BOVINE CYSTICERCOSIS: PREVALENCE, RISK FACTORS, ITS SOCIO-ECONOMIC EFFECTS ON CATTLE FARMERS IN BOTSWANA AND IDENTIFICATION OF IMMUNOGENIC ANTIGENS

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DOCTOR OF PHILOSOPHY (Ph.D.)

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by

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Thesis submitted in partial fulfilment of the requirements for the degree of

DOCTOR OF PHILOSOPHY (Ph.D.)

at

Botswana University of Agriculture and Natural Resources

Faculty of Animal and Veterinary Sciences

Department of Animal Science and Production

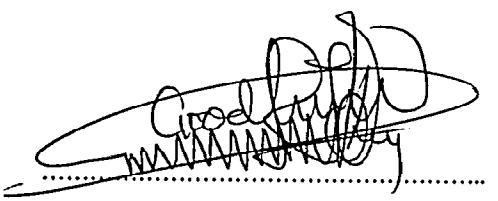
By

GOODHEAD OKECHUKWU ORIZU UCHENDU

September, 2020

DECLARATION

I declare that the dissertation hereby submitted by me for the degree of Doctor of Philosophy (Animal Science and Production) at the Botswana University of Agriculture and Natural Resources (BUAN) is my own independent work and has not previously been submitted by me at another University/Faculty for the award of any other degree or diploma.



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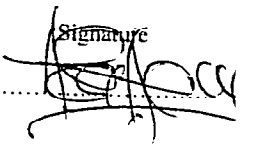
Agango A.O	28/09/2020	
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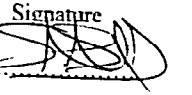
Ramahi S.S	28/09/2020	
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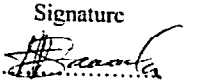
DAVIS MARUMO	28/09/2020	
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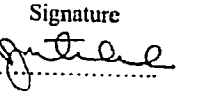
P.M. KGWATALALA	28/09/2020	
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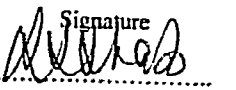
R G Chabo	07/10/2020	

ABSTRACT

Botswana's incidence of *Cysticercus bovis (C. bovis)* following passive post-mortem inspection has been increasing consistently, with attendant public health and socioeconomic implications. Most published prevalence data emanate solely from Botswana Meat Commission's data, which is not representative. The government of Botswana has practiced several traditional, prevention and control methods without achieving a decline in *C. bovis* prevalence. It is probable that either the important risk factors were misdiagnosed and/or the traditional control and prevention methods targeted at identified risk factors are inadequate. Although these risk factors are behavioural and systemic, there is no known work that associates them with the lifestyle of residents of Botswana in order to advocate for behaviour change. Vaccines and vaccination have shown to be effective in *C. bovis* prevention and control. There is no known commercial vaccine for *C. bovis*. The objective of this research was to determine the prevalence and risk factors of *C. bovis*; its effects on the socio-economy of beef farmers in Botswana and to designate the potential immuno-dominant epitopes. Non-participatory structured questionnaire, interviews, physical enumeration and measurements were used to study the demographic profile of cattle, cattle ownership & cattle farming systems in Botswana as a means of delimitating important study area and understanding the non-climatic factors affecting Botswana's cattle population. A combination of traditional method of passive post mortem abattoir inspection and a novel use of structured non-participatory questionnaire administered directly to farmers were used to determine *C. bovis* prevalence and hotspots. Results obtained from the study of both methods were compared. This combination provides more representative results than using only the abattoir inspection method. Likert scale format was used to collect survey data on risk factors of *C. bovis*. Descriptive statistics of median and inter quartile range were used to determine the most probable response for the risk factor in each particular population and level of polarity about the particular risk factor respectively. Chi-square was used to determine the quantitative relevance (role) of each risk factor, while binomial logistic regression was used to test the significance of individual risk factors in the prediction of the prevalence of *C. bovis*. Effects of C. bovis on the socioeconomy of farmers were determined by subjecting 14 objectively verifiable socioeconomic indicators to binomial regression, while examining for gender and age differences. In addition, financial losses were estimated using simple arithmetic. Through a one-dimensional electrophoresis using Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) the constituent proteins of *C. bovis* were teased out. Polyclonal primary antibody of bovine origin

and Rabbit anti-Bovine IgG (H+L) secondary antibody Horse Radish Peroxidase (HRP) were used to challenge antigens derived from cysts fluid and whole cysts of *Cysticercus bovis* isolated from beef slaughtered at the Multi Specie Abattoir Gaborone Botswana. Analysis of results showed an abattoir prevalence of 17.17% and survey prevalence of 42.35%; both of which are higher than the published prevalence of 13.5%. At 0.025 ($P<0.05$) both are significantly different from each other. In addition to delimitating novel hotspots in Botswana, this study showed significant difference of 0.00 ($P<0.05$) in prevalence among abattoirs as well as significant differences among and within districts and regions. The higher survey prevalence and the significant difference between abattoir and survey prevalence results demonstrates that BMC data is true representative of *C. bovis* prevalence in Botswana. Of the eighteen (18) tested risk factors, fourteen (14) contributed significantly ($P<0.05$) to the prevalence of *C. bovis*., with 'access to contaminated feed being the single most important risk factor. There is strong agreement (high consensus) among respondents that the factors of, 'access to contaminated feed, water' and pasture'; 'failure to deworm herd boys'; 'proximity to uncontrolled human defecation'; 'grazing of animal'; 'absence and or distanced pit latrine in the farm' are major contributors to the prevalence of *C. bovis*. Seasonal index showed close association between increased *C. bovis* incidences and outset of rainfall in the summer month of December; harvesting of Mopane worms in April and cutting of grass for roof thatching in October. Identified novel risk factors are political, behavioural, systemic and sociocultural. Results of financial and socioeconomic study indicate that devaluation or condemnation of carcass resulted in various levels of financial loss. The 'ability to save money' was most significantly affected at 0.007 ($P<0.05$) and 'experience of emotional disturbance' was significantly affected at 0.000 ($P<0.05$). The 'ability to provide food for family' was equally significantly affected at 0.097 ($P<0.10$). Also affected were farmers' ability to provide food, healthcare, education, rental for family. Furthermore, *C. bovis* caused farmers not to employ new workers; to diversify or abandon farming business. Reduced income caused 'farmers' inability to meet social, religious and family obligations'; leading some to borrowing money for upkeep of family and/or farming business. There were gender and age differences in *C. bovis* effects, which were not statistically significant ($P< 0.05$). The farmers who experienced *C. bovis* in their farms were 10.02 times more like not to save money compared to farmers who did not. Equally, farmers who experienced *C. bovis* in their farms were 29.30 times and 7.29 times more likely to experience emotional disturbance and inability to provide food for their family respectively. Severity of *C. bovis* effects on farmers' socioeconomy were dependent on the magnitude of the

infestation, the scale of production of farmers and presence or absence of some survival strategies. Farmers' response to financial & socioeconomic effects ranged from outright closure of farms, to reduction in farm capacity, to diversification of businesses. Some farmers experienced psycho-social effects. Most effective government interventions were provision of re-stocking seed calves; payment for cold treatment of infested carcass and installation of socio-economic amenities. All these played vital roles in cushioning the adverse effects of *C. bovis* on the livelihood pattern of the farmers. Electrophoretic profiling of the *C. bovis* identified nineteen (19) proteins with molecular weights of 4, 6, 14, 17, 22, 25, 28, 32, 38, 44, 50, 67, 75, 100, 115, 135, 150, 190, 245kDa. Immunoblotting under reducing conditions yielded eight (8) immunogenic proteins with molecular weights at 14, 22, 25, 50, 98, 135, 190 and 245kDa. These immunogenic proteins where further confirmed to be specific for *C. bovis*. This was done by carrying out a western blot using serum from Rabbit experimentally exposed to *C. bovis* as a source of primary antibody and Goat anti-Rabbit IgG (H+L) Secondary Antibody HRP to produce antibody-antigen complex. Chilling of samples did not cause significant difference ($P<0.05$) in quantity of proteins contained in samples however, immunogenic proteins were lost after 7 days of chilling. This study recommends that prevention and control measures should target a paradigm shift in the politics, method of cattle farming and socio-cultural lifestyle (behaviour) of Batswana. While this may be far-fetched, production of vaccines using the identified immunogenic proteins would be the best approach on the long run. Development of antemortem diagnostic kits using monoclonal antibodies and silver dyes as markers is equally recommended. Results of this study have provided information that is relevant for development of effective interventions and policy advocacy for improved beef production, increasing farmers' income, ensuring food security, and adequate management of *Taeniasis saginata* in man and bovine cysticercosis in cattle in Botswana. These guarantees attainment of sustainable development goals numbers 1,3, 5, 8, 10 and 12 in Botswana.

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I thank God because he gave me life, wisdom and strength to carry out this work. When I almost gave up, by word of knowledge he said to me, "son, increase the concentrations of your antibodies and change your colorimetric agent". I thank my Uncle, Prof. O. N. Ama for inviting me over to Botswana and upon my arrival in 2016 I encountered the Botswana Vaccine Institute, from when I rekindled my passion in vaccinology. I thank my immediate family for always calling to check on me and spurring me on with this work. I acknowledge that but for the supervision, encouragement, harassments and advice of Prof. A. O Aganga, this work would not be in your hands today. I thank Prof. Ama who painstakingly read through the studies on prevalence, risk factors and socio-economy and Dr. Marumo as well as Prof Ama for teaching me biostatistics. I thank Dr. Ramabu for reading through the study on profiling of *C. bovis* proteins and identification of immunogenic ones. I acknowledge that Prof. Nsoso kindled my interest to study bovine cysticercosis, while Prof. Madibela paid for transportation cost for survey. I thank Prof. Kgwatalala for being the first academic friend who always taught me to laugh through the tough times. I acknowledge that Mrs. Mogkwati, the University's Dean of Students took me in as her son. She literally gave me food everyday even when I had toothpicks in my mouth. I thank my sons and daughters, members of IPDYI and students of BUAN, who always asked me lots of questions about my research and made me sit up. I thank all the staff of Botswana Vaccine Institute (BVI) particularly those who worked with me in the OIE laboratory and those who cheered me on especially the gate men who kept me company when I worked late into the night. I may not mention all of you but I must mention Boss, Mr. Elliot F.; Senior Boss, Dr. George Mathlo; Dr. Hyera; Misses Thato, Kupo, Abi. Mr. Modise of the Veterinary unit and his crew. I can't forget all the staff of the Multi Specie Abattoir Botswana (MSAB), especially Dr. Steve Mugwanya, who assisted me with over 100 cysts samples across 30 abattoir visits. I thank Dr. Olupot who wrote the first letter permitting me to visit Botswana abattoirs.

Most importantly, I thank my family in Botswana, the members of RPC, in no particular, Alister, Real five, CEO, Bishop Isaac, Apostle, Pastor Dennis the Nyirenda family, the Macholos, the Thipes, and Mona-Lisa. I thank my mentor who always called to pray with me and encourage me at my trying times.

Finally, I acknowledge that this work is original; carried out by me and that the contents have not been used either inpat or full for award of any other degrees in any other university.

DEDICATION

ıvork is dedicated to my daughter, Emmanuella Goodhead Uchendu, who has been ıted from me by events of life and distance. Nana, Daddy loves you specially and shall ɔe back

dedicate this work to all the cattle and cattle farmers in Botswana and beyond who have ıg looked forward to the end of the scourge of bovine cysticercosis. I say to you, we shall ind a lasting solution to this menace.

LIST OF TABLES

LIST OF FIGURES

LIST OF ACRONYMS

AAP: American Academy of Paediatrics

AASR: Annual Agricultural Survey Report

ANOVA: Analysis of Variance

APA: American Psychological Association.

APS: Ammonium Per sulphate

BMC: Botswana Meat Commission

BTO: Botswana Tourism Organization

BUAN: Botswana University of Agriculture and Natural Resources

BWP or P: Botswana Pula

C. bovis: *Cysticercus bovis*

CBPP: Contagious Bovine Pleuropneumonia

CDC: Centres for Disease Prevention and Control

CEDA: Citizen Entrepreneurial Development Agency

CSO: Central Statistics Office

DNA: Dibonucleic Acid

DTT: Dithiothreitol sample buffer

Et. al.,: And Others (as par authors)

ETP: Ethiopian Birr

EU: European Union

EX: Exponential (as used in Statistics table)

FMD: Foot and Mouth Disease

FQ: Frequency (as used in Statistics table)

GDP: Gross Domestic Product

GNP: gross national product

HRP: Horse Radish peroxidase

IgG: (H+L) Immunoglobin G

IMF: International Monetary Funds

IQR: inter quartile range

KUP: Kuppuswami Scale

MES: common name for 2- ethanesulfonic acid

MSAB: Multi Species Abattoir Botswana

NIAD: National Institute of Allergy and Infectious Diseases

NCIRS: National Centre for Immunization Research and Surveillance

NAMPAADD: National Master Plan for Arable Agriculture and Dairy Development

NHS: National Health System

NNI: Net national income

OIE: International Organization for Animal Health

PDF: portable document format

PhD: Doctor of Philosophy

PVDF: Polyvinylidene fluoride membrane

RPC: Rhema Prophetic Centre

RV: Reverse Vaccinology

SADC: South African Development Community

SDS-PAGE: Sodium dodecyl Sulphate Polyacrylamide Gel Electrophoresis

SDG: Sustainable development Goals

SPSS: Statistical Package for Social Science For Windows

TSA: Taenia saginata Antigen

UNICEF: United Nations International Children's Fund

UNDP: United Nations Development Programme

USAID: United States Agency for International Development

USD: United States Dollar

WHO: World Health Organization

LIST OF ABBREVIATIONS

Bid: two times daily

C. bovis: Cysticercus bovis

Kg: Kilo grams

Mg: Milli grams

Mm: Milli Meters

Nd: No date (as found in citations and references)

NO. : Number

T. saginata :Taenia saginata

t-test: students' *t*-test

Viz: these include

°C: Degrees Celsius

SEVENTEEN SUSTAINABLE DEVELOPMENT GOALS

Goal 1: End poverty in all its forms everywhere

Goal 2: End hunger, achieve food security and improved nutrition and promote sustainable agriculture

Goal 3: Ensure healthy lives and promote well-being for all at all ages

Goal 4: Ensure inclusive and equitable quality education and promote lifelong learning opportunities for all

Goal 5: Achieve gender equality and empower all women and girls

Goal 6: Ensure availability and sustainable management of water and sanitation for all

Goal 7: Ensure access to affordable, reliable, sustainable and modern energy for all

Goal 8: Promote sustained, inclusive and sustainable economic growth, full and productive employment and decent work for all

Goal 9: Build resilient infrastructure, promote inclusive and sustainable industrialization and foster innovation

Goal 10: Reduce inequality within and among countries

Goal 11: Make cities and human settlements inclusive, safe, resilient and sustainable

Goal 12: Ensure sustainable consumption and production patterns

Goal 13: Take urgent action to combat climate change and its impacts

Goal 14: Conserve and sustainably use the oceans, seas and marine resources for sustainable development

Goal 15: Protect, restore and promote sustainable use of terrestrial ecosystems, sustainably manage forests, combat desertification, and halt and reverse land degradation and halt biodiversity loss

Goal 16: Promote peaceful and inclusive societies for sustainable development, provide access to justice for all and build effective, accountable and inclusive institutions at all levels

Goal 17: Strengthen the means of implementation and revitalize the Global Partnership for Sustainable Development

Table of Contents

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background of the Study

Botswana is a landlocked country with a human population of 2,316,960 in 2018 (Statistics Botswana, 2019). In Botswana, as in most African countries, the agricultural sector is vital to socio-economic development of the nation. It employs about 26.43% of total employment and 42.5% of rural population (Statistics Botswana, 2016; Trading Economics, 2017). The agricultural sector has experienced decline in its contribution to national GDP; from 42.7% in 1966 to 1.9% in 2008 (Ministry of Finance and Development Planning, 2010 in United Nations Development Programme [UNDP], 2012); later rising to about 3% in 2011; of which the livestock component (beef export) was 2.6% (Burgess, 2006). Real values contribution to GDP decreased from P1,562.45 million (P7.76 = 1USD) in 2011 to P1,447.70 million (P9.8 = 1 USD) in 2015 to P1,433.30 million (P10.00 = 1USD) in 2016 (Trading Economics, 2017).

Botswana's livestock sector is more developed than plant sector; its department being the largest department in the Ministry of Agriculture; employing about 47.6% of entire staff strength in ministry of Agriculture (World Bank, 2010). By 2012, the livestock sector represented 87% of the agriculture's contribution to the GDP (FAOSTAT, 2012 in FAO, 2014). The Beef industry has been described as the largest component of the Agrifood System (Engelen, *et. al.*, 2013); contributing about 70% of agriculture's share of Gross Domestic Product due to export to European Union markets (Moreki & Ntshese, 2008). It employs over 70% of the rural population especially at subsistence level (UNDP, 2012); thus, serving as a tool for rural income transformation & redistribution and poverty alleviation.

Botswana is Africa's largest beef exporter (Engelen, *et. al.*, 2013; Burges, 2006; Tshiamo, 2015). This sector has been highlighted as strategic within the country's economic diversification agenda (Pablo, *et. al.*, 2014). Cattle population grew progressively from 2.25 million in 1996 to 3.10 million in 2005 to over 3.5 million cattle in 2012 (Burgess, 2006). Cattle population has dropped progressively since 2102 from 3.5 million to about 1.1 million (traditional only) in 2017 (Statistics Botswana 2019). Unlike in most other countries of the world, where cattle farming is an exclusive occupation of few farmers, in Botswana, cattle ownership is a pride factor; as every family owns cattle (Thornton *et. al.*, 2003 in Patti *et. al.*

2010). This is the natural advantage that the nation has over other nations in the beef industry (Pablo, *et. al.*, 2014). Nonetheless, cattle population dropped to about 1.7 million in 2015 to 1.9 million cattle in 2019; with a 45 percent decline in households that raise cattle, from about 75,500 in 2004 to 39,000 in 2015 (Statistics Botswana, 2019).

The cattle industry is symbolic for the farming community, as a potential unifier and as an important source of food (Pablo, *et. al.*, 2014; Tshiamo, 2015).

The beef industry has been adversely affected by *Taenia saginata*/cysticercosis infestation. The larval stage of a tape worm, *Taenia saginata (T. saginata)* resides obligately in human intestine. This zoonosis constitutes both an important public health hazard as well as an economic loss to cattle farmers. Infected humans pass out the eggs of this parasite along with feces. Cattle become infected when they pick up these eggs while grazing. The oncosphere hatch from the eggs and migrate to skeletal tissues developing into cysts or bovine cysticercosis commonly referred to as beef measles. Humans become infected when they consume infected beef which had not been appropriately treated either by freezing or cooking (Urquhart et. al., 1996). Botswana's incidence of bovine cysticercosis following passive post-mortem inspection, were 12-15% in 1985 (Mosienyane, 1986); 11.045% in 2008; 10.78% in 2009; 8.03% in 2010 and 23% in 2014 in villages with high density of human population and poor hygiene status (Farmers' Magazine, 2016). Beef from carcasses identified during meat inspection procedures as having beef measles cannot be exported to the European Union. This resulted in annual loss of export earnings of about one million (P1M) (P1.00 = 0.5 USD) in 1978 (Grindle, 1978); five million pula (P5M), (P1.00 = 0.68 USD) in detained and/or condemned carcasses per year as at 1985 (Mosienyane, 1986), and about P35 million in 2008 (Aganga, 2009). Statistics show that in 2009, 2010, and 2012, beef exports worth P99,645,780 (P10.56 = 1USD) (International Monetary Fund [IMF], nd.), P100, 477, 260 (P6.33 = 1USD), and P83, 289, 960 (P7.78 = 1USD) respectively, could not be sold to the EU markets (Tshiamo, 2015).

In its determination to control this public health hazard and economic loss, the government of Botswana has practised mass literacy on proper disposal of human defecate, adequate and efficient meat inspection among several other traditional, prevention, control and intervention measures (Oladele & Lesotho, 2010). These efforts have not sufficed in reducing incidence of bovine cysticercosis. Going forward, the government has equally proposed mass treatment of humans with the use of anthelmintic.

The probable pitfalls of mass treatment of humans would include drug failure, and/or patients' non-adherence to treatment regimen, among other factors. Secondly, although literature claims that the parasite is obligate in man (Urquhart et. al., 1996), assuming higher primates like

baboons are carriers of this worm, even if all human beings were treated, eradication of bovine cysticercosis may be impossible since untreated primates, which are in close proximity to humans and cattle in Botswana, would serve as reinfection source.

Meanwhile, the occurrence and spread of bovine cysticercosis have been maintained by risk factors (determinants) which are socio-cultural (behavioural) and systemic, but avoidable (Aganga, 2017: personal communication). These factors seem to have defied the aforementioned traditional, control and prevention efforts, since prevalence rates continue to rise. It is probable that interpreting the risk factors of this zoonosis in relation to lifestyle (behaviour) of the locals shall provide adequate knowledge requisite for effective control and prevention.

Literature shows that some developed countries have adopted the use of vaccines, which have been effectively used to control bovine cysticercosis (Lightowlers, *et. al.*, 1996). A practical vaccine to prevent infection with the parasite in cattle would be valuable and would assist in controlling transmission of the parasite to humans. Young calves have been protected against infection by vaccination with non-living antigens from the parasite, especially antigen derived from the oncosphere which is contained within the egg (Rickard & Adolph 1976; Rickard *et. al.*, 1981; Rickard and Brumley 1981). From these oncosphere antigens were derived epitopes, which were further identified and categorised as immune-dominant epitopes (Lightowlers, *et. al.*, 1996).

Development of vaccine was conceived practicable in Botswana because the nation has a well-established and reputable Botswana Vaccine Institute (BVI), universities of agriculture and other research institutes whose expertise and environment were available for the identification of vaccine candidate antigens.

It was therefore the aim of this research to study the demography of cattle and cattle farmers, as means of describing the cattle industry; the hot spots and prevalence of bovine cysticercosis as a means of demonstrating the occurrence and severity of bovine cysticercosis the risk factors of bovine cysticercosis as a means of establishing bovine cysticercosis determinants as forces maintaining its high prevalence; to determine the effect of bovine cysticercosis on the socio-economy of cattle farmers in Botswana as a means of assessing the suitability of the existing intervention measures. Furthermore, this work profiled the bovine cysticercosis proteins and identified immunogenic protein. The identified proteins shall become the basic inputs for vaccine development and production of antemortem test kits.

1.2 Statement of the Problem

Botswana's cattle population dropped from 3.5 in 2012 to 1.7 million in 2015 to 1.9 million cattle in 2019; with a 45 percent decline in households raising cattle, from about 75,500 in 2004 to 39,000 in 2015 (Statistics Botswana, 2019). Available records on cattle population are usually obtained from Botswana government data, which contain only information about cattle numbers across geographical strata. Observed decline of Botswana cattle population is attributed to unthrifty climate (temperature, rainfall), unavailability of feed during cold dry winter seasons, poor funding, lack of basic farm inputs, etc. However, some sociocultural and political factors bear more serious effect on the cattle population. These information are either unresearched or undocumented.

Most of Botswana's bovine cysticercosis prevalence figures emanate from data available at the Botswana Meat Commission (BMC) (Modisa, 2014). The BMC, the country's national export abattoir, anticipated the possibility of losing the patronage of the EU beef market because of increasing rates. Consequently, the BMC streamlined sourcing of cattle by avoiding designated hotspots; while providing containment measures for hotspots; like, sponsoring professionally managed paddocks/kraals/pens and maintaining strict inspection policies in accordance with the provisions of the Livestock and Meat Industries Act (2007). Heavily infested carcasses were confiscated without compensations to farmers (personal communication). These efforts helped to down-regulate prevalence rates emanating from the BMC. However, farmers who suspect that their cattle harbour the *Taenia saginata* cysts avoided the BMC; preferring the low throughput abattoirs (Aganga, personal communication, March 8, 2017). Because, unlike the BMC, which maintains strict inspection policies, most low-throughput abattoirs do not. The reason being that the low throughput abattoirs lack enough or no competent meat inspectors. As a result, cases from low throughput abattoirs are not reflected in the documented national prevalence rates for bovine cysticercosis. Similarly, cases of bovine cysticercosis from other regions of the country, outside of BMC, and other slaughter houses and backyard slaughter were either un-captured or un-reported; a common occurrence in most developing countries (FAO, 2013). Consequently, deriving the national prevalence rate relying solely on the BMC data which was the status quo (Modisa, 2014) may not be very informative.

The prevalence of *T. saginata* in man and bovine cysticercosis in cattle has increased progressively despite government efforts. It is probable that either the important risk factors and hotspots of this infestation were misdiagnosed and/or the current traditional control and prevention measures are ineffective in reducing prevalence. Either way, there was need to

uncover novel risk factors and update information on the existing hotspots and known risk factors of bovine cysticercosis in Botswana.

