting for 81% of the infection were observed on the head, shoulder and heart (OIE, 2018).The results differ from, this study where 14% of the animals had infection in the head, heart and the rest of the carcass. Dissection of the predilection sites was considered to improve the sensitivity for detection of bovine cysticercosis (Jansen et al., 2018),

The observed significantly lower ($P < 0.05$) prevalence of bovine cysticercosis determined by meat inspection compared to Ag ELISA could be attributed to the higher sensitivity of the latter test as reported by previous authors (Dorny et al., 2002; Asaava et al., 2009). The present findings agree with those of Da Silva et al. (2015), who reported that meat inspection inadequately detects mildly infected carcasses. Allepuz et al. (2012) reported that prevalence

determined by meat inspection is approximately 50 % lower than seroprevalence which is in concordance with findings of the present study, where apparent prevalence obtained through meat inspection was twenty times less than by Ag-ELISA. The Ag ELISA detects circulating parasite antigens and presents some diagnostic advantages since it demonstrates active infections (Dorny et al., 2003)

The apparent prevalence of meat inspection (1.90%) detected is almost similar to that obtained by Sungirai et al. (2014) of 1.6% and Matabeleland North province had a higher prevalence than other provinces agreeing to the research previously done by Sungirai et al., 2014. Bulawayo being a metropolitan province had a prevalence of 1.89% on meat inspection and 35.9% on Ag-ELISA. Most cattle from Bulawayo were coming from feed lots and there is need to further investigate the reason why there is bovine cysticercosis in the feedlots. A study carried out in South Africa indicated that bovine cysticercosis in feedlots was contributed by sourcing cattle from dry areas where cattle and humans share the same limited water source (Verwoerd, 2017) and a study in Zambia revealed that cattle are already infected upon arrival in the feedlot as most cattle are purchased from traditional farms where there is poor sanitation and lack of latrines (Dorny et al., 2002). Matabeleland South had the highest apparent prevalence by Ag-ELISA as opposed to the results obtained through meat inspection and the results obtained by Sungirai et al., 2014 and this may be due to the low sensitivity of meat inspection (<15%) resulting in some positive cases being missed during inspection (Jansen et al., 2016).

According to a study carried out in Belgium, the B158/B60 Ag ELISA has a sensitivity of 40% and a specificity of 100% (Jansen et al., 2017) and Ag-ELISA tests are mostly used for epidemiological purposes (OIE, 2014), as in the case of this study. The Ag- ELISA test is not a gold standard test, but it better estimates the prevalence of bovine cysticercosis than meat inspection (Jansen et al., 2016; Allepuz et al., 2012), as observed in this study.

4.5 Conclusion

Despite the accuracy of meat inspection in detecting bovine cysticercosis observed in this study, it still has a low sensitivity as compared to Ag-ELISA. Despite Ag-ELISA not being the gold standard test for diagnosing bovine cysticercosis, it is still useful in estimating the prevalence of bovine cysticercosis. Results obtained in the study revealed presence of viable cysts, hence a

need to further study the presence and prevalence of taeniasis in humans. There is need for investigating the reasons the source of bovine cysticercosis in feedlots located in Bulawayo metropolitan province.

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Chapter 5: General discussion, conclusion and recommendations

5.1 General Discussion

The study sought to find out the possible risk factors of bovine cysticercosis in Matabeleland region and determine knowledge, practices and attitudes towards bovine cysticercosis. In chapter 3, communal cattle farmers' knowledge, attitudes and practices on the risk factors of bovine cysticercosis in the Matabeleland region of Zimbabwe were investigated. About (316/377; 83.3%) of farmers in Bulilima district slaughter their animals at home, of which (263/377; 69.8%) of the home slaughters had farmers observing the meat and they hardly knew anything about bovine cysticercosis as (366/377; 97.08%) did not know about the disease. Home slaughter in the absence of meat inspection and poor meat inspection policies can contribute to possible increase in human taeniasis cases, which is of public health concern (Alemneh, Adem and Akeberegn, 2017; Tsotetsi-Khambule et al., 2017). Majority of the participants (366/377; 97.0%) had not heard about bovine cysticercosis. Lack of knowledge can lead to poor practices concerning the prevention and control of the disease amongst farmers ultimately contributing to the high prevalence of bovine cysticercosis in the Matabeleland region. About forty-percent (149/377; 39.5 %) of the participants had no access to toilets and (358/377; 95%) of the participants sometimes defecated in the bush when in places with no toilets. Despite the presence of toilets, some participants had a habit of using the bush, thereby increasing the risk of bovine cysticercosis. Poor sanitation practices increase the risk of bovine cysticercosis as cattle get infected by grazing pastures or drinking water contaminated with *T. saginata* eggs shed in human faeces(McBrien and Courcier, 2013; Bruschi and Gómez-Morales, 2017).