Infested carcasses constituted financial loss to the local farmer and the national economy. Should the EU ban importation of Botswana' cattle, the macro-economic loss would be the loss of the entire revenue generated from export to EU, which is projected beyond P500 million annually [P10.3 = USD1] (Aganga, 2009; Statistics Botswana, 2015). Additionally, this shall create adverse socio-economic and psycho-social impact on cattle farmers and their families, who represent over 70% of the entire national population.

Furthermore, since traditional control and prevention methods have not sufficed in reducing prevalence of bovine cysticercosis and since vaccine has been effectively used to control beef measles in some developed countries (Lightowlers, *et. al.*, 1996), identification of immunodominant antigens as vaccine candidate, which would be employed in vaccine production in Botswana, became imperative.

1.3 Objective of the Study

The overall objectives of this research was to reduce prevalence and risk factors of bovine cysticercosis in Botswana's cattle to alleviate poverty and increase farmers income. Studies carried out were to profile the demography of cattle, determine politics and gender dynamics of cattle ownership and farming in Botswana, as a means of describing the non-climatic limiting factors of the cattle industry. The effect of this includes promoting gender equality, sustained growth of the livestock industry; particularly underdeveloped sectors of the industry (addressing SDG 2 5 & 8); to determine the prevalence of bovine cysticercosis as a means of demonstrating the occurrence and hotspots of bovine cysticercosis, which will guide location targeted prevention and control efforts to achieve reduced bovine cysticercosis in animal and *T. saginata* taeniasis in man and ensure food security (addressing SDG 2 & 3). In addition, to identify and correlate risk factors of bovine cysticercosis with the lifestyle of Batswana as a means of establishing them as determinants maintaining high prevalence of bovine cysticercosis, in order to advance informed control measures that can cause a sustained paradigm shift in Batswanas (addressing SDG 2, 3 & 8). Furthermore, to determine the effects of bovine cysticercosis on the socio-economy of cattle farmers in Botswana as a means of assessing the suitability of the existing intervention measures so as to develop more efficient intervention strategies, resulting in increasing farmer incomes & reduce poverty (addressing SDG 1, 2 & 8) and to identify immunogenic proteins, which may be potential vaccine candidates (addressing SDG 1).

The specific objectives included the following:

1. Determined the demography of cattle and cattle farming, thereby describing the non-climatic limiting factors of the cattle industry in Botswana.
2. Determined the prevalence of bovine cysticercosis in Botswana using data obtained from passive abattoir and active population survey.
3. Identified and quantitatively analysed risk factors of bovine cysticercosis as contributors to the increasing prevalence of bovine cysticercosis in Botswana.
4. Determined the effects of bovine cysticercosis disease on the financial, socio-economic and psychosocial wellbeing of cattle farmers in Botswana thereby queried the effectiveness of the existing intervention measures while recommending more efficient intervention strategies
5. Profiled *Taenia saginata* cysticercosis proteins and identified immuno-dominant epitopes as potential vaccine candidates.

1.4 Justification of the Study

Most published data show progressive decline on cattle population, which is commonly attributed to unthrifty climatic conditions. However, some feminists' researchers speculate that some sociocultural, political and gender-related factors bear more negative effect on cattle population than unthrifty climate (Dahl, 1987; Talle, 1987; Curry, 1996; Broch-Due & Hodgson, 2000; Hodgson, 2000; Njuki & Sanginga, 2013). It was imperative to understand how these non-climatic factors like, the demographics of cattle owners, farming systems (dairy, beef or dairy and beef), politics and gender dynamics of cattle ownership affect Botswana's cattle population.

BMC prevalence data is usually accepted as national prevalence data (Modisa, 2014). Currently, BMC avoids cattle from known bovine cysticercosis hotspots. Although this strategy regulates bovine cysticercosis prevalence, it makes BMC data non-representative. Because, other slaughter houses and backyard slaughter are either un-captured or un-reported; a common occurrence in most developing countries (FAO, 2013). Thus, the need to conduct a more holistic study, which in addition to collating prevalence records available at BMC, would elicit prevalence/incidence from low-throughput abattoir across other regions of Botswana and from farmers.

Bovine cysticercosis is preventable and treatable (Lightowlers, *et. al.*, 1996). Despite current traditional efforts by government, the disease remains prevalent in Botswana. Its increasing prevalence rate caused policy maker to speculate that either the major risk factors have been

misdiagnosed or the existing control and prevention strategies targeted at known prevalence are inefficient. It was imperative to carry out a study that would corelate the lifestyle of Batswana with the risk factors and uncover some novel and undocumented risk factor of bovine cysticercosis. Consequently assessing the suitability of existing control methods in mitigating the infestation.

Taenia saginata cysticercosis infested carcasses are either devalued, if having less than 10 cysts, or condemned, if heavily infested. Affected farmers do not receive compensation for destroyed carcass. Annually the government of Botswana support farmers with intervention measures. Apart from a few qualitative and subjective assertions regarding the effectiveness of these intervention measures, there is no known work that has adopted objectively verifiable indicators (OVI) to assess socioeconomic and financial effects of the bovine cysticercosis on cattle farmers. This study set out to ascribe quantitative worth to the socio-economic and financial effects of the bovine cysticercosis and by extension query the efficiency of the current government intervention measures.

In Australia, Lightowlers, *et. al.*, (1996) have utilised recombinant DNA techniques to clone oncosphere antigens of *T. saginata* (Lightowlers, *et. al.*, 1996). However, it is unknown whether these particular vaccines can be adopted for immunization of cattle in Botswana. Production of vaccines for immunization of cattle to control cysticercosis is relevant and feasible in Botswana. Botswana possesses valuable structures in place upon which this research can leverage. The Botswana University of Agriculture and Natural Resources (BUAN) farm, and the Botswana Vaccine Institute are valuable Institutions that shall provide the requisite research environment for development of this vaccine.

This work set out to profile Taenia saginata/cysticercosis proteins and identify its immunogenic and immune-dominant epitopes. These immune-dominant epitopes are essential inputs for the development of vaccines.

1.5 Significance of the Study

Studying the **prevalence and hotspots of the disease** across geographical regions of Botswana resulted in a more holistic prevalence results, that delaminated bovine cysticercosis hotspots and guided a coordinated bovine cysticercosis risk factors study. The systematic study of **risk factors of bovine cysticercosis and their association with the lifestyle of Batswana** (socio-cultural, economic, religious) showed a strong association between the two. These identified lifestyles are the remote driving forces of the infestation. Knowledge of these association informed evidence-based prevention and control strategies.

Furthermore, studying **socioeconomic and financial effects of the bovine cysticercosis on farmers showed** the types and magnitude of effects that bovine cysticercosis has on the livelihood patterns of cattle farmers in Botswana. This knowledge informed recommendations for guided decisions on the magnitude and nature of intervention (e.g., grants, amenities) to be directed to the affected regions. Furthermore, immunodominant epitopes were identified. These are pre-requisite inputs for the production of vaccines for bovine cysticercosis. Findings and recommendations of these studies provided tangible policy statements that addresses sustainable development goals (SDG) numbers 1,2,3,5, 8 and 12. These findings if implemented will transform the Botswana livestock industry into a vibrant, profitable and sustainable enterprise Ultimately, methods and findings of this research shall be adopted for teaching both undergraduate and graduate students; while hoping that the research shall open up novel research interest areas.

1.6 Scope of the Work

This research concentrated on bovine cysticercosis in cattle only. It did not sample goats, sheep and man. Study areas was Botswana Meat Commission (BMC) export abattoir in Lobatse, rural farmers, local abattoirs/slaughter houses and cattle Ranches located within Botswana. The study profiled *Taenia saginata* cysticercosis proteins and identified immunogenic one which are vital inputs for developing the vaccine.

1.7 Limitations of the work:

Human respondents were selected using convenience sampling method because the population is sparse and some respondents were unresponsive. Due to limited funds, only two districts from each region were selected giving 150 human respondents, with one invalid response. This number was representative for Botswana as worked out in Methods and Materials. For the survey there was limited published materials on the studied subjects, information was mostly retrieved through personal communication. Some government and private abattoir operators were frugal with data even with written permission from the Director of Veterinary Services. Poor infrastructure and lack of skilled man power at the Botswana University of Agriculture and Natural Resources (BUAN) mitigated against running the laboratory research within the school. Botswana Vaccine Institute provided the requisite laboratory environment, where the work was carried out with some delay. Due to lack of materials a ponceau-stained nitrocellulose gel was not run.

1.8 Research Questions

The following questions guided the research; viz:

1. What are the non-climatic factors affecting the cattle industry in Botswana?
2. How do these non-climatic factors affect the population, ownership and farming of cattle in Botswana?
3. What is the prevalence of bovine cysticercosis in Botswana?
4. Where are the hotspots of bovine cysticercosis in Botswana?
5. Is the prevalence of bovine cysticercosis in Botswana significant?
6. Are there significant differences between prevalence of bovine cysticercosis within and among districts and regions in Botswana?
7. Is there a difference in the mean prevalence rates obtained from passive abattoir meat inspection and rates obtained from survey within and across districts?
8. What are the risk factors (determinants) of bovine cysticercosis in Botswana?
9. Are there undiagnosed risk factor which maintain the high prevalence of bovine cysticercosis in Botswana
10. What are the measurable effects of bovine cysticercosis on the financial, socio-economic and psychosocial well-being cattle farmers in Botswana?
11. What vaccine candidate epitopes and immuno-dominant epitopes can be identified for Taenia saginata/cysticercosis

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CHAPTER TWO

2.0 REVIEW OF LITERATURE

This chapter is organized under the following sub-headings, viz: conceptual framework, theoretical framework and experimental (empirical) studies.

2.1 CONCEPTUAL FRAMEWORK

2.1.1 Concept of bovine cysticercosis -Historical background

Infections with Taeniidae family are unique among helminth zoonoses because their life cycles are dependent upon humans, dogs and cats as the definitive host (for adult worms) (Urquhart et. al., 1996; Murrell *et. al.*, 2005). The intermediate stage of this tapeworm, found in the muscles, heart and some other visceral organs of cattle, presents economic and food security issues to the beef industry and it is a public health hazard (Urquhart et. al., 1996 Harrison *et. al*, 1989). In summary, the life cycle of *T. saginata* is dependent on the link between humans and cattle. Any interruption of this link can result in the elimination of the parasite. While this appears to imply that prevention and control of taeniosis and cysticercosis should be straightforward and practical, the reality is that they have proved nearly intractable in many areas because of the highly successful dissemination and reproductive features of the parasite and because of well-entrenched cultural factors of the human hosts (Murrell *et. al.*, 2005).

2.1.2 Description of *Taenia saginata/cysticercosis*

The adult tapeworm, is flat, opaque white or yellowish (Murrell *et. al.*, 2005); length ranges from 5.0-15.0 M. The scolex, the attachment organ, different from other species of *Taenia,* possesses neither rostellum, nor hooks. The scolex is the size of a pin-head, and is followed by a short and undivided region, the neck, from which a long chain of proglottids or segments (termed the strobila) proliferate, thus the strobila has the appearance of a ribbon and may consist of more than a thousand proglottids (Harrison & Bogitsh 1991). These gradually increase in size so that the posterior end of the tapeworm has the broadest, longest and oldest distal proglottids gravid with eggs (Andreassen, 1998; Murrell *et. al.*, 2005). The gravid segment has 15-30 lateral branches on each side of the central stem in contrast to that of *T. solium* with only 7-12 lateral branches (Schmidt, 1986; Urquhart et. al., 1996)

The neck and strobila are markedly flattened, while the scolex has a radial symmetry. (Smyth & McManus, 1989 and Urquhart et. al., 1996). Mature segments are hermaphroditic, and contain several hundred testes, connected by fine sperm ductules that anastomose to form the sperm duct or vas deferens, which ends in the genital pore, forming the highly muscular cirrus (Smyth & McManus, 1989). The female sexual system consists of one bi-lobulated ovary,

connected to an oviduct. The vagina is a slightly sinuous tube which flows from the genital atrium to the oviduct; the vitelline glands are also connected to the oviduct. The oviduct, where fertilization takes place, transforms into the central sac or uterus, once the gonads and their ducts have attained maturity. Gravid proglottids resemble sacs full of eggs (between 50,000 and 80,000 each) and are approximately 0.5 cm wide by 1-2 cm long (Hoberg, *et. al.*, 2001; Scharf, 1988 in Murrell *et. al.*, 2005). The egg-containing uterus develops seven to 32 lateral branches, depending on the species. This feature allows identification if the proglottids belong to *T. solium* (seven to 11 branches) or *T. saginata* with 15-32 branches (Schmidt, 1986; Urquhart et. al., 1996). The most conspicuous feature of tapeworms is the lack of a mouth and digestive tract. The outer cellular cover, the tegument, functions in absorption, digestion, protection, and, seemingly, traction. The tegument envelops the entire worm, the most external layer of which is a brush-border formed by microtriches that are microvilli-like structures covered by a glycocalyx (thin layer of glycoproteins and mucopolysaccharides) (Smyth & McManus, 1989; Urquhart et. al., 1996).

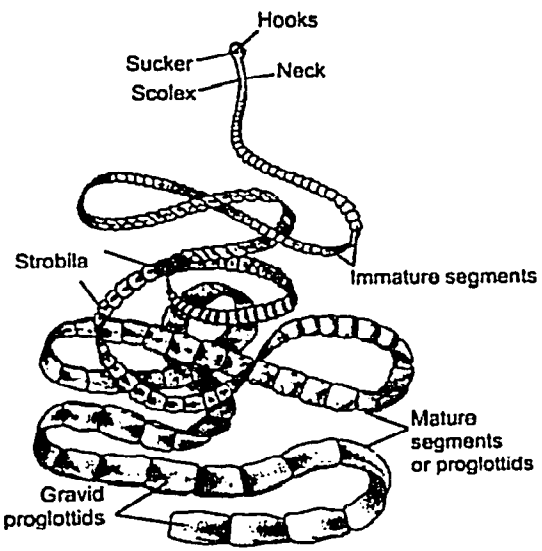

Fig 1: mature Taenia Saginata. Source: Global Health, Division of Parasitic Diseases. CDC. Page last reviewed: January 10, 2013

2.1.3 Life Cycle of *Taenia saginata/Cysticercus bovis*

Humans, definitive host of the parasite, pick up infection after consuming beef infested with *Taenia saginata* cysticercosis which eventually develops into the adult form - *Taenia saginata*.

An infected human may pass millions of eggs daily, either free in the feces or as segments each containing about 250,000 eggs and these can survive in the pasture for months. After ingestion by susceptible bovine (intermediate host) the oncosphere travels via blood to striated muscles. The oncosphere becomes infective to man at about 12 weeks when it has reached its full size of 1.0cm (Solusby, 1982; Pawlowski & Murrel,1996). By then it is enclosed by the intermediate host in a thin fibrous capsule. The longevity of the cysts ranges from weeks to years. After death, they are replaced by a caseous, crumbly mass which may become calcified. Development of patency takes 2-3 months (Urquhart et. al., 1996).

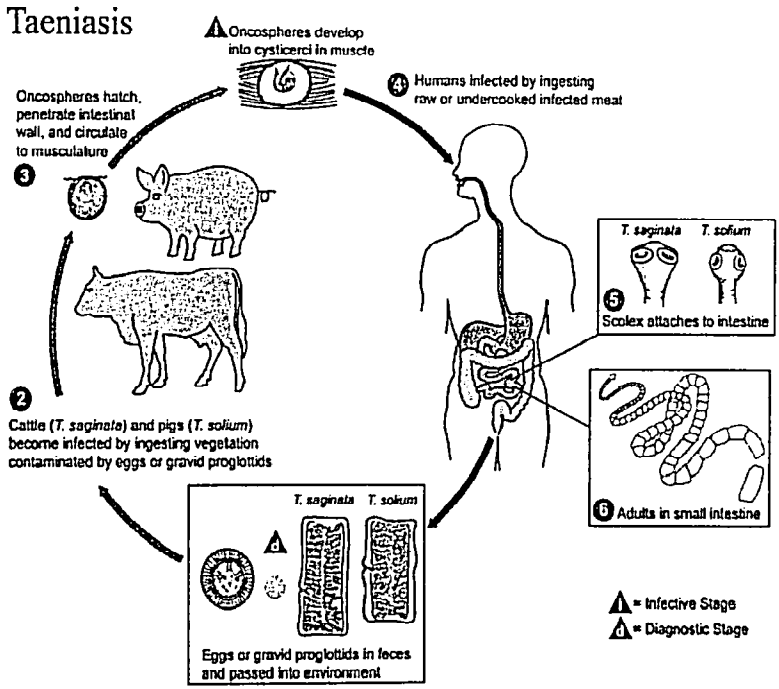

Figure 2: the Life cycle of Taeniasis (solium and saginata). Source: Science photo library, Global Health. Division of Parasitic Diseases. CDC. Page last reviewed: January 10, 2013. http://www.dpd.cdc.gov/dpdx

2.1.4 Pathogenesis and Clinical Signs

Under natural conditions, the presence of cysticerci in the muscle of cattle is not associated with clinical signs although experimentally, calves given massive infection of *T. saginata* eggs have developed severe myocarditis and heart failure associated with developing cysticerci in

the heart. In man, the adult tapeworm may produce diarrhea and hunger pains, but the infection is usually asymptomatic and is mainly objectionable on aesthetic grounds (Urquhart et. al., 1996).

2.1.5 Epidemiology and World Distribution

Globally, there are 77 million human carriers of *Taenia saginata* out of which 40% live in Africa (Megersa *et. al.*, 2010). There are two quite distinct epidemiological patterns found in developing countries and developed countries respectively.

2.1.6 Developed Countries

In developed countries where the standards of sanitation are high, with careful meat inspection, the prevalence of cysticercosis is low; lower than 1% of carcass inspected at the abattoir (Wanzala, *et. al.*, 2003; Megersa *et. al.*, 2010).

2.1.6.1 Developing Countries

In many developing countries, cattle are reared on extensive system; in poor human sanitation condition. In these circumstances the incidence of human infestation with *T. saginata* is as high as 7% (Wanzala, *et. al.*, 2003); or even as high as 12-20% (Mosienyane, 1986; Tshiamo, 2015). Consequently, non-grazing calves are usually infested in early life, often within the first few days after birth, from infected stockmen whose hands are contaminated with Taenia eggs. Prenatal infection of calves, though rare, may also occur. Passive prevalence study following routine carcass inspection is about 13-16% though the real prevalence is considerably higher (Urquhart et. al., 1996). The economic losses accruing from the condemned and downgraded carcasses due to treatment of carcasses before human consumption are substantial (Dewhirst *et. al.*, 1967; Onyango *et. al.*, 1996; Fan, 1997). Between the late 80's and the early 90's such losses were estimated at P25 million per annum in Kenya and P12.5 million per annum in Botswana (Grindle, 1978; Gracey, 1992) (P1.85 = 1USD) (Department of Treasury, n.d.). For the African continent, an annual loss between 1980-1982, P18 billion (Mann, 1983) under an overall infestation rate of 7%. In 1996, in South America, overall infestation rate estimated at 2.0%, for bovine and porcine cysticercosis caused an annual loss of P4,280 million (Fan, 1997) (P2.7 to 1USD) (IMF, n.d.).

2.1.6.2 Diagnosis of *Taenia saginata*/cysticercosis

At the abattoir, incisions made for the detection of cysticerci take into consideration the preservation of the economic value of the meat. Thus, careful incisions are made at the masseter muscle, tongue and heart; or at the intercostal muscles and diaphragm and triceps muscles (Urquhart et. al., 1996). Further examination points include, the esophagus; stomach and

intestine, general surfaces of the carcass; muscles of the shoulder behind the elbow, the chuck and the fillet (Livestock and Meat Industries Act, 2007). Table1 below details the sites and methods of examination for *Taenia saginata*/cysticercosis

Table1 Methods and sites of collection of *Taenia saginata*/cysticercosis cysts

Site of examination	Procedure and method of examination
Cheek muscles	Two deep linear incisions are made parallel to the mandible from its upper muscular insertion; incision sites are parted and by visual search cysts are identified and collected
The tongue	Upon palpation along the long axis of the tongue with hands and scrapping with knife, the cyst is felt and by incising lengthwise on the lower surface from base to root, cysts are identified.
The heart	The heart is split from base to the apex and further incisions made into the musculature to probe for cysts.
Triceps brachii	Three deep, adjacent and parallel transverse incisions were made above the point of the elbow.

Source: Livestock and Meat Industries Act, 2007

2.1.7 Prevention, Control and Treatment

Control measures differ between the developed and the developing countries. The developed countries emphasize high standard of human sanitation, compulsory meat inspection and a general practice of thorough cooking of meat beyond 57 °C, the thermal point of cysticerci. In Botswana, an developing country, treatment regulations according to the Livestock and Meat Industries Act, (2007) require infested carcasses having less than 10 cysts to be frozen at -10°C for at least 10 days. This treatment is sufficient to kill the cysticerci, although the practice reduces the economic value of the meat. Whereas Botswana permits about 10 cysticerci some European countries permit relatively low infestation of less than 5 cysticerci; beyond this limit the carcass is destroyed. Another vital control measure is stoppage of use of human sludge in cultivated field except when it is ensured that cattle will not graze the field for at least two years (Urquhart et. al., 1996; Pam, *et. al.*, 2015). Additional control measure practiced in developing countries is education of communities on good hygiene and sanitation. (Urquhart et. al., 1996; Townes & Knohn, 2004).

More recently, an increase in the number of imported beef from developing countries, infested with cysticercosis has made the eradication of the disease a primary health concern worldwide (Gredagh *et. al.*, 2011) Improvement in sanitation and public healthcare is essential for preventing the further spread of bovine cysticercosis. Altering the infrastructure to keep cows from roaming freely and contacting human feces can help to reduce human-cattle transmission. Effective measures to control and regulate meat inspection at slaughter houses has been

extremely effective in Europe and North America but not in many African countries. Pam. *et al*. (2015) recommends that programs to ensure proper compensation for the infested livestock, which are either destroyed or detained must be developed in order to discourage the underground trafficking of livestock by local farmers in endemic regions. This very vital in Botswana

2.1.8 Anthelmintic Treatment

Praziquantel and albandazole are the two anti-cysticercal drugs used to treat patients diagnosed with cysticercosis in the brain and skeletal muscles. Treatment with praziquantel (50-100mg/kg/dx30d) and albandazole (400mg bid for 8-30d) completely eliminated cysts in 80% of treated patients with an additional 10% of patients experiencing a significant reduction in the number of cysts present (Nigatu, 2004). Some investigations recommend 10mg/kg/day in 3 divided doses for one day and then 50mg/kg/day in 3 doses for 29 days of praziqualtel. Neither drugs is toxic, however a percentage of patients undergoing therapy experience advance side effects such as headache, nausea, vomiting, dizziness and increased pressure on the brain. Therefore, treatment with either praziquantel or albendazole is often administered concomitantly with corticosteroids to prevent excessive inflammation (Nigatu, 2004).

2.1.9 Vaccinology

The fifth objective of this research work was to identify immunodominant epitopes, which can be adopted as vaccine candidates for the production of vaccines against *Taenia saginata*/cysticercosis. It is therefore imperative to review relevant literature on vaccines and vaccinology

2.1.10 What are Vaccines?

A vaccine is a biological preparation that provides active acquired immunity to a particular disease. A vaccine typically contains an agent that resembles a disease-causing microorganism and is often made from weakened or killed forms of the microbe, its toxins or one of its surface proteins (National Centre for Immunization Research and Surveillance [NCIRS], 2013). Vaccines administration has proven effective in prevention, eradication or restriction of diseases. It can be prophylactic; to prevent or ameliorate the effects of a future infection or therapeutic when used against an existing disease (WHO, 2012; NCIRS, 2013). However, the efficacy or performance of the vaccine is dependent on a number of factors; these include: the disease itself; the strain of vaccine; vaccination schedule; idiosyncratic response of patient to vaccination. These patient factors are such as ethnicity, age, or genetic predisposition (World Health organisation [WHO], 2012). Due to possibility of complications with vaccine and

vaccine administration, the Centres for Disease Control (CDC) and prevention alongside other stake holders have outlined important considerations in achieving the effectiveness of a vaccination program (NIAD, 2012; CDC, 2013; Maglione, *et. al.*, 2014).

2.1.11 Types of Vaccines

Vaccines have been broadly categorized as dead or inactivated organisms or purified products derived from them. The National Institute of Allergy and Infectious Diseases [NIAD] (2012) outlined types of vaccines; these are: (i). **inactivated:** containing previously virulent, micro-organisms that have been destroyed with chemicals, heat, radiation, or antibiotics; (ii) **attenuated:** containing live, attenuated microorganisms. Many of these are active viruses that have been cultivated under conditions that disable their virulent properties, or that use closely related but less dangerous organisms to produce a broad immune response (Sinha & Bhattacharya, 2014); (iii). **toxoid:** these are made from inactivated toxic compounds that cause illness rather than the micro-organism (NIAID, 2012). (iv) **Subunit:** rather than introducing an inactivated or attenuated micro-organism to an immune system (which would constitute a "whole-agent" vaccine), a fragment of it can create an immune response (NIAID, 2012); (v). **conjugate:** certain bacteria have polysaccharide outer coats that are poorly immunogenic. By linking these outer coats to proteins the immune system can be led to recognize the polysaccharide as if it were a protein antigen (NIAID, 2012). Vaccine can immunize against a single antigen (univalent or monovalent) or immunize against two or more antigens (multivalent) (Dorland Dictionary, 2012; WHO & UNICEF, 2005).

2.1.12 Vaccination as prevention and treatment agents

While traditional vaccines are designed to prevent disease, researchers have developed therapeutic vaccines, vaccinations that treat an existing illness (Griffin, 2020). Vaccines aimed at preventing *Taenia saginata*/cysticercosis shall play a vital role to control the spread. Cattle do not require long-time immunity, therefore vaccines which provide only short term resistance may be sufficient to prevent the spread of infection to humans. To date, the most effective experimental vaccines have involved the expression of recombinant Oncosphere antigen TSOL18 and TSOL45 in *E. coli*. TSOL18 is more effective, inducing greater than 99% protection in the vaccine trials undertaken thus far. In Australia, Lightowlers, *et. al.*, (1996) have utilized recombinant DNA techniques to clone oncosphere antigens of *T. saginata* and in vaccine trials in cattle. Vaccination with a combination of two antigens, designated TSA-9 and TSA-18, induced up to 99.8% protection against experimental challenge infection with *T. saginata* eggs. Current efforts are focused on developing the method necessary to make the

vaccine widely available and successful on a practical & commercial scale. The use of recombinant vaccine in cattle combined with anti cysticercal chemotherapy appears to have potential to control and eradicate the disease (Gredagh *et. al.*, 2011).

Apart from this afore-described traditional method, reverse vaccinology (RV) has been applied to develop vaccines. Developing vaccine through RV involves designing vaccines using the pathogen's sequenced genome. This method depends on new wealth of genomic information, as well as technological advances. Reverse vaccinology is much more efficient than traditional vaccinology, because RV does not requires growing large amounts of specific microorganisms or extensive wet lab tests (Alessandro & Rino, 2012). Production of therapeutic and preventive vaccines for *Taenia saginata*/cysticercosis is possible and doable.

2.1.13 Development of immunity as a key consideration in Vaccine development

The body immune system recognizes vaccine agents as foreign, destroys them, and "remembers" them. When the virulent version of that agent is encountered, the body recognizes the protein coat and thus is prepared to respond either by (1) neutralizing the target agent before it can enter body cells, and (2) recognizing and destroying infected cells before that agent can multiply to vast numbers. It is therefore imperative to target the antigens that elicit the highest and best immune response from the host. These antigens are the most immunodominant epitopes (Sutter, *et, al.*, 1999; Kanesathasan *et.al.*, 2001). This study profiled *Taenia saginata*/cysticercosis proteins and identified immunogenic ones. The immunodominant proteins shall be utilized for *Taenia saginata*/cysticercosis vaccine production

2.1.14 Scheduling of vaccination

In order to provide the best protection, vaccination should commence immediately the immune systems of the patients (calves) are sufficiently developed to respond to particular vaccines. Additional "booster" doses may be necessary to achieve "full immunity" (NHS, 2009; AAP, 2011; CDC, 2013). Full bovine cysticercosis vaccine study will include development of vaccination schedules.

2.1.15 Economics of Development

Vaccine development is time consuming and expensive. Many of the diseases that mostly demand a vaccine, exist in poor countries who cannot afford the production cost. Consequently, pharmaceutical firms and biotechnology companies have little financial incentive to develop such vaccines (Goodman, 2005). Vaccine producers requires funding from government, universities and non-profit organisations (Olesen *et. al.*, 2009; Jit *et. al.*, 2013; Newall, *et. al.*, 2014). *Taenia saginata*/cysticercosis constitutes high economic loss in addition to the public

health implication across all developing countries. A collaborative effort of stake holders shall be vital to finance the research and drive the production of *Taenia saginata*/cysticercosis vaccine.

2.1.16 Production of Vaccines by traditional method

As earlier mentioned the development of new vaccines starts with the identification of unique components of the organism capable of generating a protective immune response (Junqueira, *et. al.*, 2014). This is the immunogenic antigen; particular interest is the immunodominant epitopes (Adu-Bobie, *et. al.*, 2003; Goodman, 2005; Scarselli *et. al.*, 2005; Ferrerira and Porco, 2008; The Washington Post, 2009). Following identification of immunodominant antigen (epitopes), necessary adjuvants, stabilizers and preservatives are added to create vaccine (Bae, *et. al.*, 2009; Muzumda & Cline, 2009). Typical traditional vaccine production utilize *in vivo* methods; involving wet labs and complex manipulation of organisms. The study employed traditional method to profile bovine cysticercosis proteins and identify immunogenic epitopes.

2.1.17 Plants as Bioreactors for Vaccine Production

Transgenic and complex plants have been identified as promising expression systems for vaccine production. Genes inserted into tobacco, potato, tomato, banana and some other complex plants produced vaccines usable for humans (Sala, *et. al.*, 2003); and animals (Ostachuk, *et. al.*, 2009; Aguireeburualde, *et. al.*, 2013). Bananas have been developed that produce a human vaccine against Hepatitis B (Kumar *et. al.*, 2005). Another example is the expression of a fusion protein in alfalfa transgenic plants for the selective directioning to antigen presenting cells, therefore increasing vaccine potency against Bovine Viral Diarrhea Virus (BVDV) (Ostachuk, *et. al.*, 2009; Aguireeburualde, *et. al.*, 2013). At the Botswana Vaccine Institute, the Tobacco Mosaic is currently in use as an expression system for the production of the Foot and Mouth Disease (FMD) Vaccine. The purified FMD virus is inserted into the Tobacco and allowed to grow into useable vaccine for vaccination of cattle (Mpolokang, 2019).

2.1.18 Production of vaccine by reverse vaccinology

Reverse vaccinology (RV) is an improvement on traditional vaccinology. It employs bioinformatics, which was pioneered by Rino Rappuoli (Pizza *et, al.*, 2000). Scientists have adopted the reverse vaccinology methods for producing vaccines for many bacterial pathogens (Rappuoli, 2000). Reverse vaccinology has been used to establish a genome-based vaccinology (Alessandro & Rino, 2012), which enabled the production of Streptococcus and B Streptococcus vaccines. It has also been used to develop vaccines for antibiotic-resistant

Staphylococcus aureus and *Streptococcus pneumoniae* (Alessandro & Rino, 2012; Kanampalliwar, *et. al.*, 2013).

Advances in sequencing technology and bioinformatics have resulted in an exponential growth of genome sequence information that has contributed to the development of software that aids genomic analysis in a short period of time and at a low cost. Reverse vaccinology applied to the genome of a pathogen aims to identify *in silico* the complete repertoire of immunogenic antigens that an organism is capable of expressing without the need of culturing the microorganism. Additionally, RV can help to discover novel antigens that might be less abundant, not expressed *in vitro*, or less immunogenic during infection that are likely to be missed by traditional approaches (Adu-Bobie, *et. al.*, 2003; Rinaudo *et. al.*, 2009; Mustafa, 2013; Vivona *et. al.*, 2006; Bertholet. *et. al.*, 2014).

The RV process begins with the proteomic information in a database; then, the selection of vaccine candidates by means of different bioinformatics tools that analyze the properties of each protein and the host immune response generated by them (Adu-Bobie, *et. al.*, 2003; Ferrerira & Porco, 2008; Scarselli *et. al.*, 2005; Rapin, *et. al.*, 2010). Good vaccine candidates do not present homology with host proteins, to avoid potential autoimmune response (Chaudhuri, *et. al.*, 2014); They possess extracellular localization, signal peptides, and B-cell epitopes (Woelk, *et. al.*, 2011). These candidates must also lack transmembrane regions, in order to facilitate their expression. In addition, it is necessary to analyze the lack of cross-reaction among other pathogenic antigens (Bertholet, *et. al.*, 2014). Furthermore, good vaccine candidates possess good antigenic and adhesin properties, which are important for the pathogenesis of the microorganism and protection against the disease (Vivona *et, al.*, 2006; He & Xiang, 2013). Extracellular or cell surface localized proteins are good vaccine candidates due to their increased accessibility to the immune system (Bertholet, *et. al.*, 2014; Chaudhuri *et. al.*, 2014). Software used to simulate immune response exist; they aid search for novel vaccine candidates (Rapin, *et. al.*, 2010; Alessandro & Rino, 2012).

2.1.19 Summary of Conceptual Literature

This research profiled *Taenia saginata*/cysticercosis proteins and identified immunogenic ones. Instead of vaccinating animals using whole cyst, cyst fluid or cyst shell, identified immunodominant proteins of *Taenia saginata*/cysticercosis shall be purified and used to produce the vaccine. The final vaccine product of this research shall be monovalent specific for *Taenia saginata*/cysticercosis. Subsequent studies on bovine cysticercosis vaccine production shall include development of vaccination schedules. Due to high cost of vaccine

production, which individual research cannot afford, collaborative efforts of stake holders shall be vital to finance the research and drive the production of bovine cysticercosis vaccine. Before production of vaccine, immunodominant epitopes identified using traditional methods shall be confirmed using reverse vaccinology method.

2.2 THEORETICAL FRAMEWORK

This section deals with scholarly theories which are relevant to the research.

2.2.1 Theories on socioeconomic impact assessment

Socio-economy is the relationship between economic activity and social life. Socio-economic factors, often called socioeconomic factors are used to compare social life and economic activity. This includes education, wealth and employment factors which are social experiences and realities that help mold one's personality, attitudes and lifestyle

2.2.2 Factors that determine the socio-economic status of an individual

2.2.2.1 Education

Level of education determines attitude and general life perception. Education level plays vital role in farmer's ability to access credit, expand capacity, and adopt cutting age technologies (Alene & Manyong (2007) p. 157 in Ayşegül, 2011). These correlates directly with the development in agriculture, economic and social growth. Higher education can increase farmer's earning capacity, which in turn contributes to quality of life (eHow, 2017) and better decision-making process (Linda, *et. al.*, 2015). American Psychological Association. [APA], (2007) claims that higher levels of education are associated with better economic and psychological outcomes (i.e.: more income, more control, and greater social support and networking).

2.2.2.2 Occupation/Employment and Income

Occupation and corresponding income are factors that can contribute to farmers' socio-economic status (eHow, 2017). In society, occupation and income corelates with social stratification, acceptance, and respect (Linda, *et. al.*, 2015). Level of income affects place of residence and social circle

2.2.2.3 Place of residence

Place of residence is a vital socioeconomic factor because the types of house farmers live in as well as the region and neighbourhood in which they reside determine their circle of association and influence. People with similar incomes often live, school associate together, leading to similar backgrounds (Linda, *et. al.*, 2015).

2.2.2.4 Culture/ethnicity

Culture and/or ethnicity are socioeconomic factors that can contribute to our thoughts and attitudes. They have impact on farmers' upbringing, core values, sense of familyhood and tradition. History of ethnicity, special holidays, and cultural beliefs are generational; shaping farmer's identity (Linda, *et. al.*, 2015).

2.2.2.5 Religion

Often closely tied to culture is the socioeconomic factor of religion. Whole social networks are built around churches, temples and mosques. Church barbecues, games, overseas missionaries, outreach groups and other religious experiences influence social adaptation (Linda, *et. al.*, 2015).

2.2.2.6 National Wealth

National wealth is measured by gross national product (GNP), net national income (NNI) and gross domestic product (GDP). Whereas the first two measure absolute national wealth, GDP relates national wealth with wellbeing of citizens. Higher GDP, means better the standard of living for citizens.

2.2.2.7 Health and Access to Medicare

Availability of basic social amenities and accessibility of health care services affect healthiness of citizens. Nations that invest in public health initiatives inspire healthy behaviors, which result in lower mortality rates, stronger population and in turn higher productivity.

2.2.3 Tools for socio-economic assessment

Socioeconomic assessment is the basis of social policy. It compares the effects that social and economic factors have on each other. Tools for socioeconomic assessment are basically a combination of social and economic instruments (Lyle, 2011).

2.2.3.1 Kuppuswami Scale (KUP)

In the past two decades, Kuppuswami scale (KUP) method has been used for determining the socioeconomic status of individuals, particularly in hospitals (Patel, *et. al.*, 2007). This scale is a composite score of *per capita* monthly income, education of the head of the family and profession of the head; giving a maximum score of 29 and a lowest of 3 (Kuppuswami, 1981 in Saleem 2019; Patel, *et. al.*, 2007). The KUP scale primarily measures socio-economic status of urban population, lays emphasis on professional education and occupation of the head of the family. However, it fails to capture the change in economic conditions of individuals and family members; the economic status of the rural populations. Thus, an uneducated, unskilled member of the family with a high income from a family business is likely to be in the upper

low category, even though he has good standard of living and can afford good health care. Conversely well-educated and skilled persons may remain jobless with poor incomes and poor standard of living but rank high using the KUP scale. Therefore, KUP does not necessarily reflect the standard of living or other human development indicators such as sanitation and health. The Kuppuswami scale is also cumbersome to remember (Mahajan and Gupta, 2003 in Patel, *et. al.*, 2007). It has values for 3 different parameters that make up a composite score which then is classified into various classes such as low, upper low, low middle, upper middle and upper. Moreover, the consumer price index has to be determined to multiply the income groups to get the appropriate groups for that year (Mishra & Singh in Patel, *et. al.*, 2007). Accurate measurement of family income is also difficult. Since family income is personal, people tend to understate or inaccurately state their family income due to previous high taxation levels or due to subsidies offered to lower income earners (Patel, *et. al.*, 2007). Based on the pitfalls of KUP scale it is not suited for determining the socioeconomy of farmers in Botswana thus, not adopted in this study

2.2.3.2 Cross-referencing

The basis of socioeconomic assessment are data classes such as income, age, race, gender, ethnicity, education, criminality, place of residence, occupation and location. Data sets are available from a wide variety of authoritative sources. Cross-referencing this wealth of data from institutional archives helps to generate insightful socioeconomic assessments (Lyle, 2011). Cross-referencing can assess a wider range of data classes unlike the Kuppuswami scale, which assesses only-composite score of per capita monthly income, education of the head of the family and profession of the head. Consequently, cross-referencing is a more appropriate tool for socioeconomic assessment.

2.2.3.3 Appropriateness of socioeconomic impact assessment for determination of impact of bovine cysticercosis on farmers

Estimations about the possible financial and economic cost/losses caused by bovine cysticercosis in Botswana are fraught with uncertainty. There have been little or no studies on the microeconomic or macro-economic effects of the infestation. The economic implications of bovine cysticercosis depend on three parameters; affected individuals, cost of control and prevention measures (cost), and the wider impact on confidence; for example, threat from EU to ban importation of Botswana beef. Direct economic costs include: losses of revenue due to condemnation or detention and devaluation of infested carcass. Such loss impacts not only on

farmers but also on upstream and downstream sectors such as Botswana Meat Commission, the medium throughput abattoirs, meat shop operators, feed mills, breeding farms etc.

In conclusion, although prevalence studies determined the hotspots, geographical spread and statistics of the bovine cysticercosis infection, the socio-economic impact assessment determined the real impact bovine cysticercosis on farmers' wellbeing. It highlighted the *nitty-gritty* of the impacts of bovine cysticercosis which are not perceptible through prevalence study. This study also assessed the effectiveness of the existing intervention programmes in cushioning effects of bovine cysticercosis on livelihood patterns of affected farmers

2.3 EMPIRICAL STUDIES

Some empirical studies have been carried out on the occurrence, spread and effects of *T. saginata* in man and *Taenia saginata*/cysticercosis in cattle in Botswana and other African countries. This section reviewed some studies that are related and relevant to this present study.

2.3.1 The Ethiopian Experience

In Ethiopia, the rural communities raise cattle under extensive husbandry practices. High population density, consumption of raw meat, low awareness of bovine cysticercosis, poor hygiene and sanitary infrastructure are closely linked to the man-animal transmission in the rural areas (Tolosa, 2010). Prevalence of taeniasis and cysticercosis vary across localities; taeniansis ranges from 10% to 70% while cysticercosis vary from 3% to 27%. Prevalence of cysticercosis were relatively lower; at about 3.1%, in 2000, in Central Ethiopia (Tembo, 2001 in Tolosa, 2010); 4.9% in 2004 at Gonder (Dawit, 2004 in Tolosa, 2010) and 7.5% in 2003 in Addis Ababa (Nigatu, 2004 in Tolosa, 2010). High prevalence of 17.5% was recorded in East Shoa in 2004 (Hailu, 2005 in Tolosa, 2010); 21% at Nekemt in 1990 (Ahmed, 1990 in Tolosa, 2010); 26.25% at Awassa in 2006 (Abunna *et. al., 2007* in Tolosa, 2010) and 30% from several rural abattoirs (Hailemarian, 1980). The prevalence has dropped across years following adoption of better practices.

Dawit *et. al.*, (2012) analyzed the public health and economic significance of bovine cysticercosis in Southern Ethiopia. Employing methods of active abattoir survey, questionnaire survey and inventory of pharmaceutical shops across periods of 2009 to 2011, the result showed prevalence of 2.6% for bovine cysticercosis and prevalence of 62.5% for *T. saginata* infestation among human respondents. Prevalence of *T. saginata* was significant ($p<0.05$) with age groups; adults having higher odds of acquiring taeniasis (OR=31.8) than lower age groups. Assessment of the economic effects of Taeniansis using inventory of Pharmaceutical shops (Pharmacies and Rural drug vendors) showed that in between 2009 to 2011 a total of 29,952

adult doses worth 40,201.8 ETB (2,407.2 USD) was spent for treatment of human Taeniasis per annum (Dawit *et. al.*, 2012). However, earlier in 1990, Ahmed claimed that the average annual expense (loss) due to taenicidal drugs used for treatment in Ethiopia was estimated to be 4,937,583.21 Ethiopian Birr (approx. M2.0 USD) (Ahmed, 1990 in Dawit *et. al.*, 2012).
In Ethiopia, apart from segmented records, there is no known macroeconomic estimate of national annual loss due to non-export of bovine cysticercosis infested carcasses. There is no known record of annual losses due to devaluation or treatment of infested carcasses.

2.3.2 The Nigerian Experience

In Nigeria, like in most other sub-Saharan Africa, the prevalence of cysticercosis and human Taeniansis are high, particularly in the rural areas. Rabi'u & Jegede (2010) studied the incidence of bovine cysticercosis in 2009 in Kano State, North-Western Nigeria using passive abattoir investigation. Incidence was 2.67%, obtained in Kano was higher than prevalence of 1.9% and 2.1% obtained in 2009 and 2010 respectively in Bauchi Zone, North-Eastern Nigeria. The North-Central (Guinea) zones of Nigeria, Kaduna and Zaria had a prevalence of 4% each between 1979 to 1980 (Dada, 1980 in Rabi'u & Jegede, 2010). Qdeer (2008) studied the prevalence of bovine cysticercosis in Jos abattoir, Nigeria and obtained a prevalence of 13.4% in 2007. Unlike Rabi'u & Jegede (2010) that observed tongue as major predilection site of the cysts, Qdeer (2008) found the heart as major predilection site. Both authors agree that human taeniasis and bovine cysticercosis have major economic implication and that poor hygiene and improper sanitary measures are most important risk factors of bovine cysticercosis (Qdeer 2008; Rani'u & Jegede, 2010).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 The Design of the Study

This study was cross sectional, making use of both survey and experimental approaches. The survey approach was used to determine the demography of cattle, cattle farming and cattle farmers: risk factors of bovine cysticercosis; prevalence and socio-economic effects of bovine cysticercosis on cattle farmers in Botswana. Survey was carried out through non-participatory interviews of respondents using structured questionnaires, personal observation of researchers, active and passive abattoir meat inspection and review of relevant literature.

Experimental approach was utilized to profile the proteins of bovine cysticercosis and identify immunogenic ones.

3.2 The Study Area

These studies were carried out in Botswana. Botswana is a land-locked country, with South Africa to the east and south, Namibia to the west and north, Zambia to the north and Zimbabwe to the north-east. It lies between approximately 20° to 29.15°E and 18° to 27°S (figure 3). The country covers approximately 582,000 square kilometers. Mean altitude above sea level is approximately 1000m. **Climate:** Botswana is close to the sub-tropical high pressure belt of the southern hemisphere. The climate is variable (World Bank, 2010) but driven by two distinct climate zones with the majority falling under the Zaire Air Boundary climate zone to the north, which brings the summer thunderstorms and heavy downpours of rain. A small part, mainly in the south-west, is influenced by the South Atlantic Oscillatory climate system which moves in and out of the country from the west and south west, generally bringing very cold spells and winter rain (Burgess, 2006; Botswana Tourism Organization [BTO], 2019).

Rainfall: The relatively flat nature of the country, with very few large open surface water bodies result in few orographic effects generating rainfall, except in a few localized areas, particularly in the south east. The country has an arid, summer rainfall climate between October/November and April (World Bank, 2010). This consist generally of scattered, high intensity, short-duration thunder showers. Mean annual rainfall ranges from 650 mm in the extreme north-east to 250 mm in the extreme south-west, with variations of 550 mm in the higher areas in the south-east and 350 mm on the lowest areas of the Limpopo valley. There are some anomalies like, prolonged general rain, in the east, in summer and in the west in the winter. There is also a commonly occurring, mini-drought period, from about mid-January to late February, although the country is prone to drought (Burgess, 2006).

Temperature: Temperature variations are extreme throughout the year. They also vary greatly within the daily cycle and according to location, vegetation cover, wind reach, and the presence of any large water bodies. In **winter**, from around mid-May to mid-August, temperatures range from about 0°C, in the early hours of the morning to 20°C in the mid-day. In **summer**, temperatures vary from 12-15°C during the morning to 30-40°C by late afternoon in the hot, dry season (generally from mid-September to late October), but the maximum temperatures remain 25-30°C, during the **rainy** season. Temperatures in the northern and in the western desert areas can rise to about 40-45°C in the late dry season, prior to the rains (Burgess, 2006; World Bank, 2010)

Humidity: The country experiences very few cloudy days, having around 290-300 sunshine days per year. Humidity is therefore extremely low, particularly in the dry months, (10%), rising to an average of around 65 %, in the rainy season. Average annual evaporation is 2,000 mm, which exceeds annual precipitation by a factor of 4 to 8 (depending on the location) (Burgess, 2006).

Population: By 2011, Botswana's population was 2,024,904 and the projected population for 2016 was 2,226,040 at a growth rate of 1.7 % (Statistics Botswana, 2015). Current population as at 2019 is 2,316,960 (Statistics Botswana, 2019).

A large part of the country; about 17% (104,460 km^2), is National Parks, Game, Forest and Private Reserves. The pastoral land includes virtually all that is outside of National Parks, Game and Forest Reserves, major cities and towns (Burgess, 2006).

Agricultural practices in Botswana can be summarized to include the following; large stock (beef ranching, traditional 'Cattle Post' and free range production systems, game ranching, feedlots, dairying); Crops and forestry; Specialist systems: (Apiculture, Ostriches, Fisheries, Aquaculture, Crocodile farming); Small stock and poultry: (Small stock, Pigs, Poultry); Subsidiary industries (Slaughterhouses & Abattoirs, Tanneries) (Burgess, 2006). The entire agriculture sector of the economy is driven largely by the international beef markets particularly South Africa and the European Union.

The livestock sector is divided into traditional systems, mixed small holder systems and commercial producers. There is very little commercial crop production, and most crops are produced for subsistence, or for local sale.

Farm tenure systems vary from tribal/traditional land use holdings, leasehold and freehold. In the case of tribal land, fields and boreholes may be passed down from one generation to the next. Whereas leasehold land is normally held for 50 years, with option to renew after that period, while freehold land is held in perpetuity, or for 999 years (Burgess, 2006).

Fig 3: Political Map of Botswana; Source: Maps of the World.com

3.2.2 Socio-economic and Marketing Issues

The major socio-economic issues affecting farmers are the remoteness of farms from major centres and the difficulty in obtaining water, access roads, telephones, and supplies. These increase marketing costs. Access to credits is difficult if not impossible because traditional farmers do not own collateral, so they operate at low input costs.

3.2.3 Livestock farming

Traditional systems are dominated by the cattle-post system, where a farmer, or group of farmers, water livestock at a central watering point (most often a well, or a borehole), and the livestock wander freely over the grazing land around the watering point. There are some areas where transhumance is still practised, mainly in the eastern Hardveld. Farmers in this region usually practice crop and livestock production. Arable land is fenced, but livestock are permitted to graze farm land after crops have been harvested. Family members migrate from villages to their lands during the ploughing, planting and harvesting period, but return home

during the fallow season leaving livestock with herd boys in the grazing areas (Burgess, 2006; Pablo, *et. al.*, 2013).

Mixed small-holder systems are an integral part of the traditional livestock sector. People who live in areas where surface water is available, either on a year-round, or a seasonal basis, have some livestock and some small fields.