The study investigated the prevalence of bovine cysticercosis using both meat inspection and B158/B60 monoclonal antibody-based Ag ELISA. The study confirmed cysticerci identified

during meat inspection as those of *T. saginata* using polymerase chain reaction. Chapter 4 aimed to determine the prevalence of bovine cysticercosis using antigen enzyme-linked immunosorbent assay (Ag ELISA) and confirm the identity of cysts detected during meat inspection using polymerase chain reaction (PCR) and sequencing in cattle slaughtered in three abattoirs in Matabeleland region of Zimbabwe. Ag-ELISA had a prevalence of (140/369; 37.9%), which was significantly higher ($P < 0.05$) than that of meat inspection (7/ 381; 1.83 %). Ag-ELISA detects viable cysticerci and thus active cattle infections with *T. saginata* cysticerci, demonstrating the possibility of introducing infected carcasses into the food chain and thus the risk of human taeniasis cases. All the *T. saginata* cysticerci identified during meat inspection were confirmed positive on PCR despite the low sensitivity of detection of metacestodes by meat inspection (Laranjo-González *et al.*, 2016). Most of the cysticerci identified during meat inspection were viable and located in the masseter. Humans get infected when they consume undercooked or raw beef and viscera containing viable cysticerci (FAO, 1994). To prevent the inadvertent introduction of infected cattle carcasses into the human food chain, it may be essential to augment meat inspection with Ag ELISA in the detection of bovine cysticercosis during slaughter of cattle.

Zimbabwe has a meat inspection control policy which states that no one is allowed to sell, keep, transport or expose for sale any meat or offal unless it has been inspected by a meat inspector (Public Health Regulations 50 of 1995). Despite the presence of the control policy, there is still consumption of uninspected meat as the study revealed that only (6/377; 1.6%) of the participants in the survey slaughter either in abattoirs or designated slaughter points. About two-fifths of the participants had no access to toilets at home and (358/377; 95%) of the participants sometimes practised open defecation, thereby increasing the likelihood of bovine cysticercosis as, infected humans defecating in or near cattle grazing areas may result in high cases of bovine cysticercosis (Laranjo-González *et al.*, 2016).

Bovine cysticercosis is a disease of socio-economic importance (Braae *et al.*, 2018) and the presence of the disease can result in economic losses to the communal farmer as about (300/377; 79.6%) of the participants relied on livestock as their source of income and (242/377; 64.2%) kept cattle as their source of income and (223/377; 59.20%) chose cattle as their livestock of choice.

5.2 Conclusion

Ag-ELISA is more sensitive than meat inspection, therefore better estimating the prevalence of bovine cysticercosis than meat inspection. The competency of the meat inspector is important in accurately diagnosing bovine cysticercosis . Home slaughter, inadequate or absence of meat inspection, consumption of raw or undercooked meat with viable cysticerci, inadequate or lack of toilets and open defecation are risk factors for bovine cysticercosis and taeniasis.

5.3 Recommendations

Due to the presence of viable cysticerci observed in the study and positive results on Ag-ELISA, there is need to investigate further on presence and prevalence of taeniasis cases in humans. There is need to educate the public on bovine cysticercosis and encourage communal livestock farmers to construct and regularly use toilets, so as to reduce the incidences of open defecation and Department of Veterinary Services to provide affordable meat inspection facilities/ services.

5.4 References

Alemneh, T., Adem, T. and Akeberegn, D. (2017) 'Mini Review on Bovine Cysticercosis', *J Healthc Commun*, 2(2), p. 15. DOI: 10.4172/2472-1654.100055.

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FAO (1994) *Manual on meat inspection for developing countries*. Available at: https://www.fao.org/3/t0756e/T0756E00.htm#TOC (Accessed: 12 August 2022).

Hassan El-Sayad, M. *et al.* (2021) '*Cysticercus bovis* in cattle slaughtered in North Egypt: Overestimation by the visual inspection method'. DOI: 10.14202/vetworld.2021.155-160.

Laranjo-González, M. *et al.* (2016) 'Epidemiology, impact and control of bovine cysticercosis in Europe: a systematic review'. DOI: 10.1186/s13071-016-1362-3.

Lateef, M. *et al.* (2020) 'Epidemiology of *Taenia saginata* taeniasis with emphasis on its prevalence and transmission in a Kashmiri population in India: A prospective study', *International Journal of Infectious Diseases*. Elsevier B.V., 98, pp. 401–405. doi: 10.1016/j.ijid.2020.06.088.

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Sungirai, M., Masaka, L. and Mbiba, C. (2014) 'The prevalence of *Taenia saginata* cysticercosis in the Matabeleland Provinces of Zimbabwe', *Tropical Animal Health and Production*, 46(4), pp. 623–627. DOI: 10.1007/s11250-014-0538-0.

Appendices 1: Research Ethics Committee

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Faculty of Veterinary Science

Research Ethics Committee 16 November 2021

LETTER OF APPROVAL

Ethics Reference No	**REC039-21**
Protocol Title	**Prevalence and associated risk factors of bovine cysticercosis in Matabeleland region of Zimbabwe**
Principal Investigator	**Dr ZK Ncube**
Supervisors	**Dr T Tshuma**

Dear Dr ZK Ncube,

We are pleased to inform you that your submission conforms to the requirements of the Faculty of Veterinary Sciences Research Ethics committee.

Please note the following about your ethics approval:

1. Please use your reference number (REC039-21) on any documents or correspondence with the Research Ethics Committee regarding your research.
2. Please note that the Research Ethics Committee may ask further questions, seek additional information, require further modification, monitor the conduct of your research, or suspend or withdraw ethics approval.
3. Please note that ethical approval is granted for the duration of the research as stipulated in the original application (for Post graduate studies e.g. Honours studies: 1 year, Masters studies: two years, and PhD studies: three years) and should be extended when the approval period lapses.
4. The digital archiving of data is a requirement of the University of Pretoria. The data should be accessible in the event of an enquiry or further analysis of the data.