Landless systems are barely feasible in these modern times. This is because there is an increasing pressure for allocated land from people with expectations of a higher standard of living than is afforded under the traditional village life. The few scattered small groups of semi-nomadic people have tended to become sedentary around boreholes provided by Government. They are provided with primary healthcare, and education for their children.

Commercial systems are practiced mainly on leasehold and freehold land. Most commercial farmers are relatively wealthy. They have access to finance and the commercial marketing sector. Within the commercial sector, production systems include stall-feeding, and tethering (Burgess, 2006).

The effects of drought on cattle population experienced in early to mid-1980s led to the death of about 1,000,000 cattle, followed by another brief but extreme drought of early 1990s, which accounted for another die-off of 1,000,000 heads of cattle (FAO, 1991). However, cattle population rose from 1,650,000 in 2007 to 2,260,000 in 2011 before dropping to 1,986,000 in 2012 and 1,100,000 in 2017 (Statistics Botswana, 2019). There are more cattle holdings (90%) but less actual cattle numbers (30-40%) with the traditional farming sector than commercial farmers, with less cattle holdings (10%) but more cattle heads of about 60-70% (CSO, 1996).

Beef production is generally off the range, where animals are weaned into fattening camps, and either sold directly to the abattoir at around 2-3 years, or finished in feedlots. All bull-calves, and unthrifty heifers are weaned and grown out for slaughter; while the best heifers are kept as replacements. Cull cows are fattened on summer grazing, and sold before the dry season.

In commercial herds where young stocks are removed from the range after weaning, more range is available for breeding cows. Usually, production rates are higher than standard off-range production systems. The negative side to this latter system, is the vulnerability of the herd to drought, which can cause slaughter of a large proportion of the breeding herd, at below premium beef grades and prices (Burgess, 2006).

The commercial beef sector favours rotational grazing and rotational-rest systems, by which an area is grazed until there is very little forage left before cattle are moved to a new paddock, or camp. The cattle are kept in separate herd categories, so that breeding cows are kept apart from young, immature bulls and steers, and heifers. Breeding generally occurs in two seasons,

with the majority of calves arriving in the mid rainy season, and a smaller, secondary crop arriving in late summer (Burgess, 2006; Pablo, *et. al.*, 2013).

In communal lands livestock production systems, "dual grazing" exist; in that, some borehole owners raise cattle for commercial gain, without fencing; they have private ranches as well as boreholes, and transfer stock from one to the other depending upon grazing conditions.

Traditional cattle production systems provide milk and draught in addition to beef. Cattle are used for barter, for bride-price negotiations, for feasts at marriages, funerals, and other important functions. Historically, cattle were used far more for transport and ploughing but this has been largely replaced by mechanized system with the use of the tractor (Burgess, 2006).

Some limitations to livestock production can be summarized as follows: i. irregular fodder production, resulting from highly variable rainfall and drought spells that occur every 2-3 years, over the past 20 years (1998-2018). ii. drought leading to less livestock unit in drought prone areas. iii. other factors affecting the grazing resource include soil types; sandy soils, which result in trampled plants being physically removed from the soil and competition for grazing from gerbils and harvester termites. iv. the impact of harvester termites on a grazing field, especially during drought, is quite phenomenal; an area of several hundred hectares can be completely denuded of grass in just a few days. v. several poisonous plants have been identified as being responsible for high mortality rates at certain times of the year. vi. livestock diseases play the most important restriction to commercial livestock production. The most important diseases include; the Foot and Mouth Disease (FMD) and Contagious Bovine Pleuropneumonia (CBPP), which are endemic in northern Botswana. Other economically important diseases of livestock are Blackwater, Heartwater, Tuberculosis, Botulism, and Human Tapeworm. The effects of these diseases are the loss of cattle heads due to death and deliberate culling of infected and affected animals, compensation paid to farmers, rejection of meat from international market etc. (Burgess, 2006; Pablo, *et. al.*, 2013).

Due to inbreeding and breeding of cross-bred animals the traditional herd is highly variable in colour, size and shape. The beef sector has a highly cross-bred herd. This is due to the introduction of European, African and Asian breeds in order to enhance 'improved' production traits into the national herd. Some local breeds include: Tswana, and Tuli, the Afrikaaner, the Brahman are relatively hardy in the arid and semi-arid conditions. They are tick-proof, and tends to feed by browsing grass and other herbaceous plants. European beef breeds that have been introduced include; Hereford, Simmental, Aberdeen Angus, to Charolais and Limousin. Breeds that are both Dairy and beef include: Red Sussex, Red Poll, Brown Swiss, Murray Grey,

and Pinzgauer. Common dairy breeds are Holstein, Friesland, Ayrshire, Jersey and Guernsey (Burgess, 2006; Pablo, *et. al.*, 2013).

3.3 General information on the study area as adopted in this study

Agricultural classifications of Botswana are region, districts and animal holding. However, politically, Botswana is classified into districts, without regions.

Statistics Botswana (2015, table 2.4 pg. 24-26) details **agricultural classification** of Botswana into **regions, districts** and **Animal Holdings**. There are six (6) regions; Southern, Gaborone, Central, Francistown, Maun and Western Region. For the purposes of this study the **Western;** region with highest cattle population and **Central Regions;** region with lowest cattle population were selected. Western region constitute of Ghanzi, Hukuntsi and Tsabang. From which **Ghanzi** and **Hukuntsi** were selected. The Central region constitute of Mahalapye East, Mahalapye west, Palapye, Serowe, Bobonong Letlhakane and Selibe-Phikwe. From which **Selibe-Phikwe, Palapye** and **Mahalapye East** and **Mahalapye West** districts were selected.

Politically, Botswana is divided into **districts,** without regions. In order for this study to maintain uniformity between the agricultural and the political mapping of Botswana, the Selibe-Phikwe district in the central region (agricultural categorization) was considered the North East District (political categorization). The Palapye and Mahalapye and Multi Specie Abattoir (agricultural categorization) were categorized as Central and Kweneng Districts (political categorization).

3.4 MATERIALS AND METHODS FOR DEMOGRAPHIC PROFILING OF CATTLE AND CATTLE FARMERS IN BOTSWANA

3.4.1 Data source and data collection

Primary data was sourced through on-site direct observation, farm enumeration and face to face interview using structured questionnaires. Information was collected from cattle farmers and their family; herd boys, meat shop operators; veterinarians; extension officers, village chiefs. Information collected included, biodata and socioeconomic description of farmers, farm location (Regions, districts and villages), farm age, farm capacity, livestock species, breeds of cattle, farming systems, uses of cattle, farmers' knowledge and experience of bovine cysticercosis, types and magnitude of government intervention measures.

Secondary data was sourced from relevant published and un-published documents available at the libraries and archives of the Botswana University of Agriculture and Natural Resource (BUAN) and Botswana Meat Commission (BMC)

3.4.2 Calculation of Sample

Formula for sample size calculation:

$$n = Nx \frac{Z^2 x\, p\, x\, (1-p)}{e^2} / N - 1 + \frac{Z^2 x\, p\, x\, (1-p)}{e^2}$$

Where: N = Population size,
Z = Critical value of the normal distribution at the required confidence level,
p = Sample proportion,
e = Margin of error

N = Population size = 100,000 (since population size is large and not known and sample size does not change much for population larger than 100,000)

Z = Critical value = 1.96 (at 95% confidence level, the critical value is 1.96)

p = Sample proportion = 0.10

e = Margin of error = 5% = 0.05

$$n = 100{,}000\, x \frac{1.96^2 x\, 0.1\, x\, (1-0.1)}{0.05^2} / 100{,}000 - 1 + \frac{1.96^2 x\, 0.1\, x\, (1-0.1)}{0.05^2} = 144$$

The sample of 144 was approximated to 150 respondents

TOTAL NUMBER OF STUDIED HOUSEHOLD: In each household only one respondent was sampled. So, total number of studied household in number of respondents, which is one hundred and fifty (150).

3.4.3 Administration of questionnaire

The multistage sampling technique was used to enumerate the population because the population is stratified into region, districts and animal holdings (Statistics Botswana, 2015). The purposive sampling technique was used to select two agricultural regions; one with the highest cattle population; the central region and the second with the lowest cattle population; the western region.

From the Central region, the Central district that has highest cattle population was selected. Using the convenience sampling method in order to make up a representative sample, some respondents were taken from Kweneng districts. This resulted to a combined district designated as Central + Kweneng district. Secondly, district with the lowest cattle population, which is North East was selected.

From the western region, the district with the highest cattle population, the Ghanzi district and the district with the lowest cattle population, the Kalagadi district were selected.

Because human and animal population are sparse convenience sampling technique was used to enumerate individual respondents.

The questionnaires were administered directly by the researcher with the help of a local interpreter for non-English speaking respondents. One hundred and fifty (150) respondents were sampled for the entire survey, (Table 1); one (1) questionnaire was invalidated because the respondent declined vital questions, giving one hundred and forty nine (149) questionnaire.

Table 2 sample size according to districts of respondents

DISTRICTS	SAMPLE SIZE
North East	30 + (1 invalid)
Central +Kweneng	31
Kalagadi district.	36
Ghanzi	52
Total	149 (+1)

3.4.4 Data analysis: The data studied and analyzed are demography of famers

Descriptive statistics was used to lay out and describe the data. Percentages of variables were compared to show spread of answers

3.5 MATERIALS AND METHODS FOR DETERMINING THE PREVALENCE OF *Taenia saginata*/CYSTICERCOSIS IN BOTSWANA USING PASSIVE ABATTOIR MEAT INSPECTION

3.5.1 Data sources and data collection –

Primary data was sourced through post-mortem meat inspection at export and local abattoirs, slaughter slabs, butcheries

Information collected are; number of animals slaughtered, number of animals harbouring *Taenia saginata*/cysticercosis, site of cysts

3.5.2 Sampling procedures and population

Abattoir sampling for bovine cysticercosis prevalence study involved multistage, purposive sampling and convenience techniques.

Abattoir sampling procedure was determined following the procedure in section 3.4.3

Convenience Sampling Technique was used to select a total of 15 meat premises including BMC. BMC prevalence served as standard because its prevalence is published annually and adopted as national prevalence.

NOTE: Meat premises sampled in Molepolole, Mahalapye, multispecies abattoir (MSAB) and BMC Lobatse were all grouped together giving Central + Kweneng District. The sampled meat premises are shown in Table 3

Table 3 sampled abattoirs according to regions and districts

Regions	Central Regions		Western Region	
Districts	Central & Kweneng Districts	North East District	Ghanzi District	Kalagadi District
Meat Premises	1. BMC Lobatse 2. Multi-Specie Abattoir Botswana 3. Tsholeta slaughter slab 4. Kubu Slaughter Slab 5. Maruping Slaughter Slab	1. Selibe-Phikwe Town Council Abattoir 2. Mmadinare Abattoir 3. Sandy's Meat 4. Botshabelo Meat Shop 5. Lesongwane Meat Market	1. Thothonu Meat Place 2. Rhodes (Meg Farm) 3. Tithe Complex	1. Cecil Waters 2. Kang Meat Market

3.5.3 Data Analysis-

1. Descriptive Statistics was used to lay out and describe the data
2. Data from passive abattoir post mortem examination was subjected to the Analysis of Variance (One-Way ANOVA) in order to check for significant difference in means of prevalence among the abattoirs.
3. Results of means of prevalence within and between Districts using questionnaire were compared for any significant difference and relevance

3.6 MATERIALS AND METHODS FOR DETERMINING THE PREVALENCE OF BOVINE CYSTICERCOSIS IN BOTSWANA USING DATA OBTAINED FROM ACTIVE POPULATION SURVEY

3.6.1 Data source and Collection:

Primary and secondary data was sourced and collected as described in section 3.4.1

3.6.2 Questionnaire sampling (Survey)

Sample size determined as worked out in section 3.4.2.

The multi-stage and purposive sampling techniques as described in section 3.4.3 were used to select the regions and districts for administration of questionnaire.

Convenience sampling technique was used to enumerate individual respondents because human and animal population are sparse. One hundred and forty nine (149) respondents were sampled (Table 2 page 38).

3.6.3 **Data Analysis-** Basic data analysis is same as described in sections 3.4.4

3.6.4 Comparison of prevalence rates obtained using abattoir inspection and population survey

Botswana's published official prevalence data arise from BMC. BMC data is not representative because it does not capture prevalence arising from low throughput abattoirs. Data obtained from survey using active questionnaire administered directly to farmers were compared to prevalence data obtained through passive abattoir investigation. The objective of studying prevalence using novel population survey method was to query BMC data, and compare

prevalence results obtained from passive abattoir meat inspection and survey method within and across same sample (districts).

3.6.5 Calculations

1. Paired sample *t*-test was used to compare means of bovine cysticercosis prevalence from abattoir and survey methods
2. District abattoir bovine cysticercosis prevalence was worked out by calculating the mean values of prevalence obtained from sampled abattoirs within a district
3. District survey bovine cysticercosis prevalence was worked out by calculating the percentage of farmers who answered "yes" to the question, "Did you record bovine cysticercosis in your carcass in 2017

3.7 MATERIALS AND METHODS FOR IDENTIFICATION AND QUANTITATIVE ANALYSIS OF RISK FACTORS OF BOVINE CYSTICERCOSIS IN BOTSWANA.

3.7.1 Data sources and data collection

The primary data was sourced through direct observation and face to face interview using questionnaires structured in Likert scale format. Information was collected from cattle farmers and family; meat shop operators; veterinarians; extension officers; the general public (locals and migrants)

The secondary data was sourced from relevant published and un-published documents available at the Libraries and Archives of the Botswana University of Agriculture and Natural Resource (BUAN); Botswana Meat Commission (BMC)

3.7.2 Administration of questionnaire

Sample size was determined as worked out in section 3.4.2.

The multistage sampling technique was used to enumerate the population because the population is stratified into region, districts and animal holdings (Statistics Botswana, 2015).

The purposive sampling technique was used to select agricultural regions and districts as described in section 3.4.3

Convenience sampling technique was used to enumerate individual respondents because human and animal population are sparse. Likert scale formatted questionnaire used to collate information about risk factors. One hundred and forty nine (149) respondents were sampled, (Table 2; page 38).

3.7.3 Data analysis:

The dependent variables is answer to occurrence of bovine cysticercosis in the carcass. This could be 'Yes' or 'No'. The independent variables are the 16 risk factors.

1. Descriptive Statistics was used to lay out and describe the data.
2. Percentages of variables were compared to show spread of answers
3. The analysis of median was used to determine the most probable response for a risk factor in this particular population.
4. Chi-square was used to establish relationship between risk factor and occurrence of bovine cysticercosis
5. Computation of mean ratings of the risk factors was used to show the relative importance of each risk factors in prevalence of bovine cysticercosis
6. One sample *t*-test was used to test significance of the mean ratings of the risk factors in the prevalence of bovine cysticercosis
7. The inter quartile range was used to determine the level of polarity about the particular risk factor. Thus, large IQR shows that people are divided but a small IQR shows consensus among respondent either negatively or positively.
8. Logistic Regression was used to quantitatively rank risk factors thus, determine relevance of each risk factors to occurrence of bovine cysticercosis.

Binary logistic regression was selected as the appropriate regression analysis because the dependent variable "OCCURRENCE OF bovine cysticercosis" has dichotomous answer (binary). It is used as a predictive analysis to describe data and to explain the relationship (interplay) between the dependent binary variable and one or more nominal, ordinal, interval or ratio-level independent variables (the risk factors) to lead to an effect. In other words, logistic regression is used for quantitative ranking of the risk factor (Ama *et, al.*, 2010).

Let P be the probability of having the prevalence of bovine cysticercosis and 1-P, the probability of not having the prevalence; the ratio (P/1-P) defines the odds in favour of having the bovine cysticercosis. So, the Binary Logistic regression models the natural log of the odds ratio as a function of the independent variables:

$$\text{Ln}\,(P/1\text{-}P) = b_1 + b_2R_1 + b_3R_2 \ldots\ldots b_nR_n \qquad (1)$$

Where:

- Ln is the Natural log and n is the number of variables
- b_1 is the intercept (constant) that gives the value of log of (P/1-P) when the other factors are absent

- b_2 to b_n represent the change in the dependent variable for a unit change in the independent variable given that the other variables are constant:
- R_1 to R_n are the risk factors

These risk factors are:

Preference of rare to cooked meat; absence or distance of pit toilet; open grazing of animals; access to contaminated pasture (Dorny, *et. al.*, 2000; Boone, *et. al.*, 2007); sharing machineries (Calvo-Artavia, *et. al.*, 2013); visitors to the farm (Dupuy, *et. al.*, 2014); Dairy animals (Eichenberger, *et. al.*, 2011); use of sewage for organic manuring (Dupuy, *et. al.*, 2014); proximity to uncontrolled human defecation (Calvo-Artavia, *et. al.*, 2014); access to contaminated water (Flutsch, *et. al.*, ., 2008; Allepuz, *et. al.*, 2009); access to contaminated feed (Dupuy, *et. al.*, 2014); Being Female (Zdolec *et. al.*, 2012); beef sold in non-licensed places; absence of meat inspectors (Deschamps *et. al.*, 2013); procuring of infected meat; consumer prefer cheap meat; failure to de-worm herd boys (Zdolec *et. al.*, 2012); poor awareness campaign; non-adherence to fencing policy (Allepuz, *et. al.*, 2009).

3.7.3.1 Computations

Questionnaire used for risk factor study adopted the Likert scale format (Appendix 1, part B). Each question could have one correct answer out of five (5) possible options. These options were: strongly disagree, scored as 1; disagree with scored as 2; neutral with scored as 3; agree with scored as 4 and strongly agree with scored as 5. For the logistic regression, these five answers were collapsed into two categories of "agree"; comprising of 'Agree', 'strongly Agree' and "disagree"; comprising of 'strongly disagree', 'Disagree' and 'neutral'.

Finally, the logistic regression table contained 149 responses sub-categorized as either "agree" or "disagree".

Logistic regression was run to show the contribution of each risk factor to the probability of the prevalence. Because the sample size of 149 is small, the binary logistic could not be run for all 16 risk factors. Eight (8) risk factors with the top ranking mean were selected for the binary logistic regression to show relative contribution proportion of each factor to prevalence of bovine cysticercosis in Botswana

3.7.3.2 Application of the Pareto Principle to delimitate the top 20% contributors

Pareto principle, also known as the 80/20 rule, states that 80 percent of the output from a given situation or system is determined by 20 percent of the input. It also means that in solving a major problem, solving 20% of the important problems will give 80% success. Following mean ranking of risk factors, top 20% contributors were used for running Pareto analysis. By this

model, eliminating top 20% of important risk factors will result to 80% drop in incidence rate of bovine cysticercosis.

All analysis was carried out using Microsoft Excel and statistical package for social science (SPSS) for windows.

3.8 MATERIALS AND METHODS FOR IDENTIFYING NOVEL RISK FACTORS OF *Taenia saginata*/cysticercosis USING NON-PARTICIPATORY INTERVIEW AND VISUAL OBSERVATION METHODS

Risk factors listed in structured questionnaire used in this research were derived from available literatures (Dorny, *et. al.*, 2000; Boone, *et. al.*, 2007; Flutsch, *et. al.*, ., 2008; Allepuz, *et. al.*, 2009; Zdolec *et. al.*, 2012; Calvo-Artavia, *et. al.*, 2014; Dupuy, *et. al.*, 2014). Some risk factors do not apply to Botswana. However, certain sociocultural practices and lifestyles peculiar to different regions and districts in Botswana constitute bovine cysticercosis risk factors. These risk factors are un-documented. It was the objective of this interview and observation exercise to uncover these novel and un-documented risk factor and document same.

3.8.1 Data sources and data collection

The primary data was sourced through direct observation and face to face interview of respondents using structured questionnaire. Relevant information was obtained from cattle farmers and family; meat shop operators; veterinarians and extension officers, politicians and traditional leaders.

3.8.2 Personal observation and administration of interview

The multistage sampling technique was used to enumerate the population because agriculturally the population is stratified into region, districts and animal holdings (Statistics Botswana, 2015).

The purposive sampling technique was used to select 2 agricultural regions and four districts as described in section 3.4.3

Convenience sampling technique was used to enumerate individual respondents because human and animal population are sparse

Whereas one hundred and forty nine (149) respondents participated in risk factor study using questionnaire only fifty (50) respondents were engaged in oral interview used to undercover novel risk factors (table 4).

3.8.3 Interview questions for novel risk factors study

1.0 In addition to documented risk factors, please indicate other determinants of the bovine cysticercosis?

2.0 Have you noticed any seasonal index of bovine cysticercosis ?

3.0 In which seasons of the year is incidence of bovine cysticercosis highest

4.0 Can you relate incidence of bovine cysticercosis to any lifestyle of practices in your locality

5.0 What one thing can the government do to put an end to bovine cysticercosis

Table 4 sample size of interviewed respondents according to districts of respondents

DISTRICTS	SAMPLE SIZE
North Central	10
Central +Kweneng	20
Kalagadi.	10
Ghanzi	10
Total	**50**

3.9 MATERIALS AND METHODS FOR DETERMINING EFFECTS OF THE BOVINE CYSTICERCOSIS ON THE FINANCIAL, SOCIO-ECONOMIC AND PSYCHOSOCIAL WELLBEING OF CATTLE FARMERS IN BOTSWANA

3.9.1 Data sources and data collection

The primary data was sourced through direct observation and face-to-face interview using questionnaires. Relevant information was obtained from cattle farmers and family; meat shop operators; veterinarians; extension officers; the general public (locals and migrants)

The secondary data was sourced from relevant published and un-published documents available at the Libraries and Archives of the Botswana University of Agriculture and Natural Resources (BUAN) and Botswana Meat Commission (BMC).

3.9.2 Administration of questionnaire

Sample size determined as worked out in section 3.4.2.

The multistage sampling technique was used to enumerate the population because agriculturally the population is stratified into region, districts and animal holdings (Statistics Botswana, 2015).

The purposive sampling technique was used to select 2 agricultural regions and four districts as described in section 3.4.3

Convenience sampling technique was used to enumerate individual respondents because human and animal population are sparse

One hundred and forty nine (149) respondents were sampled, (Table 2; page 38).

3.9.3 Data analysis

The independent variables is answer to occurrence of bovine cysticercosis in the carcass. This could be 'Yes' or 'No'. The dependent variables are the 14 socioeconomic indicators of effects of bovine cysticercosis on farmers

1. Descriptive statistics was used to lay out the data
2. The Cross-referencing Method: a comparative approach was used to assess the socio-economic effects of bovine cysticercosis on the farmers. Socioeconomy of affected farmers were compared during and after the experience of bovine cysticercosis, and compared with non-affected farms across these same periods of time
3. **The binomial logistic regression,** which uses binary variables was used to analyze the effects of the occurrence of bovine cysticercosis on the socio-economy of the farmers.

Regression Defined: Regression studies the interplay of factors to lead to an effect. It studies the effects that one independent variable has on several dependent variables. As a predictive analysis it helps to describe data and to explain the relationship (interplay) between the dependent variable and one or more nominal, ordinal, interval or ratio-level independent variables to lead to an effect (Ama, *et, al.,* 2008).

Binary Logistic Regression estimates the probability that a characteristic is present, given the values of explanatory variables, for example in a single categorical variable; $\pi = Pr(Y = 1|X = x)$. To estimate the probability of having a particular outcome in a population, X is considered the predictor variable that might contribute to the outcome. Naturally since all sections of the populations do not have equal probability of success, the probability of success will depend on the level of X.

In the analysis of the effect of bovine cysticercosis on the socio-economy of affected farmers the equation was;

- Let Y be a binary response variable

  $Y_i = 1$ *if the trait is present in obervation*

  $Y_i = 0$ *if the trait is NOT present in observation*

- $X = (X_1, X_2, \ldots., X_k)$ be a set of explanatory variables which can be discrete, continuous or a combination. X_i is the observed value of the explanatory variables for the observation i, which can be expressed as

Model: $\pi = \Pr(Yi = 1 - Xi = xi) = \frac{\exp(\beta_0 + \beta\_1x\_1)}{1 + \exp(\beta_0 + \beta_{1x_1})}$ Equation 1

To achieve a linear relationship between the dependent and the independent variables, the log of the proportions are used. So the equation becomes:

Equation: $\log\left[\frac{P}{1-P}\right] = \beta_0 + \beta_1 X_1$ Equation 2

Where **P** is the **probability of having** the socioeconomic effect of bovine cysticercosis and **1-P**, is the **probability of not having** the socioeconomic effect of bovine cysticercosis on affected farmers

the ratio **(P/1-P)** defines the **odds in favour of having** the socioeconomic effect of bovine cysticercosis on affected farmers

This equation can be reduced to

$y = \beta_0 + \beta_1 X_1$ Equation 3

Where y is the dependent variables which has fifteen categories of the socio-economic effects of the bovine cysticercosis on the affected farmers

β_0 is the **constant (intercept)**, which implies that if there is no independent variable, the odd of the socio-economies would be the value of the constant

β_1 is the parameter which is the **effect or the odd of the independent** variable on the dependent variable

X_1 is the **independent variable,** which is the occurrence of bovine cysticercosis

These categories of the dependent variables are:

S1 =Ability to provide food for family;

S2 = Ability or difficulty accessing Healthcare;

S3= Ability to provide education for wards;

S4 = Inability to afford house rent;

S5 = Inability of difficulty to save money;

S6 = Borrowed money for family upkeep;

S7 = Borrowed money for business;

S8 = Lay off workers;

S9 = Unable to employ new workers;

S11 = Unable to meet social responsibility;

S12 = Unable to meet religious responsibilities;

S13= Suffered emotionally because of negative effect of bovine cysticercosis on farming business;

S14 = Diversify Business because of uncertainty of Cattle business;

S15 = Abandon cattle farming because of bovine cysticercosis

These fifteen categories of the dependent variable were captured as binary variables which take answers as Yes = 1 or No = 0. The independent variable, which is the occurrence of bovine cysticercosis in the farm was captured as binary variables which take answers Yes = 1 or No = 0. The independent variable, was tested with each dependent variable.

3.9.4 Dependent variables and data studied

a. **Effect of bovine cysticercosis on**: mean farms sizes; income of farmers
b. **Ability of farmers to:** Employ new workers; meet social responsibility; meet religious responsibilities; provide education for wards; accessing HealthCare; afford house rent; provide food for family; save money; diversify business
c. Psycho-social reactions by Affected Farmers

All analysis was carried out using, Microsoft Excel and Statistical Package for Social Science (SPSS) For Windows.

3.10 MATERIALS AND METHODS FOR PROFILING *Taenia saginata*/cysticercosis PROTEINS AND IDENTIFICATION OF IMMUNOGENIC ONES

Methods employed in profiling bovine cysticercosis proteins and identifying immunogenic ones included; samples collections, samples preparations, reagents preparations, existing and novel laboratory protocols. Details of the materials and methods employed in profiling bovine cysticercosis proteins and identifying possible vaccine candidate antigens are contained in Appendix II; pages 195 - 214. Some operations in this study are novel and on-going, for continuity sake ancillary studies section is contained in Appendix III, pages 198 -217

CHAPTER FOUR

4.0 RESULTS

4.1 DEMOGRAPHIC PROFILING OF CATTLE AND CATTLE FARMERS IN BOTSWANA

4.1.1 Background of the Study

In Botswana like in most other African countries, the agricultural sector is vital to the socio-economic development of the nation (Statistics Botswana, 2016a; Statistics Botswana, 2016b). The nation is Africa's largest beef exporter (Burges, 2006; Engelen, *et. al.*, 2013; Tshiamo, 2015); with the beef industry described as the nation's third highest foreign earner, and largest component of the Agrifood System (Engelen, *et. al.*, 2013). This sector has been highlighted as strategic within the country's economic diversification agenda (Pablo, *et. al.*, 2014). Cattle population had grown progressively from 2.25 million in 1996 to 3.10 million in 2005 (Burgess, 2006) to over 3.5 million cattle in 2012 (Statistics Botswana, 2013), but dropped to about 1.7 million in 2015; with a 45 percent decline in households raising cattle, from about 75,500 in 2004 to 39,000 in 2015; 1.9 million cattle in 2019 (Statistics Botswana, 2019). Unlike in most other countries of the world, where cattle farming is an exclusive occupation of few farmers, in Botswana, cattle ownership is a pride factor; a potential unifier and an important source of food (Tshiamo, 2015) as, every family owns cattle (Thornton *et. al.*, . 2003 in Patti *et. al.* 2010). This is the natural advantage that the nation has over other nations in the beef industry (Pablo, *et. al.*, 2014).

Total traditional cattle holding has decreased progressively from 75,037 in 2007 to 74,664 in 2011 to 37,755 in 2015. Total commercial holdings increased from 527 in 2007 to 1,061 in 2010 before declining to 765 in 2013. Similarly, total traditional cattle population has not been steady; increasing from 1,649,642 in 2007 to 2,260,262 in 2011 but progressively decreased from 1,985,595 in 2102 to 1,596,605 in 2014. Total commercial cattle population also increased from 138, 736 in 2007 to 399,478 in 2010 but decreased to 238,981 in 2013 (Statistics Botswana, 2017). Total cattle population, traditional and commercial as at 2019 was estimated at 1,596,605 (Statistics Botswana, 2019).

Most published materials have attributed the decline in Botswana's cattle population to unthrifty climatic conditions like, temperature and rainfall, unavailability of feed, poor funding of the agricultural sector due to change in government policies favouring non-agricultural sectors of the economy etc. However, some sociocultural and political factors bear more serious

effect on the cattle population than aforementioned. The aim of this demographic and characterization study was to understand the population and spread of cattle; cattle breeds distribution across population strata; analysis of farming systems (dairy, beef or dairy and beef); polities and gender dynamics of cattle ownership and farming in Botswana. This study also established relationship between these socio-cultural-characteristics of the cattle industry and cattle population. Results of this study provides information based on accurate knowledge of actual operators of the industry necessary for policy advocacy that enable achievement of SDG 1, 2, 3, 5, 8, 12

4.1.2 Materials and Methods for Demographic Profiling of Cattle and Cattle farmers in Botswana

Information about questionnaire administration for Demographic profiling of cattle and cattle farmers was discussed in section 3.4.22 & 3.4.3. Data analysis for the study as shown in sections 3.4.4. One hundred and forty nine respondents were sampled for the study as shown in table 2

4.1.3 Categorizing farmers' age groups

The age groups adopted in this work are 16-25, 26-35, 36-45, 46-55, 56-65, 66-75 and >75 years. The lowest margin is 16 because this is the average high school leavers' age. Some of these school leavers who do not proceed to college enter the farming industry at age 16. The highest age bracket is >75 (stretching up to 100). The life expectancy in Botswana is 68.3 for women and 62.94 for men. By experience, at age 75, most people have retired from active civil service back to farming. An age gap of 9 was arbitrarily adopted to divide out the brackets between 16 and 75

4.1.4 Results of Demographic Profiling of Cattle and Cattle farmers in Botswana

4.1.4.1 Demographic description of farmers according to gender

Table 5 demographics of farmers according to gender

Demography	Description	Male	Female	Sub total
Marital Status of Farmers	Unmarried	67.21	32.79	43.00
	Married	72.82	27.18	49.30
	Divorced	63.64	36.36	7.70
Educational Level of Farmers	Primary	82.46	17.54	11.40
	Junior School	75.68	24.32	22.20
	Senior School	68.24	31.76	14.80
	Diploma	67.54	32.46	26.80
	First degree	55.95	44.05	16.80
	Masters	35.00	65.00	2.00
	Others	0.00	100.00	1.30
	None	100.00	0.00	4.70
Level of Involvement of Farmers	Full time	74.22	25.78	41.90
	Part time	65.06	34.94	58.10
Other Occupation of Farmers	Trading	69.23	30.77	9.10
	Civil service	57.11	42.89	39.40
	Professional	50.00	50.00	1.40
	Others	84.21	15.79	13.30
	No other occupation	73.02	26.78	36.70
Family Size of Farmers	No child	70.69	29.31	11.60
	1-5 children	66.72	33.28	67.30
	6-10 children	85.25	14.74	18.30
	>10 children	25.93	74.07	12.70
Districts of Farmers	North East	59.90	40.10	20.20
	Central	64.42	35.78	20.80
	Kalagadi	83.06	16.94	24.20
	Ghanzi	67.71	32.29	35.30
Age of Farm	<1 year	61.11	38.89	3.60
	1-5 years	76.63	23.37	29.10
	6-10 years	65.68	34.32	23.60
	>10 years	66.14	33.86	44.00
Capacity of farm	>500	70.15	29.85	20.10
	100-499	75.37	24.63	13.40
	50-99	77.98	22.02	33.60
	10-49	66.04	37.96	21.60
	5-9	50.00	50.00	8.20
	< 5	39.39	60.61	3.30
Type of farm practice	Beef only	68.70	31.30	52.40
	Dairy only	68.42	31.58	1.90
	Dairy and Beef	40.74	59.26	45.70
Farmer's knowledge of bovine cysticercosis	Yes	70.06	29.94	94.20
	No	50.00	50.00	6.80
Record of bovine cysticercosis in farm or butcheries	Yes	74.47	25.53	42.30
	No	34.74	65.26	30.80
Financial effect of bovine cysticercosis on farmers	Yes	74.90	25.10	50.20
	No	64.69	35.31	45.60
Effects bovine cysticercosis on farmers' economy	Yes	72.63	27.37	36.90
	No	63.90	36.10	57.70

4.1.4.2 Demographic description of farmers according to age

Table 5b demography of farmers according to age

Age	Score No (%)	level of involvement of farmers		Marital status of farmers		
		Full time No (%)	Part time No (%)	Unmarried No (%)	Married No (%)	Divorced No (%)
16-20	1(0.67)	0 (0.0)	1(100.0)	1(100.0)	0(0.0)	0(0.0)
21-25	4(.03)	2 (50.0)	2(50.0)	4()100.0	0(0.0)	0(0.0)
26-30	8(5.4)	3(37.5)	5(62.5)	8(100.0)	0(0.0)	0(0.0)
31-35	19(12.8)	7(38.9)	11(61.2)	13(72.2)	5(27.8)	0(0.0)
36-40	21(14.1)	6(27.3)	15(72.7)	17(81.0)	3(13.3)	1(4.8)
41-45	21(14.1)	10(47.6)	11(52.4)	9(45.0)	9(45.5)	2(10.0)
46-50	24(16.1)	8(33.3)	16(66.7)	5(23.8)	12(57.1)	4(19.1)
51-55	26(17.5)	6(24.0)	19(76.0)	2(8.3)	19(79.2)	3(12.5)
56-60	12(8.1)	7(63.6)	4(36.4)	1(9.0)	9(81.8)	1(9.0)
61-65	6(4.0)	6(100.0)	0(0.0)	0(0.0)	5(100.0)	0(0.0)
66-70	2(1.3)	2(100.0)	0(0.0)	0(0.0)	2(100.0)	0(0.0)
71-75	3(2.1)	3(100.0)	0(0.0)	0(0.0)	3(100.0)	0(0.0)
>75	2(1.3)	2(100.0)	0(0.0)	0(0.0)	2(100.0)	0(0.0)
Total	149(100.0)	62(100.0)	84(100.0)	60(100.0)	69(100.0)	11(100.0)

4.1.4.3 Ownership of different species of livestock according to gender

Table 6 ownership of different species of livestock according to gender of farmers

Other Livestock In The Farm	Gender Of Farmers		Sub-Total
	Male (%)	Female (%)	
Sheep	30.30	69.70	1.40
Goats	36.14	63.86	11.10
Horse	30.30	69.70	1.40
Donkey and Horse	46.51	53.49	2.10
Sheep and Goats	66.67	33.33	7.60
Sheep, Goats, Donkey and Horse	83.92	16.08	9.00
Sheep, Goat and Horse	38.04	61.96	8.30
Goat, Donkey and Horse	100.00	0.00	4.20
Goat and Donkey	52.63	47.37	4.90
Sheep, Goats and Chicken	34.48	65.52	7.60
Goat and Chicken	39.74	60.26	6.90
Sheep, Goats, Donkey and Chicken	52.63	47.37	4.90
Goats, Donkey and Chicken	12.82	87.18	2.80
Goats and Horse	46.51	53.49	2.10
Sheep, Goats and Donkey	59.52	40.47	9.00
Chicken	46.51	53.49	2.10
No Other Animal	55.12	44.88	13.20
Horse and Sheep	100.00	0.00	0.70
Sheep, Horse and Donkey	100.00	0.00	0.70

4.1.4.4 Ownership of different breeds of cattle according to gender

Table 7 ownership of different breeds of cattle according to gender of farmers

Combination of breeds owned by farmers	Gender of Farmers		Total
	Male (%)	Female (%)	
Tswana Breed	46.38	53.62	33.60
Brahman	100.00	0.00	3.40
Simmental	63.93	36.07	3.40
Charolais	100.00	0.00	0.70
Tswana Cross Brahman	0.00	100.00	0.70
Brahman Cross Simmental	0.00	100.00	0.70
Brahman, Simmental, Beef Waster	100.00	0.00	0.70
Brahman, Red Sussex	100.00	0.00	1.30
Tswana, Brahman, Simmental	55.54	44.85	10.10
Tswana, Brahman, Simmental, Brown Swiss	0.00	100.00	0.70
Tswana, Afrikanner, Brahman, Hereford, Simmental, Red Sussex, Brown Swiss	46.34	53.66	2.00
Brahman, Simmental	100.00	0.00	2.00
Tswana, Brahman	43.87	56.13	14.80
Tswana, Brahman, Charolais	30.30	69.67	1.30
Tswana, Afrikanner, Brahman, Simmental, Charolais	74.56	25.44	10.10
Tswana, Red Sussex	30.65	69.35	2.70
Tswana, Simmental, Pinzgauer	0.00	100.00	0.70
Tswana, Brahman, Charolais, Brown Swiss	100.00	0.00	0.70
Brahman, Musi	30.30	69.67	1.30
Tswana, Brahman, Simmental, Charolais	56.86	43.14	2.70
Tswana, Brahman, Brahman Cross Tswana	100.00	0.00	0.70
Tswana, Brahman, Brown Swiss	0.00	100.00	0.70
Tswana, Limousin	30.30	69.67	1.30
Brahman, Simmental, Beef Master	0.00	100.00	0.70
Tswana And Afrikanner	100.00	0.00	0.70
Tswana, Hereford, Brahman, Simmental	100.00	0.00	0.70
Brahman, Simmental, Aberdeen Angus, Friesland	100.00	0.00	0.70
Tswana, Simmental, Afrikaaner	30.30	69.67	1.30

Table 8 socio-economic effects of bovine cysticercosis across gender and ages of farmers

Socio-economic Effects	Category	Gender		Age of farmer						
		Male (%)	Female (%)	16-25 (%)	26-35 (%)	36-45 (%)	46-55 (%)	56-65 (%)	66-75 (%)	>75 (%)
Ability to provide food for family	Yes	23.2	13	80	29.2	12.2	12.5	27.8	40	0
	No	76.8	87	20	70.8	87.8	87.5	72.2	60	100
Ability to provide healthcare for family	Yes	18.2	10.9	60	25	9.8	6.3	33.3	20	0
	No	81.8	89.1	40	75	90.2	93.8	66.7	80	100
Ability to provide education	Yes	17.3	13	40	25	7.3	10.4	27.8	25	25
	No	82.7	87	60	75	92.7	89.6	72.2	75	75
Ability to afford house rent	Yes	11.2	15.2	20	25	9.8	4.2	22.2	25	0
	No	88.8	84.8	80	75	90.2	95.8	77.8	75	100
Ability to save money	Yes	48	43.5	60	44	43.9	37.5	66.7	100	25
	No	52	56.5	40	56	56.1	62.5	33.3	0	75
Borrowed money for family upkeep	Yes	23	9.1	40	20	15	14.9	27.8	40	0
	No	77	90.9	60	80	85	85.1	72.2	60	100
Borrowed money for business upkeep	Yes	16	11.1	20	16	10	16.7	16.7	20	0
	No	84	88.9	80	84	90	83.3	83.3	80	100
Laid off workers	Yes	23	15.6	40	28	12.5	18.8	22.2	60	0
	No	77	84.4	60	72	87.5	81.3	77.8	40	100
Unable to employ new workers	Yes	31	28.3	40	24	19.5	31.3	50	80	0
	No	69	71.7	60	76	80.5	68.8	50	20	100
Difficulty to meet social responsibility	Yes	25.3	30.4	60	37.5	22	20.8	27.8	60	0
	NO	74.7	69.6	40	62.5	78	79.2	72.2	40	100
Difficulty to meet religious responsibility	Yes	18	6.5	60	20	9.8	6.3	22.2	40	0
	NO	82	93.5	40	80	90.2	93.8	77.8	60	100
Experienced emotional disturbance	Yes	40	45.7	60	36	34.1	37.5	66.7	60	50
	No	60	54.3	40	64	65.9	62.5	33.3	40	50
Diversified business because of *bovine cysticercosis*	Yes	27.3	39.1	20	36	31.7	21.3	50	60	0
	No	72.7	60.9	80	64	68.3	78.7	50	40	100
Abandoned business because of *bovine cysticercosis*	Yes	3.1	2.2	0	4.2	2.5	4.2	0	0	0
	No	96.9	97.8	100	95.8	97.5	95.8	100	100	100

4.1.4.6 Farmers knowledge of and experiences of bovine cysticercosis

Table 9 percentage of farmers according to their different experiences due to bovine cysticercosis

	Knowledge (%)	Record (%)	Detained (%)	Condemned (%)	EFC (%)
Yes	94.6	42.3	28.0	14.7	18.1
No	5.4	57.7	72.0	85.3	81.9
Total	100.0	100.0	100.0	100.0	100.0

Key:
Knowledge: Farmers who had knowledge of existence of bovine cysticercosis
Record: Farmers whose carcass recorded as infested with bovine cysticercosis
Detained: Farmers who carcasses were detained because of infestation with bovine cysticercosis
Condemned: Farmers who Carcasses were condemned because of infestation with bovine cysticercosis
EFC: Farmers whose farm capacity were affected by bovine cysticercosis

4.1.4.7 The capacities of farms across to Districts

Table 10 percentage of farms capacities (measured in number of cattle heads) across districts

Districts	Capacity of Farm (Number of heads of cattle)						Total (%)
	>500 (%)	100-499 (%)	50-99 (%)	10-49 (%)	5-9 (%)	< 5 (%)	
North East	6.46	12.94	23.38	33.33	13.43	9.95	20.1
Central	9.62	16.35	41.83	25.96	6.25	0.00	20.8
Kalagadi	22.31	5.37	55.37	11.16	5.37	0.00	24.2
Ghanzi	32.66	17.19	19.20	19.20	7.74	3.73	34.9
Sub Total	20.1	13.4	33.6	21.5	8.1	3.4	100

Ghanzi district has highest population of cattle and highest number of farms with capacity > 500 heads of cattle. North East district has least number of farms that have farm capacity > 500 heads of cattle but highest number of farms having capacity of 10-49 cattle. Kalagadi and Central districts fall in between Ghanzi and North East districts, with Kalagadi having the highest number of farms with capacity of 50-99 heads of cattle (table 10)

4.1.4.8 Capacities of farms and farm practices

Table 11 farm capacities compared with farming practice

Capacity of Farm (No of Cattle)	Type of farm practice			Row Total (%)
	Beef only (%)	Dairy only (%)	Dairy and Beef (%)	
>500	50.0	3.3	46.7	20.5
100-499	35.0	0.0	65.0	13.7
50-99	53.0	6.1	44.9	33.6
10-49	58.1	3.2	38.7	21.2
5-9	83.3	0.0	16.7	8.2
< 5	50.0	0.0	50.0	2.7
Column Total	53.4	2.1	44.5	100

The modal farm capacity is 50 – 99 cattle with about 33.6%; followed by 10-49 cattle with about 21.2%. Farm capacities of >500 cattle heads contributed 20.5% of farm spread in Botswana. Beef farming only with 53.4% is more practiced than Beef and Dairy farming and Dairy farming only (table 11).

4.1.4.9 Mean capacities of farms across years

Table 12 average number of cattle heads per each holding across years

Farm Capacity across Years	No of responses	Mean cattle heads
Farm Capacity in 2009	137	200.44
Farm Capacity in 2011	140	193.36
Farm Capacity in 2013	144	194.06
Farm Capacity in 2015	145	218.53
Farm Capacity in 2017	145	243.86
Estimate percentage rise in number of cattle between 2015-2017		11.6

Highest mean farm capacity in 2017 was 243 cattle; least number of heads of cattle was 193.36, which was in 2011. Percentage rise in heads of cattle between 2015 and 2017 was 11.6% (table 12)

4.1.5 Discussions of demographic profiling of cattle and cattle farmers in Botswana

There are more male (69.8%) than female (30.2%) cattle farmers in Botswana (Table 5). This trend can be explained by the fact that in Botswana as in most African countries, cattle business is culturally designated a male-dominant farming industry while the female counterpart participate more in the crop farming industry. This result agrees with claims of Gender Researcher (2012) who remarked that "Cattle is a citadel of male power in Botswana" (Gender Researcher 2012 in Andrea, 2016). Women inability to acquire land can limit their participation in cattle farming. Historically, the Land Act of 1968 encouraged male dominance in land tenure. This was amended in 1993 and finally abolished in 2004 through the Abolition of Marital Power Act; which means that, any adult woman, married or not, can legally own (or use) land and cattle, sign contracts, and execute transactions with banks or other financial institution without the proof of her husband's or male relative's consent (Kalabamu, 2006 in Andrea, 2016). Nonetheless, some respondents claimed that it is probable that the actual process of implementing these laws still leaves gap for gender inequality and discrimination against women in the land tenure system (for cattle farming), land ownership, cattle ownership and cattle inheritance. Gulbrandsen (2012) observed that in Botswana, property claims, rights and access to cattle have been of the utmost importance for negotiating political and social relations, not the least for gender relations, where women have been excluded from cattle ownership (Kalabamu 2005 in Andrea, 2016). Having more male than female farmers as found in this research further agrees with the findings of the 'feminist political ecology approaches' that have emphasized how environmental resources in Botswana and 'elsewhere' are gendered (Hovorka 2006; Sikor & Lund 2009; Moepeng 2013). With more male than female cattle farmers, this study disagrees with Thornton *et. al.*, (2003), who said that two-thirds (66 percent) of the world's 600 million poor livestock keepers are rural women (Thornton *et. al.*, 2003 in Patti *et. al.*, 2010)

Recently scholars have also observed a shift from the status quo claiming that there are current efforts to stimulate economic and social development which focused on changing land policies for gender equality, increasing productivity, and the commercialization of cattle (UNDP, 2012 pg 4; Ransom 2011 in Andrea, 2016).

Another reason why there are more male than female respondents is that in most African homes the man (male) is considered the head of the family; as such claims ownership of the entire family; the house, the cattle and even the woman. So, even when the woman participates more directly in the cattle business, the cattle is considered a property of the man. This is in

agreement with the writings of Dr. Yirgalem Gebremeskel a Livestock Program Specialist Economic Growth and Transformation Office, USAID Ethiopia who posits that in Ethiopia, as in other African countries', although men own the cattle, the work of milking animals and dairy industry is dominated by women (Gebremeskel, 2014). In a related discourse the Head of Department of Agricultural Statistics and Research at the Ministry of Agriculture in Central Botswana, explained that out of respect, widows might refer to their personal cattle as belonging to their late husbands' (Andrea, 2016 pg 109).

It is imperative to clarify that the result of this study merely outlines ownership of cattle in relation to gender; but not gender participation in the day-to-day operations of the cattle industry. Tables 5-8 do not show that men participate more in the cattle farming industry in Botswana. Many feminist scholars challenge what they perceive as simplistic assumptions concerning the nature of male dominance among pastoral people (Dahl, 1987; Talle, 1987; Curry, 1996; Broch-Due & Hodgson, 2000; Hodgson, 2000; Njuki & Sanginga, 2013). They criticize earlier gender researches among cattle pastoralists for their exaggerated emphasis on the importance of male dominance, and instead focus attention on the complex roles, rights and relations of women in pastoral societies. They argue that beyond herding of livestock which is predominantly a male dominated sector; that if the totality of the participations of women (like milking, cleaning, selling of animals, tending of new borns) are taking into consideration, it would be obvious that women contribute more in the cattle industry (Curry 1996; Broch-Due & Hodgson 2000; Hodgson 2000; Njuki and Sanginga 2013b).

In addition to having more male than female farmers, the male farmers owned more large farms capacities ranging between 100 to >500 cattle, than their female counterparts who own farms with capacities ranging from 5 to <100 cattle. Another reason why women own less cattle than men is that women's access to credits and other inputs is insufficient for them to engage in large scale cattle farming. Similarly, women owned more small stocks and chicken than cattle. The trend is however different in some regions of Botswana especially in Ghanzi where some "Rich White Women" who are favoured by both "race and class" have shown to have easier access to credits and inputs and so own large scale farms (Andrea, 2016). **So, in Botswana, gender and racial discrimination are an important determinants of cattle ownership and farming. These anomalies can be corrected using policies that stop gender discrimination and reduce inequalities; effecting achievements of SDG 5 and 10**

Most populous breeds of cattle in their order of popularity are Tswana, Brahman, Tswana/Brahman cross, Simmental, Charolais etc. (Table 7). Women owned more local than

exotic breeds of cattle within their flock, relative to their male counterparts whom, although they equally owned more local breeds than women, owned most of the exotic breeds. The local breeds are better adaptation for the Botswana climate than the exotic breeds. Similarly, among the exotic breeds the beef breeds are more adapted than the dairy breeds. Most dairy breeds are located in the Ghanzi districts (western region) where they are confined to Ranches and crawls. Youths in the age range of 18-30 years contribute 47.4% of the entire national population (Statistics Botswana, 2013). However, their participation in cattle farming is low (table 5b); leading to loss of useable man-hour/labour. Youths perceive cattle farming as unfashionable and tedious. As a result, prefer other jobs especially white collar jobs, which further explains why their participation in cattle business is predominantly part time. It is also possible that either this is the schooling age or most of the youth do not possess cattle, land and other inputs requisite for the cattle business. This result agrees with the Monitoring & Evaluation Unit, Agricultural Planning & Statistics, which realized that there was generally lack of youth participation in agriculture in Botswana (Ministry of Agriculture, 2008). In response, and to encourage the youth, Botswana government in conjunction with Citizen Entrepreneurial Development Agency (CEDA) (Botswana) credit facility, launched called Young Farmers Fund in April 2006. This fund was aimed at enabling the youth to get credit to establish agricultural businesses in Botswana. This program proposed that since the youth had difficulty accessing land, they were allowed to lease land for agricultural businesses at low premium rates over a long grace period (Ministry of Agriculture 2008). Despite these efforts, youth participation in cattle farming remains low (Table 5b).