Ethics approval is subject to the following:

1. 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.
2. **Applications using Animals:** FVS ethics recommendation does not imply that AEC approval is granted. The application has been pre-screened and recommended for review by the AEC. Research may not proceed until AEC approval is granted.

We wish you the best with your research.

Yours sincerely

PROF M. OOSTHUIZEN
Chairperson: Research Ethics Committee

100 YEARS OF VETERINARY EDUCATION

Room 8-6, Arnold Theiler Building
University of Pretoria, Faculty of Veterinary Science
Private Bag X04, Onderstepoort 0110, South Africa
Tel +27 (0)12 529 8300
Email marie.watson-kriek@up.ac.za
www.up.ac.za

Faculty of Veterinary Science
Fakulteit Veeartsenykunde
Lefapha la Disaense tša Bongakadiruiwa

Appendices 2: Humanities Ethics

Faculty of Humanities
Fakulteit Geesteswetenskappe
Lefapha la Bomotho

15 October 2021

Dear Dr ZK Ncube

Project Title:	Prevalence and associated risk factors of bovine cysticercosis in Matabeleland region of Zimbabwe
Researcher:	Dr ZK Ncube
Supervisor(s):	Dr T Tshuma Dr MC Marufu
Department:	Production Animal Studies
Reference number:	20539852 (HUM017/0921)
Degree:	Masters

Thank you for the application that was submitted for ethical consideration.

The application was ***conditionally*** **approved** by the **Research Ethics Committee** on 2021-09-30 due to the following:
- Pending permission from the Medical Research Council of Zimbabwe

Please note that data collection may not commence. Once the outstanding documentation and sufficient clarification is submitted, full ethical clearance will be granted. To facilitate the administrative process, please log onto the Peoplesoft ethics platform and select the 'Docs due Conditional approval' tab to upload a cover letter, addressing each of the above issues, together with any supporting/ outstanding documents.

Sincerely,

Prof Karen Harris
Chair: Research Ethics Committee
Faculty of Humanities
UNIVERSITY OF PRETORIA
e-mail: tracey.andrew@up.ac.za

Room 7-27, Humanities Building, University of Pretoria, Private Bag X20, Hatfield 0028, South Africa
Tel +27 (0)12 420 4853 | Fax +27 (0)12 420 4501 | Email pghumanities@up.ac.za | www.up.ac.za/faculty-of-humanities

Appendices 3: National Animal Research Ethics Committee

National Animal Research Ethics Committee
All communications should be addressed to:
The Chairman
National Animal Research Ethics Committee
18A Borrowdale Road
P.O Box CY 551, Causeway
Harare
Vetlabs@dots.org.zw
263-24-703753, 705886/7

18 October 2021

To: Dr Z K Ncube

Ref: Protocol Title: Prevalence and associated risk factors of bovine cysticercosis in Matabeleland region of Zimbabwe (008/2021).

We have the pleasure to inform you that the National Animal Research Ethics Committee (NAREC) has granted the approval to conduct the above research, Payment of RTGs12 000 will be required for registration.

Project Name: "Prevalence and associated risk factors of bovine cysticercosis in Matabeleland region of Zimbabwe."

Institution: University of Pretoria

Principal investigator: Dr Z K Ncube

22/10/2021
Mr D. Dube
Agriculture Research Council
Vice Chair
National Animal Research Ethics Committee (NAREC)

25/10/2021
Dr P.V. Makaya
Chairman
National Animal Research Ethics Committee (NAREC)

Appendices 4: Animal Ethics Committee

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Faculty of Veterinary Science
Animal Ethics Committee

5 October 2021

Approval Certificate
New Application

AEC Reference No.:	REC039-21
Title:	Prevalence and associated risk factors of bovine cysticercosis in Matabeleland region of Zimbabwe
Researcher:	Dr ZK Ncube
Student's Supervisor:	Dr T Tshuma

Dear Dr ZK Ncube,

The **New Application** as supported by documents received between 2021-04-21 and 2021-10-01 for your research, was approved by the Animal Ethics Committee on its quorate meeting of 2021-10-01.

Please note the following about your ethics approval:

1. The use of species is approved:

Species	**Number**
Beef cattle	384 Abattoir
Samples Tissue	384
Blood	384 (collection after slaughter ONLY)

2. Ethics Approval is valid for 1 year and needs to be renewed annually by 2022-10-05.
3. Please remember to use your protocol number (REC039-21) 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.theeder@up.ac.za

Fakulteit Veeartsenykunde
Lefapha la Diseanse tša Bongakadinuiwa

Appendices 5: Medical Research Council of Zimbabwe

Telephone: 08644073772/791193
E-mail: mrcz@mrcz.org.zw
Website: http://www.mrcz.org.zw

Medical Research Council of Zimbabwe
Josiah Tongogara / Mazowe Street
P. O. Box CY 573
Causeway
Harare

APPROVAL

MRCZ/B/2188 **09 November, 2021**

Dr. Zolile Kwakhiwe Ncube
65070 Tshabalala Extension
Nkulumane
Bulawayo

RE: - Prevalence and Associated Risk Factors of Bovine Cysticercosis in Matebeleland Region of Zimbabwe

Thank you for the application for review of research activity that you submitted to the Medical Research Council of Zimbabwe (MRCZ). Please be advised that the Medical Research Council of Zimbabwe has **reviewed** and **approved** your application to conduct the above titled study.