Similarly, participation of farmers above 60 years (60-75 years) in cattle farming is low (Table 5b). This relates to Botswana's life expectancy at birth, which has progressed from 64.1 in 2009 to 68.3 in 2011 for women and from 58.32 in 2009 to 62.94 in 2010 for men (Statistics Botswana, 2012; Country Economy.com, 2016). By 2012, Botswana had a total of less than 10,000 males 'above 70 years' (Statistics Botswana, 2013). This explains why there are few farmers of age bracket (60-75) (Table 5b). Most of them retired from civil service into full time farming.

Age bracket (31-55) years are the most energetic and productive class in the society. They contribute about 15 percent of the national population (Statistics Botswana, 2013). Most are part time farmers (Table 5b). This group engage in active civil service duties or other non-farming businesses.

Overall population of active farmers has decreased across years (NBS, 2012). This study shows that a high proportion of highly skilled man-power and man-hour that could have been employed in cattle farming business are trapped in civil service and other non-cattle enterprises. Engaging this population in cattle farming would scale up productivity to achieve SDG 1, 8 and 12.

About 41.9% of farmers in Botswana are full time farmers (Table 5b). Botswana has more full time cattle farmers than Nigeria, which has less than 10% full time cattle farmers (Mafimisebi *et. al.* 2013). In Botswana cattle farming is both a traditional lifestyle and a thing of pride cutting across all tribes and sub-nations and age brackets. High percentage of the population operate either ranches or cattle post. On the contrary, in Nigeria, cattle is farmed predominantly in the northern part of the country but consumed more in the south. Only 1% of cattle in Nigeria are in ranches while the rest of the 99% are engaged in pastoral Nomadism, which is traditionally estimated at 83% pastoral, 17% village cattle and 0.3% peri-urban (Mafimisebi *et. al.*, 2013; Uchendu et. al 2015; Ducrotoy, 2016).

By 2011, Botswana average marriage age was 46-65 years (Statistics Botswana, 2012), same age range that constitutes about 12 percent of the unmarried from this study (Table 5b). Marriage age has gone up since 2010. Botswana Marriage Statistics published that the mean age at first marriage was 38 years for bachelors and (32-33) years for spinster (Statistics Botswana, 2012).

This study showed high percentage of divorce, but low percentage of marriages (Table 5). This agrees with Botswana Core Welfare Indicators Survey of 2009 and 2010. The Botswana Core Welfare Indicators Survey of 2009 and 2010 reported increase in divorce rate from 0.9% in 2002 to 2.2% in 2010; with 1.6% for female and 0.7% for male, increase in unmarried and separation, but decrease in marriages (Statistics Botswana, 2013). This observed trend agrees with the assertion of Seitshiro in 2010 that while Botswana's divorce rate has gone up, the country has been experiencing a declining marriage rate, at 18% (Seitshiro, 2010). Utlwanang (2017) observed that divorce rate in Botswana has been increasing over the years registering 56% in 2008, 60% in 2009 and 70% in 2010 (Utlwanang, 2017). These figures continues to increase since 2010. Some respondents report reported that Batswana women prefer single parenthood to marriage (Interview Reports, 2017 in Andrea, 2016)

Divorce impacts negatively on cattle ownership and farming. It leads to breakdown of family unit; causing partners to liquidate all jointly acquired assets (if in community of marriage) including home, farms and cattle etc.

Average family size was 4 person (table 5). This is within range of published figures by Botswana Core Welfare Indicators Survey of 2009 and 2010; an average family size of 4.5 persons in 1993/1994; 4.1 persons in 2002/2003 and 3.46 persons in 2009/2010 (Statistics Botswana, 2013) In traditional, un-mechanized farming setting, family size translates to available farming workforce. With an average family size of about 4 (excluding parents), Botswana possess, reasonable farming workforce, which could be harnessed in the agricultural sector. **This will achieve SDG 12**

Botswana has high literacy rate and this agrees with documented records of Statistics Botswana (2013), that an overall, 79.0% of the population had attended school as at 2012. However most educated respondents were part-time farmers. In Botswana as in most other African countries, active farming, particularly cattle farming, is considered non-elites business. Elites, comprising mostly of politicians, Afrikaners, and White women (in Botswana) (Andrea, 2016), own the bulk of the cattle. They are part-time farmers but employ herders on direct full-time basis (Mulale, 2001). This phenomenon is applicable to Nigeria where cattle is owned by a small but influential elite group known as the "Meyiti Allah" who are indirectly involved, but coordinate the activities of the Nomadic Fulani Herd Boys who roam with the animals (Ducrotoy, 2016; Integrated Regional Information Network 2009a; 2009b; 2009c; 2010a; 2010b; 2010c in Ducrotoy, 2016). Studies in northern Nigeria showed positive correlation between educational level and agricultural development (Alene and Manyong 2007). They however suggest that the impact of education is only significant in adoption of improved technology (Alene & Manyong (2007) p. 157 in Ayşegül, 2011). Other related studies show that level of education correlates directly with social status of farmers, ability of farmers to access credits, adopt cutting edge technology, which correlates directly with the development in agriculture (Schultz, 1964 in Ayşegül, 2011). Summarily, with few elites involved directly in cattle farming the level of development in the cattle industry is slow.

Whites, Afrikaners and English farmers own a reasonable percentage of large scale cattle farms in Botswana. Some locals (Batswana) have also been spotted in this category. These are either in ranches, (fenced and unfenced) cattle farms and cattle post (Masicke & Ulrich 2008 in Andrea, 2016). To this group, cattle farming is purely a commercial venture and being driven by 'rational' and 'economic' forces. However, another group of farmers referred to as the 'Communal farmers' are sometimes depicted as being less interested in commercial activity, but focused on the 'traditional' Batswana cattle exchanges and cattle accumulation per se (Burgess 2006; Masike & Ulriich 2008; Ransom 2011 in Andrea, 2016). Recently cattle

farming in Botswana has greatly moved away from traditional farming: on satisficing goals to full commercial enterprise: on profit maximizing goals, and from migratory animal to enclosed farms. This portends positive results for Botswana in the beef export industry.

Also observed is that majority of the families in Botswana possess at least one cow; leading to a popular claim that there are as much as three cattle to every Motswana (Burgess 2006 in Andrea, 2016).

The western region possesses more cattle farms and cattle population than the southern and central region individually (table 10). This finding agrees with data published by Annual Agricultural Survey Report, 2014 in Statistics Botswana, (2016 pg. 46). Ironically, majority of the cattle owners live in southern region so, although cattle population is higher in the western region, cattle household population is higher in the southern than western region (Annual Agricultural Survey Report, 2014 in Statistics Botswana, 2016 pg. 31). Going by slaughter records the southern region has more population than the western region (Annual Agricultural Survey Report, 2014 in Statistics Botswana, 2016). Reason for this disparity is that the national export abattoir, the Botswana Meat Commission, is situated in Lobatse in the southern region of Botswana. This company alone slaughters about 44 percent (110,000 out of 250,000 cattle) of all cattle slaughtered in Botswana. However, most of these cattle come from the western region. A further breakdown of the farmers' distribution according to districts shows that there are more farms but less farmers in the Ghanzi and Kalagadi districts of the Western Region than North East and the Central districts within the Central region (table 10). Additionally, the districts in the Western region possess more large scale farms than the districts in the Central region (table 10). It therefore follows that the districts in Western region would suffer more financial loss and socio-economic effects than the districts in the Central region in an event of disease outbreak or importation ban from the EU.

The cattle industry in Botswana emphasizes 'beef only', followed by 'beef and dairy'. Farming of dairy alone is rare and primordial (table 11). Dairy farming as a standalone-enterprise is not viable (Ministry of Agriculture, 2010a). Dairy products are in short supply in Botswana (Ayşegül, 2011; Moreki *et. al.*, 2011). Moreki *et. al.*, (2011) reported that as at 2010, the dairy sub-sector's contribution to the national liquid milk demand was 17%; with a per caput consumption of milk at 25.2 litres per person per year. Annual milk production was estimated at 7.70 million litres while 38.6 million litres were imported (Moreki *et. al.*, 2011). Currently about 80 percent of the milk consumed in Botswana is imported from South; making Botswana a net importer of dairy products from neighbouring countries.

Interview results of this research show that unlike the beef cattle which are allowed to hoof freely across the country, the dairy cattle are always housed. The low milk production is attributed to lack of quality feeds for dairy cattle, high feed costs and unavailability of good dairy breeds suitable for local environmental conditions (Moreki *et. al.*, 2011). In addition to housing cost, machinery and manpower requirements for dairy business are much higher than for the beef industry. Feed for dairy cattle is imported from neighbouring South Africa and are costly. **It therefore follows that local production of feed for dairy cattle industry would be a viable business in Botswana. Fodder production as a way of reducing feeding costs provides an opportunity that should be explored. This will create employment, increase farmers income and foreign earning. Do this answers to SDG of 1, 2, 3, 8, 9, 12**

Due to high input cost, scarcity of pasture and un-thriftiness of cattle caused by unfavourable climate (heat splashes), milk production level is uneconomical. The profit margin in dairy farming is minimal and unsustainable., even for large scale farms. This condition has forced many dairy farms, to shut down their dairy farms or revert to beef farming or a combination of beef and dairy (Mulale, 2001; Ayşegül, 2011; Dzimiri, 2013). In response, the government engaged consultants under National Master Plan for Arable Agriculture and Dairy Development (NAMPAADD) to advance policy recommendations that will enable Botswana produce dairy food for food security. This therefore meant that Botswana would make efforts to foster dairy development plans wherever the potential exists. In so doing the gap between production and consumption of dairy products in the country will be narrowed (Government of Botswana Publication, 2011). This achieves SDG 8 and 12.

Further processing of milk into various products such as skilm milk product and whole milk product etc is a promising sector. This should create employment to the locals and save the country foreign Exchange (Moreki et. al., 2011). As a general recommendation, the milk industry is highly recommended for further study and as a business opportunity for Botswana. Estimation of the average age of farms and cattle post in Botswana reveals that cattle farming in Botswana pre-dates independence; more than 50% of the farms are older than a decade (table 5). These finding agrees with Tshiamo (2015) and Andrea (2016) both of whom recognized cattle farming in Botswana as a cultural and generational practice. The cattle industry is symbolic for the farming community, as a potential unifier and as an important source of food (Pablo, *et. al.*, 2014; Tshiamo, 2015). Cattle is the mainstay for Botswana's economy. It is third highest single foreign earner after diamonds and tourism (Engelen, *et. al.*, 2013; Burges, 2006; Tshiamo, 2015).

Less than 30% of cattle farms and cattle post were built in the last 5 years and these are predominantly small scale farms (less than 100 cattle) (table 5). Results agrees with assertions of the Assistant Minister of Education and Skills Development, Mr. Moiseraele Goya, that in the recent past, the key national development goal has moved Botswana from a resource-driven economy (like agriculture) to a highly diversified knowledge-based economy (Olesitse, 2015). In a related lecture Prof. Roy du Pré, the EU Technical Advisor in Botswana and the SADC Region had published that the world has moved from being resource-based economies to knowledge-based economies and that Botswana has recently begun to beat the "Knowledge Economy" drum, indicating that Botswana must become a "Knowledge Society" and become part of the "Global Knowledge Economy" (Roy du 'Pre, 2018). Conclusively, while Botswana is steering towards knowledge economy, she is losing resource-based economy.

Ownership of livestock has been closely linked to economic, sociocultural and sentimental reasons. Few farmers kept 'only cattle' in their farms; majority kept at least two or more species of farm animals and two of more breeds of cattle (table 6 and 7). Farmers operate mixed livestock farming as a form of security, to serve, as multiple strings of income. Furthermore, mixed livestock farming has been linked to certain cultural and social values as a source of pride (Pablo, *et. al.*, 2014).

Goats are the most populous farm animal (table 6).

By relative population, Ghanzi has the highest number of cattle (beef, beef and dairy and dairy); it just naturally follows that more activities, responses and more positive cases would be experienced at Ghanzi. There is no record showing that the infestation of bovine cysticercosis had a direct effect on cattle population as per culling infested animals. However, financial losses due to devaluation or outright condemnation of carcasses have caused some farmers to either reduce investment scale or quit cattle farming entirely. The mean cattle size was 243 heads per farmer in 2017, which is the highest across years.

This work agrees with Statistics Botswana (2019) that there has been consistent decrease in cattle population since 2011.

4.1.6 Deliverables of the study

In summary, this work captured in one piece, the demography of cattle; cattle population, distribution and spread; ownership, available breeds and usage in Botswana. By adopting gender as an underlining criterion, this study further profiled cattle farmers in Botswana according to their biodata, scale and levels of involvement in cattle farming business, type of farming operations, knowledge of and/or experience of bovine cysticercosis; as well as

addressing some socio-cultural and political factors affecting women involvement in the cattle industry. This study uncovered some non-climatic factors affecting cattle population, especially gender, racial and sociocultural factors. The effect of this includes **promoting gender balance, reduce inequality, sustained growth** of the livestock industry; particularly **underdeveloped sectors** of the industry. Local production of feed for dairy cattle industry is a highly recommended as a viable business in Botswana. Fodder production as a way of reducing feeding costs provides an opportunity that should be explored. This will **create employment, increase farmers income and foreign earning**. This study provides policy recommendations for achievement of SDG1, 2, 3, 5, 8, 9, 10 & 12.

4.2 DETERMINATION OF THE PREVALENCE (RATE) OF BOVINE CYSTICERCOSIS IN BOTSWANA USING COMBINED METHODS OF PASSIVE ABATTOIR INSPECTION AND ACTIVE SURVEY METHODS

4.2.1 Background of the study

The prevalence (rate) of bovine cysticercosis in Botswana is high (Mosienyane, 1986; Tshiamo, 2015); and consistently increasing at rates from 12% in 1974 to 15% in 1983 (Mosienyane, 1986); 10-20% in 2006 and 18 to 20% between 2006 and 2014 (Modisa, 2014.) Most existing government published prevalence figures emanate mainly from records available at the Botswana Meat Commission (BMC) (Mosienyane, 1986; Modisa, 2014)

BMC is the country's national export abattoir, with three major slaughter plants located at Lobatse in the Southern Region; Francistown in the North East Region and Maun in the North West Region. Cumulatively, BMC slaughters about 44% (110,000 cattle) of overall national slaughter population (250,000) (Statistics Botswana, 2015). Although this percentage compared with the overall national slaughter may be numerically high, it is not statistically representative because it fails to capture the cattle population slaughtered in the other regions of the country, and cattle population slaughtered at other low throughput abattoirs, slaughter houses, butcheries and private homes. Thus, deriving the national prevalence rate relying solely on the BMC figures may not be very informative.

Another pitfall with using BMC records is that currently farmers who suspect that their cattle harbour the cysts avoid BMC but prefer the private slaughter and low throughput meat premises, being that the latter fails to conduct a thorough investigation (Aganga, 2017). Ultimately, these cases are not reflected in the BMC documented prevalence rates for bovine cysticercosis.

There is no known work that has attempted to study the prevalence of bovine cysticercosis in Botswana by analyzing figures from BMC as well as figures from middle/low-throughput abattoirs, mini slaughter houses; butcheries and at homes. Furthermore, there is no known work that has attempted to determine prevalence of bovine cysticercosis by combining and comparing figures obtained using passive abattoir inspection method and active survey (questionnaire) administered directly to farmers.

In addition to collating prevalence records available at BMC Lobatse and other selected low throughput abattoir across other regions of Botswana, this study attempted to elicit prevalence/incidence that occur at the private homes through non-participatory interview using structured questionnaires. These strategies showed to be more holistic than only passive abattoir inspection.

revalence of bovine cysticercosis obtained though abattoir sampling was 17.17%, which is 7.17% increase compared to 13.5% obtained in 2015 (Tshiamo, 2015). Prevalence rate btained through survey method was 42.3%. There is a significant difference $p = 0.025$ between ie prevalence obtained from abattoir and the prevalence obtained using survey. Significant ifference exist between means of prevalence ($P<0.05$) within and between districts. The :sults which arise from a methodological approach as applied in this study has been able to rovide more all-inclusive and reliable prevalence rates.

4.2.1.1 Results of prevalence rate study using abattoir sampling

4.2.1.2 Prevalence rate (%) results of bovine cyticercosis obtained from abattoirs through passive post mortem inspection

Table 13 prevalence rate (%) of bovine cysticercosis obtained through abattoir passive post mortem sampling

Meat Premises	Capacity/week	Positive Cases of Cysticercosis	Prevalence rate (%) (capacity/positive cases)
Central + Kweneng District			
BMC Lobatse	2700	270	10.00
MSAB	240.0	30	12.5
Kubu Molepolole I	200-230	30	14.0
Maruping Molepolole II	250-260	35	13.7
Tsholeta Mahalapye I	150	24	16.0
North-East District			
SPTC Abattoir	210	31	14.8
Madinare Abattoir	40	8	20.0
Sandy's Meat	12.0	2	16.7
Botshabelo Meat Shop	10-15	2	16.0
Lesongwane Meat Market	10-12	3	27.3
Ghanzi District			
Totonu Meat Place	15	2	13.3
Rhodes (Meg Farm)	20	3	15.0
Tithe Complex	10.0	1	10.0
Kalagadi District			
Cecil Waters	12	4	33.3
Kang Meat Market	20	2	10.0

Footnote: To achieve representative sampling, abattoirs in Kweneng and Central were selected and designated as Central

Table 13 shows that the Botswana Meat Commission (BMC) in Lobatse, which is one of the plants of the national export abattoir, has the lowest prevalence rate of 10%. Similarly, the Tithe Meat Complex, a low through-put meat place located at the Ghanzi District in the Western Region has a prevalence rate of 10%. The Multi Specie Abattoir Botswana (MSAB) located in the Kweneng district has the next lowest prevalence rate of 12.5%. The highest prevalence rate of 33.3% is recorded by the Cecil Waters Meat place, which is located at Kalagadi district in the Western Region followed by prevalence of 27.3%, which is recorded at Lesongwane Meat Market located in the North East District of the Central Region

4.2.2 Prevalence from abattoir passive sampling compared with published prevalence

Table 14 One sample statistics and test for prevalence results from abattoir meat inspection

								95% Confidence Interval of the Difference	
N	Mean	Std. Deviation	Std. Error Mean	T	Df	Sig. (2-tailed)	Mean Difference	Lower	Upper
15	16.1733	6.58510	1.70027	10.100	14	0.000	16.17333	13.5266	20.8200

Report of t-test:

Table 14 shows a one sample *t*-test for prevalence results from abattoir meat inspection. At *t* (14) = 10.10, the mean, which is the prevalence is 16.17; p = 0.000. Confidence Interval = (lower = 13.53; upper = 20.83).

This test shows that the prevalence of bovine cysticercosis obtained from abattoir is 16.17, and this is higher than the published (known) prevalence of bovine cysticercosis of 13.5 according to Tshiamo (2015).

4.2.2.1 Comparison of individual abattoir prevalence with published prevalence (standard) of 13.5% (Tshiamo, 2015)

Table 15 significant difference between the published prevalence and the individual meat premises

Premises	Capacity/Week (Observed)	Frequency (N)	Prevalence	ExFq		SE	P-Value
Botswana Meat Commission	2700	270	10	364.5	0.006576	0.005774	5.13E-08
MSAB (Kweneng)	240	30	12.5	32.4	0.022058	0.021348	0.325149
Kubu Molepolole I (Kweneng)	215	30	14	29.025	0.023305	0.023664	0.584938
Maruping Molepolole II (Kweneng)	255	35	13.7	34.425	0.0214	0.021533	0.537231
Tsholeta Mahalapye I (Central)	150	24	16	20.25	0.027902	0.029933	0.814875
SPTC Abattoir	210	31	14.8	28.35	0.023581	0.024504	0.709282
Mmadinare	40	8	20	5.4	0.054031	0.063246	0.885513
Sandy's Meat	12	2	16.7	1.62	0.098647	0.107669	0.627178
Botshabelo Meat Shop	12.5	2	16	1.6875	0.096654	0.103692	0.602049
Lesongwane Meat Market	11	3	27.3	1.485	0.103034	0.134324	0.909775
Totonu Meat Place	15	2	13.3	2.025	0.088233	0.087678	0.490958
Rhodes (Meg Farm)	20	3	15	2.7	0.076412	0.079844	0.577814
Tithe Complex	10	1	10	1.35	0.108062	0.094868	0.373012
Cecil Waters	12	4	33.3	1.62	0.098647	0.136049	0.977633
Kang Meat Market	20	2	10	2.7	0.076412	0.096825	0.933839

KEY:
Ho is that Po = 0.135; H_A is that Po ≠ 0.135; where Ho is Null Hypothesis; H_A is Alternate Hypothesis; Po is prevalence of individual meat premises;
ExFq is Expected Frequency = Observed (i.e. Number of animal slaughtered) X 0.135
SE = Square Root of Po (1-Po/N); Where Po is individual prevalence; N is national prevalence
ESE = P(1-P)/N where N is National Prevalence
P-Value = probability of rejecting Null Hypothesis when true
RESULT: BMC prevalence is significantly different (at 5.13E-08) from National prevalence of 13.5% whereas the prevalence rates from the other meat premises are not significantly different from the national prevalence

4.2.2.2 Knowledge of bovine cysticercosis and some effects of bovine cysticercosis on cattle farming

Table 16 results of questionnaire sampling about knowledge of bovine cysticercosis and some effects of bovine cysticercosis on farming (%)

	Knowledge	Record	Detained	Condemned	EFC
Yes	94.6	42.3	28.0	14.7	18.1
No	5.4	57.7	72.0	85.3	81.9
Total	100.0	100.0	100.0	100.0	100.0

Key:
Knowledge: Knowledge of bovine cysticercosis by farmers and operators of meat premises
Record: Farmers and meat premises operators who recorded bovine cysticercosis in their Carcasses
Detained: Farmers and meat premises operators whose carcasses were detained
Condemned: Farmers meat premises operators whose Carcasses were condemned
EFC: Farmers whose Farm capacity were affected by bovine cysticercosis

The prevalence rate of bovine cysticercosis obtained by administering structured questionnaire directly to farmers was 42.3%. This was obtained by calculating all respondents who answered "YES" to the question, 'Did you record bovine cysticercosis in your carcass in 2017'?
About 94.6% of respondents had knowledge of bovine cysticercosis; while 28.0% and 14.7% of respondent had their carcasses detained and condemned respectively.
Meat premises operators as used in table 16 includes middle/low-throughput abattoirs, mini slaughter houses, butcheries and at homes' backyard or private homesteads

4.2.2.3 Comparison of prevalence rates across districts obtained from passive abattoir meat inspection and questionnaire investigation

Table 17 prevalence across districts as obtained from abattoir inspection and survey

Districts of Respondents	Survey %	Abattoir %
Central + Kweneng	32.00	13.24
North East	41.01	18.96
Kalagadi	38.56	12.77
Ghanzi	57.72	21.67
Mean Prevalence	42.32	16.17

KEY: DCP: Districts Contribution to overall Prevalence. Districts survey % values were worked out by cross-tabulating each district with number of respondents who answered 'Yes' to the question, "have you recorded bovine cysticercosis in your carcass in 2017?"

Across the four districts of North East, Central, Ghanzi and Kalagadi, the prevalence of bovine cysticercosis obtained through survey were higher than the prevalence obtained through passive abattoir meat inspection.

Both by survey and abattoir methods Ghanzi district had the highest prevalence of 57.72% for survey and 21.67% for abattoir inspection method. North East had prevalence of 41.01% by survey and prevalence of 18.96% by abattoir inspection. Kalagadi district had prevalence of 38.56% by survey method and prevalence of 12.77% by abattoir inspection, Central + Kweneng district had prevalence of 32% for survey method and Prevalence of 13.24% for abattoir inspection methods (table 17).

The mean prevalence obtained through passive abattoir inspection was 16.17% while mean prevalence obtained through survey was 42.31% respectively. Both results were higher than both the published prevalence rate of 13.0% (Tshiamo 2016) and BMC prevalence rate of 10%. This higher prevalence indicate actual increase in prevalence between 2015 for Tshiamo's work and this research of 2017. This higher figures, arising from a more detailed and holistic survey also show that relying solely on prevalence figures obtained from BMC cannot be a good representative of national prevalence rates. Ghanzi is identified as hotspot for bovine cysticercosis in Botswana (table 17)

Table 18 independent samples test of prevalence from abattoir and prevalence from survey

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	T	Df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
Prevalence	Equal variances assumed	0.914	0.376	-2.971	6	0.025	-20.99750	7.06654	-38.28871	-3.70629
	Equal variances not assumed			-2.971	4.816	0.033	-20.99750	7.06654	-39.37387	-2.62113

Using a paired sample t-test the means of prevalence of bovine cysticercosis obtained from abattoir was tested against the means of bovine cysticercosis obtained using questionnaire

The prevalence results from Questionnaire was compared to prevalence results from Abattoir as a means of challenging the status quo of measuring prevalence using only data from passive abattoir post mortem examination

1. The Levene's test the Significance level at 0.376 is not statistically significant, which means that both samples come from the same population.
2. From the t-test of Equality of Means, the *t*-value is -2.971 and a significance level of 0.025 ($P<0.05$).

Analysis shows that there is a significant difference ($P<0.05$) between the prevalence obtained from abattoir and the prevalence obtained using questionnaire

4.2.2.4 Multiple comparisons of prevalence rate (%) of bovine cysticercosis across districts

Table 19 multiple comparisons of prevalence rate (%) of bovine cysticercosis across districts

Test of Homogeneity of Variances				ANOVA					
Percentages of bovine cysticercosis				Percentages of bovine cysticercosis					
:vene's Statistic	df1	df2	Sig.		Sum of Squares	Df	Mean Square	F	Sig.
				Between Groups	438.454	3	146.151	9.533	.002
1.619	3	11	0.241	Within Groups	168.636	11	15.331		
				Total	607.089	14			

1. In the Levene's test, the significance level of 0.241 is significant and shows that the samples are not the same (equal). It further shows that the data is eligible for ANOVA test of variance

2. From Analysis of variance (ANOVA) test, a significance level is 0.002, shows that there is a significant difference in the prevalence rates between the districts

Table 20 multiple comparisons of prevalence of bovine cysticercosis across districts

(I) District of meat premises	(j) District of meat premises	Mean difference (i-j)	Std. Error	Sig.	95% confidence interval	
					Lower bound	Upper bound
North East	Central	5.7200	2.4763	0.155	-1.733	13.173
	Kalagadi	-10.1900*	3.2759	0.042	-20.049	-.331
	Ghanzi	6.1933	2.8594	0.193	-2.412	14.799
Central	North East	-5.7200	2.4763	0.155	-13.173	1.733
	Kalagadi	-15.9100*	3.2759	0.002	-25.769	-6.051
	Ghanzi	.4733	2.8594	0.998	-8.132	9.079
Kalagadi	North East	10.1900*	3.2759	0.042	-.331	20.049
	Central	15.9100*	3.2759	0.002	6.051	25.769
	Ghanzi	16.3833*	3.5743	0.004	5.626	27.140
Ghanzi	North East	-6.1933	2.8594	0.193	-14.799	2.412
	Central	-.4733	2.8594	0.998	-9.079	8.132
	Kalagadi	-16.3833*	3.5743	0.004	-27.140	-5.626

Key: *. The mean difference is significant at $p < 0.05$ level. Using the Tukey HSD test to compare one district against another

The Mean prevalence of the Districts is as follows: North East = 41.01%; Central = 32.00%; Kalagadi = 38.56%; Ghanzi = 57.72%. There is a significant difference of 0.042 ($P<0.05$) between the means of the prevalence in Selibe-Phikwe and Kalagadi and a significant difference of 0.002 between the means of the prevalence in Central and Kalagadi. There a significant difference of 0.004 ($P<0.05$) between the means of prevalence in Ghanzi and Kalagadi.

4.2.2.5 Documented prevalence across years

Table 21 an outline of prevalence across years

Year	Prevalence Rate (%)
1974	12.0
1983	15.0
2002	10.0
2006	12.0
2012	18.0
2014	20.0
2015	13.5
2017	17.1

Ref: (Mosienyane, 1986; Modisa, 2014; Tshiamo, 2015)

Highest prevalence rate of bovine cysticercosis is noticed between 2012 and 2014 with prevalence of 18% and 20% respectively.

4.2.3 Discussion on prevalence of bovine cysticercosis in Botswana using passive abattoir inspection and active survey method

The BMC and MSAB, operate at higher slaughter scale than the privately owned, low throughput abattoirs (Table 13). These former abattoirs, more than the latter, observe higher compliance with provisions of Livestock and Meat Industries Act (2007) about procedures and actions to be taken regarding *Taenia saginata* taeniansis/cysticercosis. infested carcasses. The BMC and MSAB abattoirs are expected to record higher prevalence than the other slaughter facilities going by their higher capacity and mandatory compliance with standards in meat inspection. On the contrary, their prevalence is lower. This paradox can be explained by these three reasons; farmers who suspect that their cattle may harbour bovine cysticercosis cysts prefer unlicensed backyard slaughter and low throughput meat premises to the major abattoirs, being that the former fails to conduct thorough investigations (Aganga, 2017; Uchendu, 2020); the BMC shows lower prevalence figures than most abattoirs because in the recent years the BMC boycotted cattle supplied from cysticercosis hotspots; farmers' refusal to supply beef cattle to BMC, for example, has caused BMC to invest in partly owned feedlots in order to augment their demand of beef cattle.

Hotspots of cysticercosis are homogenously distributed across districts and regions, as both high and low prevalence scores were obtained from abattoirs within the same district or region. For example, both Celcil Waters, a village slaughter, having the highest prevalence of 33.3% and the Tithe Meat Place, in the city, with low prevalence of 10% are all located in the Western Region. Similarly, within the North East District, the prevalence at the Selibe-Phikwe Town Council (SPTC) Abattoir, an urban government-operated abattoir, had prevalence of 14.8% whereas Lesongwane Meat Market, a rural abattoir had prevalence of 27.3% (Table 13). Across board, an identifiable pattern was that the prevalence is generally higher in the rural and poorer communities than in the urban and elite communities even within the same district. This finding agrees with the findings of Farmers' Magazine (2016) and Hendrickx *et. al.*, (2019) both of whom claim that the prevalence and spread of bovine cysticercosis is closely linked to poverty and poor hygiene. It is also important to assert that categorizing a district as a hotspot based solely on the prevalence records obtained at the abattoir may be erroneous. Cattle slaughtered in an abattoir may be reared and purchased from another district or region, hence bovine cysticercosis could be imported. Thus, in the assessment of cysticercosis hotspots, an efficient trace back mechanism is important to tag the carcass with its original source. For instance, although the Ghanzi district of the Western Region is the highest producer of beef cattle, most

of these cattle are sold to and slaughtered at the BMC in Lobatse, which is located in the Southern Region. As such, although the positive cases are identified at the (BMC) Southern Region, the cases trace back to the Ghanzi district in Western Region. NRC, (2009) has described disease traceability, beyond diagnosis, as vital component for rapid response and disease surveillance.

The abattoir (mean) prevalence of 17.13% was higher than both the 2015 published (known) prevalence of 13.5% (Tshiamo, 2015) and the BMC prevalence of 10% which is considered the national standard. This indicates about 26.9% rise in prevalence of cysticercosis from 2015 to 2017 (table 21). And buttresses the argument that determining prevalence using a combination of BMC data and data from middle/low-throughput abattoirs are more representative than BMC data alone. This result also agrees with the findings of FAO (2013) that reliable estimates of bovine cysticercosis are lacking due to the low pathogenicity and under-reporting of this infection. At a significant score of p= 0.000, there is a significant difference in the prevalence (means) among the sampled abattoirs (table 14). Thus, the proportion of cattle with bovine cysticercosis in Botswana is significant. At 95% confidence interval, the wide difference between the upper and lower confidence interval, both of which are higher than the BMC and published prevalence (table 14), shows that there is a wide range (disparity) among the prevalence of the abattoirs (Ama, *et, al.*, 2008). It also shows that there is a significant difference between the BMC and published prevalence and the prevalence obtained through this study. This disparity holds and equally calls for a holistic prevalence study since it is apparent that relying on BMC results alone is not representative of cysticercosis prevalence in Botswana.

Although some meat premises showed higher prevalence than both the BMC and national prevalence, only the BMC prevalence is significantly different from the published prevalence (p=0.02; the reason being that most meat premises having high prevalence posseses small sample size. For example, the Cecil Waters with prevalence of 33.3% has slaughter capacity of 12 cattle per week (table 15). Although the time series analysis of prevalence of cysticercosis does not show a consistent pattern, it does appear that there is a correlation between annual temperature and prevalence of cysticercosis. Highest cysticercosis prevalence was noticed between 2012 and 2014 with prevalence of 18% and 20% respectively (Table 21). The probable explanation for the spike in prevalence is that Botswana experienced drought between 2010-2012. Drought leads to pasture scarcity, which leads to hunger and starvation. Scavenging

arising from scarcity of pasture increases contact exposure of cattle to oocysts of bovine cysticercosis contained in human feces.

Furthermore, malnourishment cause immunosuppression, germ recrudescence (Droves, 2011), that in turn allows worn burden to establish easily. Prevalence results obtained from the questionnaire investigation are higher than abattoir prevalence. Unlike the abattoir meat inspection, which is passive and investigates only the major and government abattoirs, the survey method pro-actively investigates across boards. Additionally, the survey methods investigated private abattoirs; slaughter houses and the individual farmers. It is imperative to highlight that both the abattoir and questionnaire samples were subjected to the Levene's test of significance, given a significance level of 0.376, which is not statistically significant. This means that the variances of the two samples are the same (equal) and that both samples originate from the same population (table 18 and 19). Conclusively the questionnaire (survey) method is more holistic than the passive abattoir method.

There is a significant difference (0.042) ($P<0.05$) between the means of bovine cysticercosis prevalence in North East and Kalagadi districts; and a significant difference (0.002) ($P<0.05$) between the means of bovine cysticercosis prevalence in Central and Kalagadi districts; and a significant difference (0.004) ($P<0.05$) between the means of bovine cysticercosis prevalence in Ghanzi and Kalagadi. Significant difference in means is observed between Kalagadi and Central and between Kalagadi and North East. The difference occurred in the relationship between Kalagadi and the other three districts. These differences in means informed the need to study the differences in lifestyles of locals as well as the correlation between the lifestyle, as risk factors, and the observed prevalence at the district level. Uchendu, *et. al., unpublished* 2020 has shown a correlation between the lifestyle of the farmers as risk factor and observed prevalence at the district level. For example, the Kalagadi district is home to the Basarwa tribes "bush men" who customarily do not use toilets but insist on open defecation in pastureland. They consume rarely done beef and hoof cattle through a semi-sedentary-to-nomadic pastoral system. In Ethiopia, scholars have observed these differences in prevalence of bovine cysticercosis among districts; particularly where there are differences in health status, healthcare delivery, beef consumption lifestyle and preferences (Hailemarian, 1980 in Tolosa, 2010; Tembo, 2001 in Tolosa, 2010; Dawit, 2004 in Tolosa, 2010; Nigatu, 2004 in Tolosa, 2010; Hailu, 2005 in Tolosa, 2010; Ahmed, 1990 in Tolosa, 2010; Abunna *et. al., 2007* in Tolosa, 2010). Ghanzi with a survey prevalence of 57.72% and abattoir prevalence of 21.67%

is a major hotspot for bovine cysticercosis in Botswana (table 17). Makunda extension centre in Ghanzi district has bovine cysticercosis prevalence of 43 (table 27)

Knowledge of existence and effects of bovine cysticercosis in cattle farming was commonplace (table 16). This knowledge traces back to the previous national awareness campaign programmes. Farmers' knowledge of ovine cysticercosis ranged from those who knew only the colloquial name as 'beef measles', to those who knew the cause, course, control and management of bovine cysticercosis . Unfortunately, this knowledge did not result in reduced prevalence of the cysticercosis; rather the prevalence increased over time (Mochankana & Robertson, 2018). The former minister of Agriculture, Mr. Dick Raf stated that the government awareness campaign could not empower the citizens with willpower requisite for attitudinal and behavioural changes towards personal hygiene and proper disposal of human defecate; both of which have been identified as major risk factors of bovine cysticercosis (Dick Raf, personal communication, 2017).

4.2.4 Recommendations prevalence of bovine cysticercosis in Botswana using passive abattoir inspection and active survey method

1. It is recommended that meat inspection officers should conduct thorough investigation at the major and high through-put abattoir as well as at private, low throughput abattoirs. This will ensure that infestations in cattle from farmers who deliberately avoid the major abattoirs will still be picked up at the low throughput abattoirs.
2. It is recommended that collection of prevalence data should include the use of questionnaire in addition to statutory use of passive meat inspection records from ~~in~~ public abattoirs.
3. The differences in means of prevalence across districts inform the need to study the differences in lifestyles as well as the correlation between the lifestyle of the farmers and observed prevalence at the district level.

4.2.5 Study Outcome prevalence of bovine cysticercosis in botswana using passive abattoir inspection and active survey method

1. **Representativeness:** This result is more representative than the regular prevalence obtained from BMC records alone; because it included; middle and low through-put abattoirs, butcheries, slaughter houses and household slaughters.

2. **Systematic:** It provides understanding of the prevalence of the disease at district, regional and national levels in Botswana, which is more systematic than generating only a national prevalence.
3. **Comparisons:** This study compared prevalence both at export and local abattoir and other meat places. It also compared prevalence across districts and showed significant differences.
4. **Active and more Holistic:** Using questionnaire to study the prevalence allowed for an active and more holistic study thus challenged the status quo of calculating prevalence based only on passive abattoir post-mortem inspection
5. **Risk Study Guide:** The results guided a coordinated study of the risk factors of the disease taking to consideration the established hotspots

4.3 IDENTIFICATION AND QUANTITATIVE ANALYSIS OF RISK FACTORS OF BOVINE CYSTICERCOSIS IN BOTSWANA.

4.3.1 Background of the Study

Bovine cysticercosis is an important food safety issue; having economic and public health implication (Grindle, 1978; Boone, 2007; Aganga, 2009; Tshiamo, 2015). The government of Botswana has practiced mass literacy on proper disposal of human feces (which is considered major risk factor); adequate and efficient meat inspection among several other prevention, control and intervention measures (Niels & Murrell, 2005 pp 63-72). Despite these efforts, the prevalence rate has increased (Mosienyane, 1986; Modisa, 2014). This increase has led researchers and policy makers to question the effectiveness of adopted control and prevention methods, in addressing the risk factors (determinants). It is probable that either the important risk factors have not been identified or/and the control and prevention methods targeted at identified risk factors are inadequate. Preliminary investigation for this study showed that major risk factors of bovine cysticercosis are behavioural, cultural and systemic. However, there is no known work that closely associates these risk factors with the lifestyle and cultures of Batswana in a bid to recommend behavioural changes.

Using a Likert scale format, survey data on risk factors were extracted from respondents. These data were analyzed using chi-square, binary logistic regression and other relevant statistic to determine the quantitative relevance of each risk factor in contributing to the prevalence of bovine cysticercosis. In addition to studying literature documented risk factors, this work identified novel risk factors and hot spots for bovine cysticercosis. Seasonal index of bovine cysticercosis shows close association between increased incidences and outset of rainfall in December, harvesting of Mopane worms in April and cutting of grass for roof thatching in October. Identified novel risk factors showed to be political, behavioural, systemic and sociocultural. This study has shown that interpreting the risk factors of this zoonosis in relation to the lifestyle and culture of Batswana provides adequate knowledge that can enhance its effective control and prevention. It is therefore recommended that prevention and control measures should target a behavioural change in the lifestyle of Batswana, as this is the driving force of the prevalence.

4.3.2 METHODS/METHODOLOGY

4.3.2.1 Data sources and data collection:

Primary data was sourced through direct observation and face to face interview using questionnaires structured in Likert Scale format. Details of primary and secondary data sources and data collection are discussed in section 3.7.1

4.3.2.2 Administration of questionnaire

The multistage sampling technique was used to enumerate the population because the population is stratified into region, districts and animal holdings (Statistics Botswana, 2015). Details of Questionnaire administration are shown in section 3.7.2.

4.3.2.3 Sample Size:

One hundred and forty nine (149) respondents were selected for the study. Details of sampling according to districts are show in table 2.

4.3.2.4 Data analysis

Descriptive Statistics was used to lay out and describe the data. Percentages of variables were compared to show spread of answers. Inter-quartile range was used to describe an approximation of consensus of the entire population. Chi-square was used to establish relationship between risk factor and occurrence of bovine cysticercosis Logistic Regression was used to determine how each of the risk factors relates to the occurrence of bovine cysticercosis. Pareto principle was applied to delimitate the top 20% risk factors contributors, which when eliminated would result to 80 % decrease in prevalence of bovine cysticercosis; details available at section 3.7.3.

4.3.3 RESULTS OF IDENTIFICATION AND QUANTITATIVE ANALYSIS OF RISK FACTORS OF BOVINE CYSTICERCOSIS IN BOTSWANA.

4.3.4 Analysis of respondents perception of risk factors using Likert Scale

Table 22 Likert scale analysis of risk factors (%)

Likert Scale	PRTC	ADPT	GRZ	ACP	SM	VIF	SFOM	PUHD	ACW	ACF	BF	BSNLP	ABMI	PIM	CPCUM	FD	PAC	NAFP
Strongly Disagree	10.7	1.3	2.7	2.7	4.8	2.1	6.8	2.0	2.7	2.7	15.6	8.8	9.5	10.9	10.2	2.0	12.9	4.1
Disagree	9.4	5.4	14.3	6.1	36.1	26.0	27.9	6.8	7.5	13.6	38.1	35.4	32.0	34.7	28.6	8.2	21.1	17.0
Neutral	9.4	0.7	4.1	2.0	6.8	3.4	2.0	4.1	0.7	4.1	8.2	3.4	4.1	4.1	2.0	1.4	1.4	5.4
Agree	38.3	47.0	51.7	52.4	36.7	49.3	42.9	49.7	59.2	57.1	24.5	34.7	36.1	31.3	44.2	53.7	40.8	51.7
Strongly Agree	28.9	43.6	24.5	35.4	12.2	14.4	16.3	37.4	26.5	18.4	7.5	14.3	15.6	16.3	13.6	33.3	21.1	19.0
Un-answered	2.0	0.7	2.7	1.4	3.4	4.8	4.1	0	3.4	4.1	6.1	3.4	2.7	2.7	1.4	1.4	2.7	2.7
Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0

KEYS:

PRTC = Preference of Rare to Cooked Meat; ADPT = Absence or Distance of Pit Toilet; GRZ = Gazing of Animals; ACP = Access to Contaminated Pasture; SM = Sharing Machineries; VIF = Visitors in the Farm; SFOM = Sewage for Organic Manuring; PUHD = Proximity to Uncontrolled Human Defecation; ACW = Access to Contaminated Water; ACF = Access to Contaminated Feed; BF = Being Female; BSNLP = Beef Sold in Non-Incensed Places; ABMI = Absence of Meat Inspectors; PIM = Procuring of Infected Meat CPCUM = Consumer Prefer Cheap Unfit Meat; FD = Failure to De-worm Herd Boys; PAC = Poor Awareness Campaign; NAFP = Non-adherence to Fencing Policy

4.3.5 Analysis of respondents according to their views of risk factors as contributors to prevalence of bovine cysticercosis

Table 23 percentages of respondents according to their views of risk factors as contributors to prevalence of bovine cysticercosis

Risk factors	Agree (%)	Disagree (%)	Median	IQR
1. Are the underlisted risk factors of bovine cysticercosis in Botswana	95.0	5.0	4.00	2
2. Preference of rare to well-cooked meat	69.4	30.6	4.00	2
3. Absence and or distanced pit latrine in the farm	92.5	7.5	4.00	0
4. Grazing of animal	78.3	21.7	4.00	0
5. Access to contaminated pasture	89.0	11.0	4.00	0
6. Visitors in the farm	66.9	33.1	4.00	2
7. Using sewage for organic manuring	61.7	38.3	4.00	2
8. Proximity to uncontrolled human defecation	90.8	9.2	4.00	0
9. Access to contaminated water	88.7	11.3	4.00	0
10. Access to contaminated feed	78.7	21.3	4.00	0
11. Poor awareness campaign about bovine cysticercosis to farmers and public	63.6	36.4	4.00	2
12. Non-adherence to fencing policy	72.7	27.3	4.00	2
13. Consumers prefer cheaper meat even unfit	58.6	41.4	4.00	2
14. Failure to deworm herd boys	88.3	11.7	4.00	0
15. Lack of or absence of meat inspections at butcheries or homes	53.1	46.9	4.00	2
16. Sharing of machineries and tractors	50.7	49.3	3.00	2
17. Being female (Diary animals slaughtered at very old age)	65.9	34.1	2.00	2
18. Beef sold at non-licensed areas	50.7	49.3	2.00	2
19. Butcheries procure cheaper meat which are more likely to be infested	49.0	51.0	2.00	2

The median analysis shows most probable response for the risk factor in population, while the Inter-quartile range (IQR) shows the level of polarity about a particular risk factor. A large IQR shows that people are divided but a small IQR shows consensus among respondent either negatively or positively.

Results show that respondents tend to disagree about the risk factors of 'sharing of machineries and tractors'; 'diary female animals staying longer'; 'beef sold at non-licensed areas'; 'butcheries procure infested meat because it is cheaper' contribute to the prevalence. Whereas the respondents tend to agree that the other 14 risk factors contribute to the prevalence of bovine cysticercosis. In order to determine the consensus, the agreement was further subjected to test of consensus using the interquartile range (table 23). The results show that there is strong agreement (high consensus) among respondents that the factors of, 'failure to deworm herd boys'; 'access to contaminated feed'; 'access to contaminated water'; 'proximity to uncontrolled human defecation'; 'access to contaminated pasture'; 'grazing of animal'; 'absence and or distanced pit latrine in the farm' are major contributors to the prevalence of bovine cysticercosis. There was weak agreement (low consensus) among the respondents over the other remaining 11 factors as contributors to the prevalence of bovine cysticercosis (table 23).