This approval is based on the review and approval of the following documents that were submitted to MRCZ for review: -

- Completed MRCZ 101 new application form
- Informed Consent Forms (English and Shona)
- Study protocol
- Data collection tool

- **APPROVAL NUMBER : MRCZ/B/2188**

This number should be used on all correspondence, consent forms and documents as appropriate.

- **TYPE OF MEETING : EXPEDITED**
- **APPROVAL DATE : 09 November, 2021**
- **EXPIRATION DATE : 08 November, 2022**

After this date, this project may only continue upon renewal. For purposes of renewal, a progress report on a standard form obtainable from the MRCZ offices should be submitted three months before the expiration date for continuing review.

- **SERIOUS ADVERSE EVENT REPORTING**: All serious problems having to do with subject safety must be reported to the Institutional Ethical Review Committee (IERC) as well as the MRCZ within 3 working days using standard forms obtainable from the MRCZ Offices or website.
- **MODIFICATIONS**: Prior MRCZ and IERC approval using standard forms obtainable from the MRCZ Offices is required before implementing any changes in the Protocol (including changes in the consent documents).
- **TERMINATION OF STUDY**: On termination of a study, a report has to be submitted to the MRCZ using standard forms obtainable from the MRCZ Offices or website.
- **QUESTIONS**: Please contact the MRCZ on Telephone No. (0242) 791193, 0864407377203 or by e-mail on mrcz@mrcz.org.zw

Other

- Please be reminded to send in copies of your research results for our records as well as for Health Research Database.
- You're also encouraged to submit electronic copies of your publications in peer-reviewed journals that may emanate from this study.
- In addition to this approval, all clinical trials involving drugs, devices and biologics (including other studies focusing on registered drugs) require approval of Medicines Control Authority of Zimbabwe (MCAZ) before commencement.

Yours Faithfully

.....

MRCZ SECRETARIAT
FOR CHAIRPERSON
MEDICAL RESEARCH COUNCIL OF ZIMBABWE

MEDICAL RESEARCH COUNCIL OF ZIMBABWE
2021 -11- 09
APPROVED
P.O. BOX CY 573 CAUSEWAY HARARE

PROMOTING THE ETHICAL CONDUCT OF HEALTH RESEARCH

Appendices 6: Section 20

agriculture, land reform & rural development

Department:
Agriculture, Land Reform and Rural Development
REPUBLIC OF SOUTH AFRICA

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
Reference: 12/11/1/1/6 (1944KL)

Dr. Zolile Kwakhiwe Ncube
Department of Production Animal Studies
University of Pretoria
Tel: 012 529 8227
E-mail: chris.marufu@up.ac.za; zkmadlela@gmail.com

Dear Dr Ncube

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

Your undated application signed on 5 May 2021 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 undated application signed on 5 May 2021 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. 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;
4. Only the following may be used in this research project:
   4.1. Blood samples and Taenia cysts from clinically healthy cattle at Bulawayo Abattoir in Bulawayo, Circle Y Abattoir in Bulawayo and Mvutcha Abattoir in Bulawayo;

– 1 –

4.2. Taenia cysts must be frozen in accordance with the official meat safety protocols of Zimbabwe and dissected out of the carcasses before being placed in 'Primestore'.

4.3. Blood samples and Taenia cysts must be inactivated at 56 °C for at least 30 minutes at the Provincial Veterinary Laboratory in Bulawayo;

4.4. Inactivation of blood and Taenia samples must be performed under the supervision of the person responsible for the laboratory. This person must issue written confirmation of the sample identification numbers, temperature and time at which the heat inactivation was conducted;

5. Veterinary import permits in terms of Section 6 of the Animal Diseases Act 1984 (Act no 35 of 84) must be obtained for the importation of all cattle blood samples and Taenia cycsts, prior to importation;
6. This research project must be conducted in the Research and Training Laboratories, at the Department of Veterinary Tropical Diseases, Onderstepoort;
7. All potentially infectious material utilised or generated during or by the research project is to be destroyed at completion of the research project;
8. Only waste disposal companies registered to remove biohazardous waste may be used for the removal of waste from the research project
9. It is the responsibility of the researcher and relevant laboratory or facility managers to ensure that the human safety aspects of this research project are adequately addressed;
10. Records must be kept for five years for auditing purposes;

Title of research/study: Prevalence and associated risk factors of bovine cysticercosis in Matabeleland region of Zimbabwe
Researcher: Dr. Zolile Kwakhiwe Ncube
Institutions: Research and Training Laboratories, Department of Veterinary Tropical Diseases, Faculty of Veterinary Sciences, Onderstepoort;
Permit Expiry Date: 31 December 2022
Our ref Number: 12/11/1/1/6 (1944KL)
Your ref: REC039-21

Kind regards,

DR. MPHO MAJA
DIRECTOR: ANIMAL HEALTH
Date:
2021-06-23

SUBJECT: *PERMISSION TO DO RESEARCH IN TERMS OF SECTION 20 OF THE ANIMAL DISEASES ACT, 1984 (ACT NO. 35 OF 1984) 12/11/1/1/6 (1944KL)*

Appendices 7: Section 20 amendment

agriculture, land reform & rural development

Department:
Agriculture, Land Reform and Rural Development
REPUBLIC OF SOUTH AFRICA

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
Reference: 12/11/1/1/6 (1944KL1)

Dr. Zolile Kwakhiwe Ncube
Department of Production Animal Studies
University of Pretoria
Tel: 012 529 8227
E-mail: chris.marufu@up.ac.za; zkmadlela@gmail.com; Takula.Tshuma@up.ac.za

Dear Dr Ncube

AMENDMENT OF SECTION 20 APPROVAL IN TERMS OF THE ANIMAL DISEASES ACT, 1984 (ACT NO 35 OF 1984) FOR: "*PREVALENCE AND ASSOCIATED RISK FACTORS OF BOVINE CYSTICERCOSIS IN MATABELELAND REGION OF ZIMBABWE*"

Your application dated 8 September 2021 for an amendment to a Section 20 permit refers. The following amendment is hereby granted on point 4.2 of the Section 20 permit with reference number 12/11/1/1/6 (1944KL) that was issued for the abovementioned study:

i) Taenia cysts must be frozen in accordance with the official meat safety protocols of Zimbabwe and dissected out of the carcasses before being placed in at least 70% ethanol.

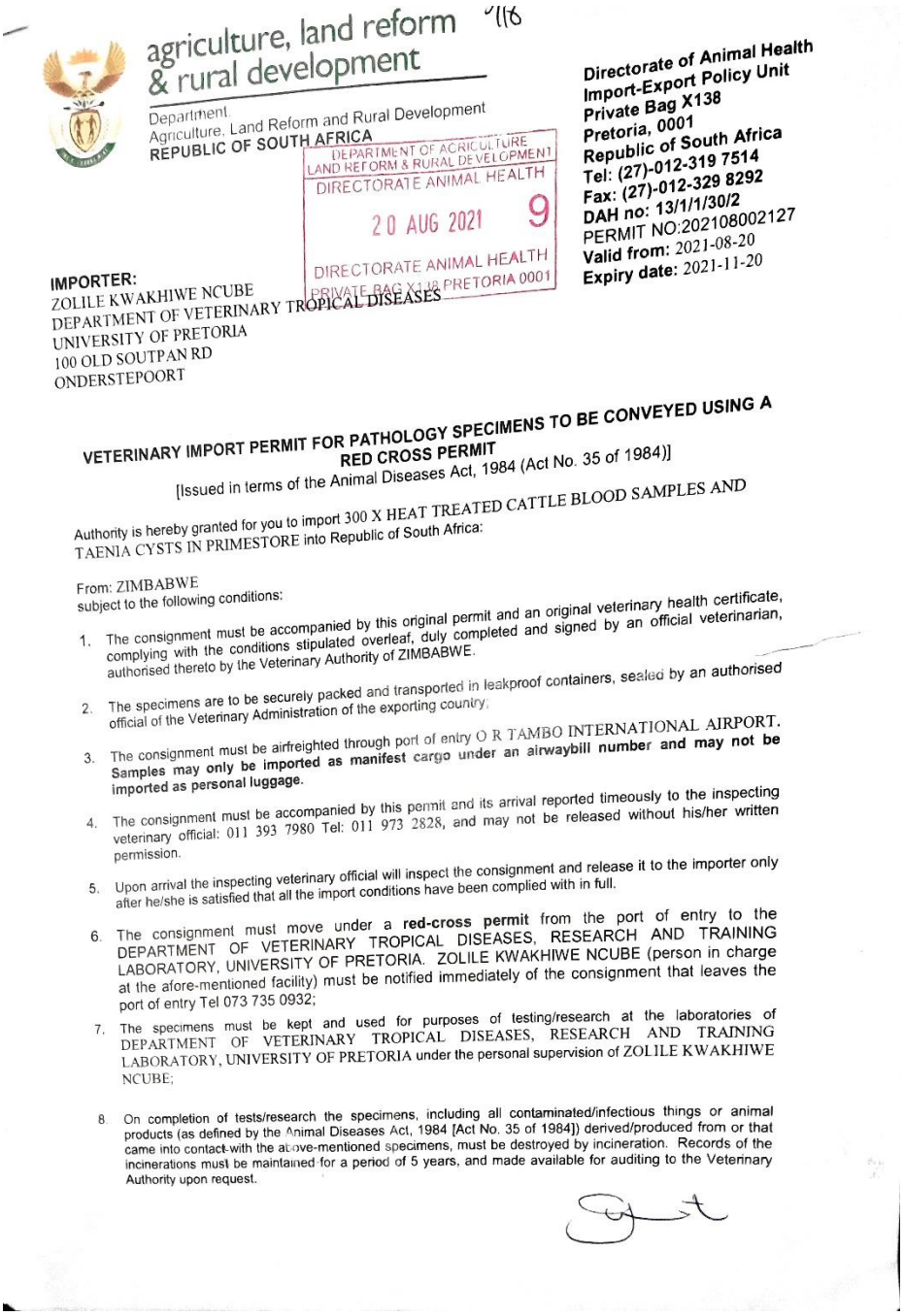
All other conditions as specified in Section 20 permit with reference number 12/11/1/1/6 (1944KL) remain in full effect.

Kind regards,

DR. MPHO MAJA
DIRECTOR: ANIMAL HEALTH
Date: 2021 -09- 28

– 1 –

Import permit 8: Import permit



agriculture, land reform & rural development

Department
Agriculture, Land Reform and Rural Development
REPUBLIC OF SOUTH AFRICA

DEPARTMENT OF AGRICULTURE
LAND REFORM & RURAL DEVELOPMENT
DIRECTORATE ANIMAL HEALTH
20 AUG 2021
9
DIRECTORATE ANIMAL HEALTH
PRIVATE BAG X138 PRETORIA 0001

Directorate of Animal Health
Import-Export Policy Unit
Private Bag X138
Pretoria, 0001
Republic of South Africa
Tel: (27)-012-319 7514
Fax: (27)-012-329 8292
DAH no: 13/1/1/30/2
PERMIT NO:202108002127
Valid from: 2021-08-20
Expiry date: 2021-11-20

IMPORTER:
ZOLILE KWAKHIWE NCUBE
DEPARTMENT OF VETERINARY TROPICAL DISEASES
UNIVERSITY OF PRETORIA
100 OLD SOUTPAN RD
ONDERSTEPOORT

VETERINARY IMPORT PERMIT FOR PATHOLOGY SPECIMENS TO BE CONVEYED USING A RED CROSS PERMIT

[Issued in terms of the Animal Diseases Act, 1984 (Act No. 35 of 1984)]

Authority is hereby granted for you to import 300 X HEAT TREATED CATTLE BLOOD SAMPLES AND TAENIA CYSTS IN PRIMESTORE into Republic of South Africa:

From: ZIMBABWE
subject to the following conditions:

1. The consignment must be accompanied by this original permit and an original veterinary health certificate, complying with the conditions stipulated overleaf, duly completed and signed by an official veterinarian, authorised thereto by the Veterinary Authority of ZIMBABWE.
2. The specimens are to be securely packed and transported in leakproof containers, sealed by an authorised official of the Veterinary Administration of the exporting country.
3. The consignment must be airfreighted through port of entry O R TAMBO INTERNATIONAL AIRPORT. **Samples may only be imported as manifest cargo under an airwaybill number and may not be imported as personal luggage.**
4. The consignment must be accompanied by this permit and its arrival reported timeously to the inspecting veterinary official: 011 393 7980 Tel: 011 973 2828, and may not be released without his/her written permission.
5. Upon arrival the inspecting veterinary official will inspect the consignment and release it to the importer only after he/she is satisfied that all the import conditions have been complied with in full.
6. The consignment must move under a **red-cross permit** from the port of entry to the DEPARTMENT OF VETERINARY TROPICAL DISEASES, RESEARCH AND TRAINING LABORATORY, UNIVERSITY OF PRETORIA. ZOLILE KWAKHIWE NCUBE (person in charge at the afore-mentioned facility) must be notified immediately of the consignment that leaves the port of entry Tel 073 735 0932;
7. The specimens must be kept and used for purposes of testing/research at the laboratories of DEPARTMENT OF VETERINARY TROPICAL DISEASES, RESEARCH AND TRAINING LABORATORY, UNIVERSITY OF PRETORIA under the personal supervision of ZOLILE KWAKHIWE NCUBE;
8. On completion of tests/research the specimens, including all contaminated/infectious things or animal products (as defined by the Animal Diseases Act, 1984 [Act No. 35 of 1984]) derived/produced from or that came into contact with the above-mentioned specimens, must be destroyed by incineration. Records of the incinerations must be maintained for a period of 5 years, and made available for auditing to the Veterinary Authority upon request.

Signature

9. On completion of tests/research the specimens, including all contaminated/infectious things or animal products (as defined by the Animal Diseases Act, 1984 [Act No. 35 of 1984]) derived/produced from or that came into contact with the above-mentioned specimens, must be destroyed by incineration. Records of the incinerations must be maintained for a period of 5 years, and made available for auditing to the Veterinary Authority upon request.
10. **This permit does not absolve the importer from compliance with the provisions of any other legislation relating to this import**
11. This permit is subject to amendment or cancellation by the Director Animal Health at any time and without prior notice being given.
12. This permit is valid for three (3) months from date of issue and FOR ONE CONSIGNMENT ONLY.

SPECIAL CONDITIONS:

IN ADDITION, THE VETERINARY HEALTH CERTIFICATE (DESCRIBED IN CONDITION 1 ABOVE) ISSUED BY A VETERINARIAN AUTHORIZED HERETO BY THE VETERINARY AUTHORITIES OF ZIMBABWE, MUST CERTIFY THAT:

1. BLOOD SAMPLES AND TAENIA CYSTS WERE COLLECTED FROM CLINICALLY HEALTHY CATTLE PRESENTED FOR SLAUGHTER
2. ALL THE BLOOD SAMPLES AND TAENIA CYSTS HAVE BEEN INACTIVATED AT 56°C FOR AT LEAST 30 MINUTES AT THE PROVONCIAL VETERINARY LABORATORY, BULAWAYO

DIRECTOR: ANIMAL HEALTH

NOTE:

- All imports for research purposes require Section 20 permission in compliance with the Animal Diseases Act.
- Any consignment imported into South Africa packed with either wood packaging material or dunnage, will require treatment to remove any pests present (by heat or methyl bromide fumigation). Treatment must be indicated as per IPPC prescript on wood packaging material. [Directorate: Inspection Services Tel: 012 309 8754 or Fax 086 732 4768 or www.daff.gov.za]

Appendices 9: Export permit

All communications should be addressed to
"CHIEF DIRECTOR"
Telephone 242 791516, 242-707683
Fax 791516
Email vetpermits@gmail.com

ZIMBABWE

Reference: VB/2/13/

DEPARTMENT OF VETERINARY SERVICES
Ministry of Lands, Agriculture, Water, & Rural Resettlement
P.O Box CY 66
Causeway
Harare

18 OCTOBER 2021

TO WHOM IT MAY CONCERN

EXPORT OF SAMPLES TO SOUTH AFRICA

This letter serves to confirm that **DR ZOLILE KWAKHIWE NCUBE** has been authorised to export the following samples:

- 384 bovine serum samples
- 37 *Taenia saginata* cysticerci

For research purposes to:

University of Pretoria,
Department of Veterinary Tropical Diseases,
Faculty of Veterinary Science,
Private bag X04,
Onderstepoort 0110,
RSA

These samples are non-infectious and do not cause a hazard to people handling them or risk of spreading disease into South Africa.

CHIEF DIRECTOR
DEPT OF VET SERVICES
REGULATORY OFFICE
13 OCT 2021
P.O. BOX CY 66, CAUSEWAY, HARARE
TEL: +263-242-706238/706603

Dr. Clementine V Mandizvidza
Veterinary Imports & Exports Officer
For: Chief Director Veterinary Services

Appendices 10: Questionnaire

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA
Faculty of Veterinary Science

Consent form for farmers to participate in the study
[Prevalence and associated risk factors of bovine cysticercosis in Matabeleland region of Zimbabwe]

Introduction
This research study is being conducted by [Dr Zolile Kwakhiwe Ncube] registered as a Masters student at the University of Pretoria, South Africa, to [determine the prevalence and associated risk factors of bovine cysticercosis in cattle and associate it with human taeniasis cases in the Matabeleland region, Zimbabwe.].

Procedure
The participants will be asked to respond to closed and open-ended questions prepared on a questionnaire. The interview will take approximately 15 minutes per individual farmer .The questions will include details about [knowledge about bovine cysticercosis, availability and usage of toilets]

Risks
There are no risks for participation in this study.

Benefits
There will be no monetary benefits from participation in this study. The information that will be obtained during the study on [Prevalence and associated risk factors of bovine cysticercosis in Matabeleland region of Zimbabwe] will [equip the farmer with knowledge about bovine cysticercosis, transmission, and prevention].

Participation
Participation of respondents in this study is voluntary. The participants may refuse to participate or withdraw at any time during the study. However, non-participation will disadvantage the community in that the results obtained from the study will not be a true representation of the data in the study area. A misrepresentation of the results will affect [the true representation of associated risk factors of bovine cysticercosis and affect possible future intervention from possible assistance from relevant stakeholders].

Confidentiality
All the information will remain confidential. The data will be recorded as group data with no identifying information and data will be destroyed should participants withdraw from the study. The data obtained from the participants will be secured in the Institutional Repository for a period of 15 years. Only the Supervisors, the researcher and other persons directly involved in the research will have access to the information.

Access to the researcher
If participants have enquiries or wish to withdraw from the study, they may contact [Zolile Kwakhiwe Ncube] at [00263782116509]

Location ..... Date .....

Signature of Participant..... Signature of Researcher.....

Prevalence and associated risk factors of bovine cysticercosis in Matabeleland region of Zimbabwe

[Prevalence and associated risk factors of bovine cysticercosis in Matabeleland region of Zimbabwe]

SECTION A (FARMER'S/ OWNER DEMOGRAPHY)

1. Are you the.....

- ◯ Owner
- ◯ Animal attendant/caretaker

2. Gender:

- ◯ Male
- ◯ Female

3. What was your age: ..... years

4. What is your marital status:

- ◯ Single (Never married)
- ◯ Married
- ◯ Divorced
- ◯ Widow(er)

5. What is your level of education: .....

6. What is your home language:

- ◯ Ndebele
- ◯ Shona
- ◯ Xhosa
- ◯ Sotho
- ◯ English
- ◯ Kalanga
- ◯ Nambya
- ◯ Tonga
- ◯ Other

» Household size

7a. Adult males

_____

7b. Adult females

_____

7c. Children males

_____

7d. Children females

_____

8. How long have you been raising animals? .....

Years

_____

9. What livestock species do you keep?

- [ ] Cattle
- [ ] Sheep
- [ ] Goats
- [ ] Chickens
- [ ] Other

9. What livestock species do you keep?

If more than 1 separate by a comma

_____

Cattle kept

_____

Sheep kept

_____

Goats kept

_____

Chickens kept

_____

Livestock importance

1st choice

- ◯ Cattle
- ◯ Sheep
- ◯ Goats
- ◯ Chickens

2nd choice

- ◯ Cattle
- ◯ Sheep
- ◯ Goats
- ◯ Chickens

3rd choice

- ◯ Cattle
- ◯ Sheep
- ◯ Goats
- ◯ Chickens

4th choice

- ◯ Cattle
- ◯ Sheep
- ◯ Goats
- ◯ Chickens

10. What are your sources of income?

- ☐ Livestock
- ☐ Crops
- ☐ Old age pension
- ☐ Salary/Wages
- ☐ Other
- ☐ Not applicable

10. What are your sources of income?

1st choice

- ◯ Livestock
- ◯ Crops
- ◯ Old age pension
- ◯ Salary/Wages

2nd choice

- ◯ Livestock
- ◯ Crops
- ◯ Old age pension
- ◯ Salary/Wages

3rd choice

- ◯ Livestock
- ◯ Crops
- ◯ Old age pension
- ◯ Salary/Wages

SECTION B (CATTLE HERD INFROMATION)

11. What breed of cattle do you keep?

- ☐ Nguni
- ☐ Tuli
- ☐ Mashona
- ☐ Non descript
- ☐ Other

Nguni kept

_____

Tuli kept

_____

Mashona kept

_____

Non-descript kept

_____

» 12. What are the sexes of your cattle:

Males (non castrate)

_____

Castrated males

_____

Females

_____

13. What is the body condition score of your herd

1 poor , 5 very good

0 5

14. Do you have calving pens (space for giving birth):

- ◯ Yes
- ◯ No

15. Do you have restraining equipment :

- ◯ Yes
- ◯ No

16. Do you have weight estimation equipment :

- ◯ Yes
- ◯ No

17. Which system of management do you adopt in your farm:

- ◯ Rural Household/Backyard
- ◯ Extensive
- ◯ Semi-Intensive
- ◯ Intensive

18. What is your cattle's major source of water

- ◯ Steam/Flowing river
- ◯ Public water
- ◯ Well
- ◯ Public Borehole
- ◯ Private borehole
- ◯ Other

19. What is your major source of cattle feed?

- ◯ Concetrate
- ◯ Grazing
- ◯ Mixed feeding
- ◯ Other

SECTION C (RISK FACTORS AND AWARENESS)

» C1: Awareness about bovine cysticercosis.

20. Have you heard about bovine cysticercosis (beef measles)?

- ◯ Yes
- ◯ No
- ◯ Unsure

21. Have you received any education on public health awareness about bovine cysticercosis (beef measles)?

- ◯ Yes
- ◯ No

22. Where did you acquire this information?

_____

23. Can you give the local name for the condition

- ◯ Yes
- ◯ No
- ◯ Unsure

24. What name is it called in your vernacular language?

_____

25. Do you know how cattle acquire cysticercosis?

- ◯ Yes
- ◯ No

26. If yes, please specify .....

_____

27. How is this disease transmitted to humans? Ingestion of undercooked beef or offals

- ◯ Yes
- ◯ No
- ◯ Unsure

27. How is this disease transmitted to humans? ingestion of raw beef or offals

- ◯ Yes
- ◯ Not
- ◯ Unsure

28. Is measled beef dangerous for human consumption?

- ◯ Yes
- ◯ No

» C2: Slaughter and meat inspection

29. Where do you slaughter your cattle?

- ◯ At home
- ◯ Designated slaughter point
- ◯ abattoir

30. When slaughtering at home, does the meat get inspected?

- ◯ Yes
- ◯ No

31. Who inspects the meat?

- ◯ Farmer
- ◯ Animal health technician
- ◯ Veterinary doctor
- ◯ Extension worker

32. What do you look for when inspecting the meat?

_____

33. Have you had any cattle diagnosed with bovine cysticercosis during meat inspection?

- ◯ Yes
- ◯ No
- ◯ Unsure

34. Who inspects the meat?

- ◯ Farmer
- ◯ Animal health technician
- ◯ Veterinary doctor
- ◯ Extension worker

35. Have you had any of your cattle condemned during meat inspection due to bovine cysticercosis

- ◯ Yes
- ◯ No
- ◯ Unsure

36. Has any of your cattle undergone cold treatment due to bovine cysticercosis at the abattoir?

- ◯ Yes
- ◯ No
- ◯ Unsure

37. What do you do when abnormalities are found on the carcass?

- ◯ Discard
- ◯ Sell
- ◯ Consume

» C3: Sanitation

38. Do you have access to a toilet at home?

- ◯ Yes
- ◯ No

39. If yes, how often do you use the toilet?

- ◯ Always
- ◯ Often
- ◯ Sometimes
- ◯ Never

40. Do you have access to a toilet at local gathering points?

- ◯ Yes
- ◯ No

41. If yes, how often do you use the toilet?

- ◯ Always
- ◯ Often
- ◯ Sometimes
- ◯ Never

42. Do you have access to a toilet in the grazing area

- ◯ Yes
- ◯ No

43. If yes, how often do you use the toilet?

- Always
- Often
- Sometimes
- Never

44. If No to any of the questions 36, 38 & 40 where do you defecate?

_____

45. When open defecating do you bury the faecal matter

- Yes
- No

46. Do you use clean water and soap to wash your hands after defecating?

- Water only
- None
- Water and soap

» C4: Medical hits

47. Have you been diagnosed with worms in the past 5 years

- Yes
- No

48. Have you been dewormed in the previous year

- Yes
- No

THANK YOU FOR YOUR PARTICIPATION

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