Figure 4 Bar chat of percentages of respondents according to their views of risk factors as contributors to prevalence of bovine cysticercosis (constituted results of Likert scale)

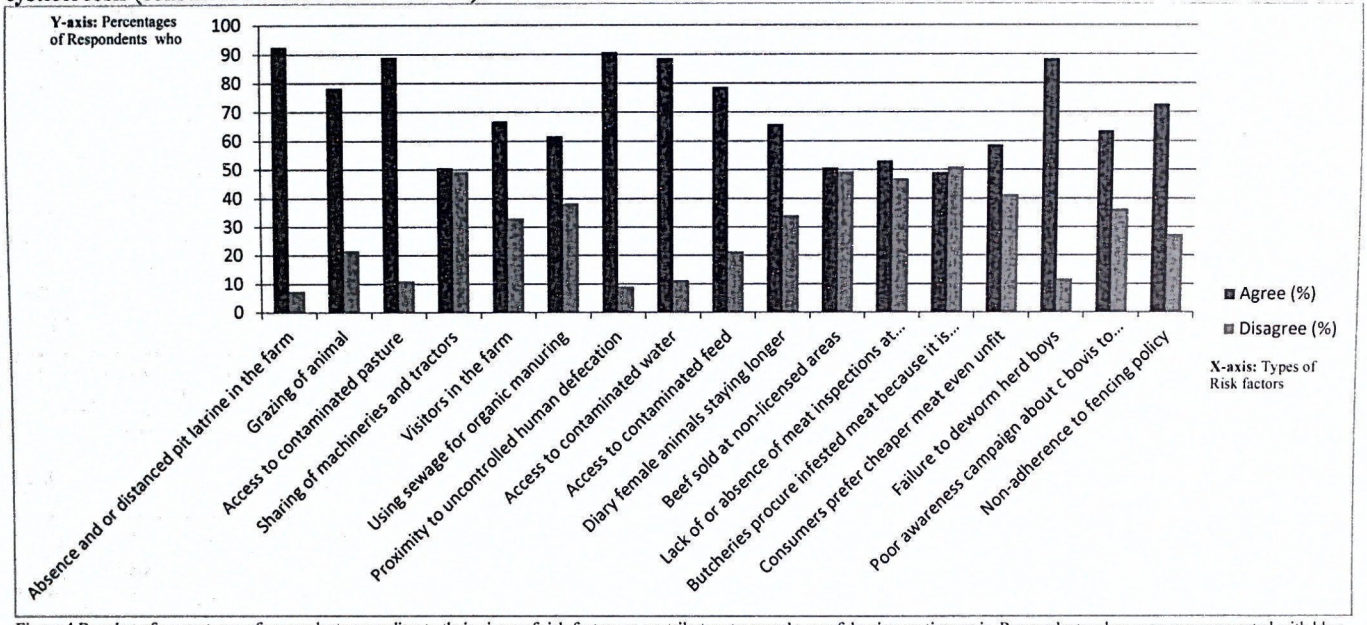

Figure 4 Bar chat of percentages of respondents according to their views of risk factors as contributors to prevalence of bovine cysticercosis. Respondents who agree are represented with blue bars while respondents who disagree are represented as orange bars

4.3.6 Determination of the quantitative relevance of each risk factor in the prevalence of cysticercosis

Table 24 Chi square determining quantitative relevance of each risk factor in the prevalence of cysticercosis

One-Sample Test	Test Value = 3							
	N	Mean	t	df	Sig. (2-tailed)	Mean Difference	95% Confidence Interval of the Difference	
							Lower	Upper
Preference of rare to done meat	144	3.67	6.218	143	0	0.674	0.46	0.89
Absence and or distanced pit latrine in the farm	146	4.29	18.379	145	0	1.288	1.15	1.43
Grazing of animal	143	3.83	9.435	142	0	0.832	0.66	1.01
Access to contaminated pasture	145	4.13	14.647	144	0	1.131	0.98	1.28
Sharing of machineries and tractors	142	3.16	1.608	141	0.11	0.162	-0.04	0.36
Visitors in the farm	139	3.5	5.338	138	0	0.504	0.32	0.69
Using sewage for organic manuring	141	3.35	3.342	140	0.001	0.355	0.14	0.56
Proximity to uncontrolled human defecation	141	4.18	15.371	140	0	1.184	1.03	1.34
Access to contaminated water	142	4.03	13.287	141	0	1.028	0.88	1.18
Access to contaminated feed	141	3.78	9.192	140	0	0.78	0.61	0.95
Diary female animals stay longer	138	2.68	-2.997	137	0.003	-0.319	-0.53	-0.11
Beef sold at non-licensed areas	142	3.11	0.97	141	0.334	0.106	-0.11	0.32
Lack of or absence of meat inspections at butcheries or homes	143	3.17	1.531	142	0.128	0.168	-0.05	0.38
Butcheries procure infested meat because it's cheaper	143	3.08	0.685	142	0.495	0.077	-0.15	0.3
Consumers prefer cheaper meat even unfit	145	3.23	2.125	144	0.035	0.228	0.02	0.44
Failure to deworm herd *boys*	145	4.1	14.192	144	0	1.097	0.94	1.25
Poor awareness campaign about c bovis to farmers and public	143	3.37	3.206	142	0.002	0.371	0.14	0.6
Non-adherence to fencing policy	143	3.66	7.179	142	0	0.664	0.48	0.85

Table 24 (page 90) shows chi square analysis of the data, which served to determine the association of the variables; as well as determine quantitative relevance of each risk factor in causing bovine cysticercosis. With test value at 3, risk factors of means above 3 were considered relevant factors of the prevalence. Risk factors with mean score of 4 and above considered relevant in maintaining prevalence of bovine cysticercosis were; 'absence and or distanced pit latrine in the farm'; 'access to contaminated pasture'; 'proximity to uncontrolled human defecation'; 'access to contaminated water' and risk factors of 'failure to deworm herd boys'. The rest of the thirteen (13) risk factors had mean ratings less than or equal to 3.

Contributions of the risk factors of, 'butcheries procure infested meat because it's cheaper'; 'lack of or absence of meat inspections at butcheries or homes'; 'beef sold at non-licensed areas', and 'sharing of machineries and tractors' to the prevalence of bovine cysticercosis are not statistically significant. But the rest of the 14 risk factor contributed significantly ($P<0.05$) to the prevalence of bovine cysticercosis (table 24). The **disagreement** among respondents that 'diary female animals stay longer' was a contributor to the prevalence of bovine cysticercosis was statistically significant ($P<0.05$) at 0.003; with negative *t*-value and mean score (table 24).

Table 25 showing likelihood ratio tests

Effect	Model Fitting Criteria	Likelihood Ratio Tests		
	2-Log Likelihood of Reduced Model	Chi-Square	df	Sig.
Intercept	127.517a	0	0	.
Non-adherence to fencing policy	128.156	0.639	1	0.424
Access to contaminated feed	129.049	1.532	1	0.216
Preference of rare to done meat	127.572	0.055	1	0.815
Absence and/or distanced pit Latrine in the farm	127.697	0.18	1	0.672
Grazing of animal	127.724	0.207	1	0.649
Access to contaminated pasture	127.992	0.474	1	0.491
Visitors in the farm	130.247	2.73	1	0.098
Proximity to uncontrolled human defecation	129.292	1.775	1	0.183
Sharing of machineries and tractors	129.098	1.581	1	0.209
Using sewage for organic manuring	127.518	0.001	1	0.973
Diary female animals stay longer	128.575	1.058	1	0.304
Beef sold at non-licensed areas	134.414	6.897	1	0.009
Lack of or absence of meat inspections at butcheries or homes	128.39	0.873	1	0.35
Butcheries procure cheaper infested meat	128.237	0.72	1	0.396
Consumers prefer cheaper meat even unfit	127.563	0.046	1	0.831
Poor awareness campaign about bovine cysticercosis to farmers and public	127.998	0.48	1	0.488

Table 25 tested how each of the variables predicts the prevalence. This shows that only factor of **'beef sold at non-licensed premises'** individually significantly predicted the prevalence of bovine cysticercosis

.3.7 **Table 26 logistic regression of risk factor in the prevalence of bovine cysticercosis**

Variables in the Equation

	B	S.E.	Wald	Df	Sig.	Exp.(B)
Preference of rare to done meat(1)	-0.284	0.415	0.47	1	0.493	0.752
Absence and or distanced pit latrine in the farm(1)	0.045	0.782	0.003	1	0.954	0.956
Proximity to uncontrolled human defecation(1)	-0.678	0.683	0.987	1	0.32	0.507
Grazing of animal(1)	0.139	0.461	0.09	1	0.764	1.149
Access to contaminated feed(1)	0.737	0.442	2.786	1	0.095	2.09
Non-adherence to fencing policy(1)	0.134	0.43	0.097	1	0.756	1.143
Constant	-0.38	0.267	2.022	1	0.155	0.684

iterature indicates **"Access to contaminated feed"** and **"Grazing of animals"** as major risk factors f bovine cysticercosis. These were used as controllers (adjusters) in the multivariant regression, while sting for five top significant risk factors. These five factors were "Preference of rare to done meat", absence and or distanced pit latrine in the farm", "proximity of uncontrolled human defecation", Non-adherence to fencing policy" (table 26). None of the risk factors is significant individually.

able 26 shows that those who answered yes to "access to contaminated feed" are 2.09 times more kely to conceive "access to contaminated feed" as risk factors contributing to prevalence of bovine ysticercosis. Those who answered yes to "Grazing of animals" and "absence and or distanced pit atrine in the farm" are 1.149 and 0.956 times respectively more likely to conceive them as contributing o the prevalence of bovine cysticercosis

lodel of log of odds of risk factors in favour of bovine cysticercosis prevalence = Ln (P/1-P) = -0.38 SE =0.267) + -0.284PRW (SE=0.415) + 0.045ADL (SE=0.782) + -0.678PUD (SE=0.683) + .139GRZ (SE=0.461) + 0.737ACF (SE=0.43) + -0.38NFP (SE =0.267) (Table 26)

Key: PRW = preference of rare to well done beef; ADL = Absence or distanced latrines; GRZ = Grazing farm animals; PUD = Proximity to uncontrolled defecation; NFP = Non-adherence to fencing Policy

4.3.8 DISCUSSIONS ON QUANTITATIVE DETERMINATION OF RISK FACTORS OF BOVINE CYSTICERCOSIS IN BOTSWANA

Fourteen (14) out of eighteen (18) tested risk factors contributed significantly ($P<0.05$) to bovine cysticercosis prevalence, with 'access to contaminated feed' being the single most important risk factor. Contributions of risk factors of, 'butcheries procure infested meat because it's cheaper', 'lack of or absence of meat inspections at butcheries or homes', 'beef sold at non-licensed areas', and 'sharing of machineries and tractors' to bovine cysticercosis prevalence were not statistically significant ($P<0.05$) (table 24). The **disagreement** among respondents that 'diary female animals stay longer' was a contributor to the prevalence of bovine cysticercosis was statistically significant ($P<0.05$) at 0.003; with negative *t*-value and mean score (table 24).

There is strong agreement (high consensus, with IQR of 0) among respondents that the risk factors of, 'failure to deworm herd boys'; 'access to contaminated feed'; 'access to contaminated water'; 'proximity to uncontrolled human defecation'; 'access to contaminated pasture'; 'grazing of animal'; 'absence and or distanced pit latrine in the farm' were major contributors to the prevalence of bovine cysticercosis (table 23). Respondents **disagree** that the risk factors of 'sharing of machineries and tractors'; 'beef sold at non-licensed areas'; 'butcheries procure infested meat because it is cheaper' contribute to bovine cysticercosis prevalence (table 23). There are rarely any non-licensed abattoirs in Botswana except may be in the remote villages where meat inspectors cannot reach due to lack of access roads. Generally, Botswana practices strict antemortem inspection especially in export abattoir (Livestock and Meat Industries act, 2007). Beef industry is closely monitored as it is third highest single foreign earner after diamonds and tourism (Engelen, *et. al.*, 2013; Burges, 2006; Tshiamo, 2015). Respondents unanimously "disagree" that 'locals procure infested and cheaper meat' instead of wholesome beef, because every family owns cattle and beef is readily available and affordable in Botswana (Thornton *et. al.*, 2003 in Patti *et. al.*, 2010).

Similarly, risk factor of 'Being female', was considered not common and not important by most respondents (table 23). This finding disagrees with Zdolec *et. al.*, (2012) who claims that female animals especially when used for breeding, stay longer than beef animals and so increase probability of spreading infestation. Although this finding disagrees with Zdolec *et. al.*, (2012), it is known that dam-to-nconate transmission of bovine cysticercosis occurs with female animals (Eichenberger, *et. al.*, 2011), but rarely with male animals.

Respondents who answered yes to "access to contaminated feed" are 2.09 times more likely to conceive "access to contaminated feed" as risk factors contributing to bovine cysticercosis prevalence. Those who answered yes to "Grazing of animals" and "absence and or distanced pit latrine in the farm" are

1.149 and 0.956 times respectively more likely to conceive them as contributing to the bovine cysticercosis prevalence (table 26).

Multinomial logistic regression analysis, which determined both individual and joint effects of risk factors on bovine cysticercosis prevalence shows that all variables (risk factors) jointly significantly ($p<0.05$) predicts the prevalence of cysticercosis (table 26). However, test on how each (individual) variables predicts bovine cysticercosis prevalence shows that only factor of '**beef sold at non-licensed premises**' significantly predicted the prevalence of cysticercosis (table 25). **It is therefore recommended that there should be a strong policy to minimize butcheries buying and slaughtering animals without proper ante-mortem and post-mortem examination.**

The paradox of non-significance of most variables individually yet jointly significant is that there could be a case of multi-collinearity. This means that some or most of the variables in the model are correlated with others; because they are basically measuring the same thing. For example, '**absence or/and distanced pit latrines in the farms**' will cause '**herd boys who have not been dewormed**' '**to defecate indiscriminately**' and encourage '**proximity of cattle to uncontrolled defecation**', which will cause '**unfenced**', '**grazing animals**' to '**consume contaminated pastures**' and '**drink contaminated water**'.

Respondents showed greater consensus in 'disagreeing' that restraining/keeping cattle perpetually within fenced farms can be practiced in Botswana. It appears that hoofing of animals has political, cultural and social driving forces, which must be preserved (table 23). In a related study the author found that EU, the main buyer of Botswana's beef insist on grass-fed beef. For Botswana to maintain this market, the cattle are allowed to roam (Uchendu, 2020)

Literature is replete with a repertoire of risk factors of bovine cysticercosis. Some of which, either do not apply to Botswana or their contribution to bovine cysticercosis prevalence is negligible. Chi square test was used to determine the mean (average) answers of respondents, which was interpreted as the quantitative relevance of each risk factor in contributing to prevalence of bovine cysticercosis. With test-value at three (3), risk factors with means equal to or above 3 (moving towards agree) were considered important whereas risk factors with mean scores less than or equal to 3 (moving towards disagree) were considered unimportant. The important risk factors were 'access to contaminated pasture', 'absence and or distanced pit latrine in the farm', 'proximity to uncontrolled human defecation', 'access to contaminated water' and 'failure to deworm herd boys'. Other important risk factors were, access to contaminated feed, grazing of animals and preference of rare to cooked meat.

Risk factors which were categorized as unimportant were, 'sharing machineries', 'visitors to the farm', 'using sewage for organic manuring', 'beef sold in non-licensed places' and 'being female'. Other

unimportant risk factors were: 'absence of meat inspectors', 'procuring infected meat', 'consumer prefer cheap meat', 'poor awareness campaign' and 'non-adherence to fencing policy'. For emphasis, the term **unimportant risk factor** does not mean that risk factors do not contribute to the spread of bovine cysticercosis, rather it designates risk factors whose means scores are less than or equal to the test-value of 3.

Chi square (table 24), determined fourteen (14) important risk factors whose contribution to the prevalence of bovine cysticercosis were statistically significant. Of these risk factors, the top 20% contributors are 'absence and or distanced pit latrine in the farm' (mean = 4.29), 'proximity to uncontrolled human defecation' (mean = 4.18), 'access to contaminated pasture' (mean = 4.13); and 'failure to deworm herd boys' (mean = 4.10). Pareto principle, was adopted to develop a control model, which posits that eliminating these top 20% important risk factors will cause 80% drop in bovine cysticercosis prevalence. Therefore, it is recommended that an efficient control and prevention strategy should focus on these top 20% important risk factors rather than a repertoire of literature-documented risk factors.

Seasonal index using secondary data and observation (section 4.3.9) showed close association between increased bovine cysticercosis incidences and 'outset of rainfall in December'; 'harvesting of Mopane worms' in April and 'cutting of grass for roof thatching' in October.

Generally, respondents showed **greater consensus** in 'agreeing' that risk factors that had **high probability** to cause bovine cysticercosis contributed more to high prevalence of bovine cysticercosis. This consensus homogenizes farmers and provides a platform from which to drive an attitudinal change; unlike when farmers were in disagreement (table 23). This can mean that identifying areas of common agreement (consensus) among farmers will provide good entry points in advancing control and prevention strategies.

Respondent's knowledge of literature-documented risk factors of bovine cysticercosis was high (table 23). This confirms claims that the widespread government awareness campaign programs was effective (Aganga, 2009, Mochankana & Robertson, 2018). However, this widespread knowledge has not translated to altitudinal and behavioural changes requisite for control/prevention of bovine cysticercosis that should in turn cause decline in prevalence of bovine cysticercosis (Dick Raf, personal communication, 2017). This realization informed search for possible novel risk factors and to corelate the risk factor with the lifestyle of Batswana.

4.3.9 IDENTIFICATION OF NOVEL RISK FACTOR OF BOVINE CYSTICERCOSIS USING INTERVIEW AND PHYSICAL OBSERVATION METHODS

4.3.9.1 Background of the study

The government of Botswana has practiced mass literacy on proper disposal of human feces; presumably considered the most important risk factors; adequate and efficient meat inspection among several other prevention, control and intervention measures. With the increasing prevalence of bovine cysticercosis, it is apparent that none of these strategies has sufficed in reducing incidences of bovine cysticercosis. It is probable that the main underlying risk factors of bovine cysticercosis in Botswana have been misdiagnosed or that the adopted control and prevention methods have not been effective in reducing the prevalence. Although these risk factors are behavioural, systemic and progressive, there is no known work that associates these risk factors with the lifestyle of residents of Botswana and climatic changes as a means of uncovering novel risk factors of bovine cysticercosis in Botswana. The objective of this study was to identify novel and undocumented risk factors of bovine cysticercosis in Botswana using interview system and observation visual methods

4.3.9.2 Materials and Methods for identification of novel risk factors and hotspots of cysticercosis in Botswana using non-participatory interview and visual observation

The risk factors listed in the structured questionnaire were derived from available literatures as described in section 3.8.1

4.3.9.3 Data sources and data collection

In addition to risk factor listed in the questionnaire, primary data for identification of novel risk factors was sourced through direct personal observation and face to face interview of respondents. Details of data source and collection are shown in section 3.8.2

4.3.9.4 Sample size

Whereas one hundred and forty nine (149) respondents filled out the questionnaire for quantitative analysis of risk factor of bovine cysticercosis only 50 of them engaged in oral interview used to identify novel risk factors table 4.

4.3.9.5 Interview Questions:

List of questions filled by 50 respondents are showed in section 3.8.3

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4.3.9.5 Interview Questions:

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4.3.10 RESULTS FROM INTERVIEW ON RISK FACTORS ACCORDING TO DISTRICTS

GENERAL NOTES ON RESULTS OF THE INTERVIEW

Risk factors identified from each districts were discussed specifically for that district. Alternatively, the discussion could have centered on risk factors with affected districts been mentioned.

Either approaches presents both merits and demerits. Whereas the latter method would be brief and direct, the first approach allows for thorough explanation of identified risk factors within each districts. As such each risk factor is discussed in their merits within the districts. Furthermore, discussing risk factors according to districts makes it easy to establish association between the risk factors and lifestyle of the locals in each district. And this is actually the basis for this study. Therefore in this study the results of the interview and observation of risk factors are discussed districts after districts. This means that one risk factors may be discussed severally as long as they exist in the districts.

4.3.10.1 RESULTS FROM GHANZI DISTRICTS

Table 27 extension areas in Ghanzi district with cattle population in 2016

Extension Area	Population of Cattle	Prevalence %
Kole	6564	15
Makunda	4329	43
Charles Hill	6882	26
Karakubis	8571	18
Kalkpontein	9885	19
Chobokwane	16,959	22
Total cattle	53,190	

Reference: Animal Stock Census, 2016. Unpublished Official Document of Department of Extension Services Ghanzi

Some undocumented risk factors of cysticercosis were identified in the Ghanzi District and these factors have shown to be more serious than the literature documented risk factors. These are:

4.3.10.1.1 Beyond Grazing: Infestation through feeding cattle with harvested contaminated grass and introducing bovine cysticercosis positive animals into the crawls

Ordinarily, cattle raised in ranches should not be infested with bovine cysticercosis since these animals do not graze freely from where they can consume pasture contaminated with human feces. However, there are sporadic incidences of bovine cysticercosis in cattle raised in crawls and ranches. The reason is that ranch operators buy already infested animals from rural farmers in the community and introduce into their ranch. These animals account for positive cases at slaughter.

Secondly, some ranches harvest grasses from open grass lands & field where the rural people defecate indiscriminately. Cattle raised on such grasses also become infested with bovine cysticercosis.

4.3.10.1.2 The Makunda experience

Makunda has the highest prevalence of bovine cysticercosis of 34% in Ghanzi district (bovine cysticercosis prevalence: Abattoir Records, 2016; unpublished official document of Department of Extension Services Ghanzi). **Makunda although has the least population of cattle in Ghanzi district is a major bovine cysticercosis hotspots (table 27) with prevalence of 43%.** Some of the identified risk factors in Makunda include:

4.3.10.1.2.1 High population density of cattle:

In Makunda cattle population concentrate in few areas. This leads to high population density of cattle. The high concentration of cattle arises from the following factors; viz:

i. **Fewer bore holes than needed:** there are few bore hole points in Makunda, this leads to high cattle and man population, sharing the water source

ii. **Communal farming leading to close proximity of man and animal**

Although Ranch farming is typical in Ghanzi district, in the Makunda Extension Area cattle farming is communal. Farmers keep their cattle within the homestead instead of cattle posts. This close proximity favours high man-animal interaction, as both man and animal share common boreholes and animal freely roam across play grounds and fields

4.3.10.1.3 Preference for bush defecation to use of toilets:

Culturally, the original Massires and Basarwa tribes that live in the Ghanzi districts prefer open air defecation to using built toilets. Efforts made to introduce this tribe to civilization has yielded minimal results. Cattle get infested with bovine cysticercosis by consuming contaminated pasture.

4.3.10.1.4 Improper and insufficient meat inspection:

Ghanzi district, with the highest cattle population has inadequate number of meat inspectors to carry out effective meat inspection. There are only three (3) meat inspectors, four (4) slaughter houses, about twelve (12) butcheries and no abattoir located in Ghanzi district. By design, majority of the cattle produced from Ghanzi, western region are sold to Botswana Meat Commission (BMC) located in Lobatse in the southern region. Refusal to license an abattoir in Ghanzi was to ensure that BMC, Lobatse being an export abattoir, receives enough cattle to allow it operate at maximum capacity. This practice has hindered Ghanzi from building its own local abattoir. The short falls of meat inspectors and absence of abattoir has led to animals being slaughtered without proper inspection. This way, cases are undetected; and without proper diagnosis and mitigation, the infestation is continuously recycled and multiplied.

4.3.10.2 RESULTS FROM KALAGADI DISTRICTS

4.3.10.2.1 Unfenced sewage and drainage systems

Sewage and drainage systems in Lehututu extension area of Hukuntsi are not cordoned. Cattle have unhindered access to sewage waste and effluents. The researcher observed that during the dry spells, herd boys purposely herd cattle to sewage dams to water their flock. Consequently, cattle is exposed to contamination and this forms a major risk factor for spread of bovine cysticercosis .

4.3.10.2.2 Lack of basic amenities-Scarcity and Inadequacy of Water

The Kalagadi district is predominantly a desert terrain. The name Kalagadi means thirst. It is known to possess deep water bed;-reaching depths of about 400-500 meters into the ground (Dick Raff, 2017 personal communication; Integrated Marketing n.d.). Several bore holes dug in the Kalagadi districts do not reach this depth and so cannot supply appreciable quantity of water. Furthermore, the underground water available in Kalagadi region is known to be extremely salty and cannot be fed to man and cattle. Salt Pans are common findings around the Kalagadi district

Due to water scarcity, farmers fetch water in jerry cans from rain-fed parchment to water animals. These rain-fed parchments, usually located in valleys, collect early rains that wash down contaminants from the uplands; contaminants like human feces and sewage effluents. These parchments are major foci for transmission of the taenia eggs to cattle that drink from them.

4.3.10.2.3 High population density due to designation of arable grazing lands for wild life

In the early 2000s large portions of grazing land were taken over by the government of Botswana and designated as Wild Life reserve Areas. These designated areas, which have been condoned off and marked 'No Grazing Area' divides through the breadth of Kalagadi district. The two divides formed by this wild life Reserves are pasture land close to human settlement, which is small and cannot sustain existing cattle population and the pasture lands beyond the Wild life Reserve Areas, which is far removed from the human settlement and cattle cannot trek across the reserve to reach it. Although the farther pasture lands contain much grass for cattle, its use is fraught with many challenges. The most obvious challenge is that cattle would have to walk long distances of about forty kilometers (40 KM) before reaching the grazing lands. Another challenge is that these grazing lands lack bore holes or any other sources of water supply. Thus, cattle trek so far to find pasture, and trek back to drink water at the boreholes within the home stead. As animals trek through this long stretch of Trans-Kalahari Express way they are exposed to pasture contaminated with human feces. Cattle also die of thirst due to long dry trek, in addition to some being killed by vehicles or wild animals

4.3.10.2.4 Lack of toilet facilities in farms and construction sites

It is recommended that pit latrines should not be dug at a distance less than 100 meters away bore holes (Water Aid, 2011 in Graham & Polizzotto, 2013). Feces sip into ground water, and into the bore hole water. In compliance with this distancing policy, toilets are built about eight hundred (800) meters to one (1) kilometer away from bore holes. Consequently the absence or distancing of toilet facilities from bore hole points' leaves the farmers and herd boys no option but to defaecate indiscriminately around the watering points. These feces are eventually washed down into the watering point and consumed by cattle.

Similarly, majority of the construction sites at Lehututu extension area lack toilet facilities (mobile and built toilets). Engineers and work men resort to indiscriminate defecation around the nearby bushes. This was identified as major risk factor for the spread of Cysticercosis in Lehututu.

4.3.10.2.5 Inadequate meat inspection due to lack of access roads and slaughter houses

Hukuntsi lacks access (good) roads linking villages with farm and cattle posts. Secondly, there is insufficient number of slaughter slabs and meat inspectors. For example, there is only one government owned slaughter slab and one meat inspector in their entire Hukuntsi, except for a privately owned butchery licensed as Kang Slaughter slab or Kang Meat Market. These factors leave farmers with no option than to slaughter their cattle at home and in farm settlements. This situation constitutes a major risk factor for the spread of cysticercosis.

4.3.10.2.6 High illiteracy and poor funding

Rural farmers do not understand the concept of incidence, prevalence and risk factors of cysticercosis. Some respondents attributed cysticercosis to witchcraft attack. Most of the rural farmers in Hukuntsi are poor and operate at subsistence level. These poor farmers cannot afford bore holes, farm machineries and deworming of herd boys. Access to credits is extremely difficult if not impossible because farmers lack collateral and other conditions to qualify for credits.

Sharing of farm implements is common practice and this was identified as a major risk factor of cysticercosis. These factors have contributed to the poor management and control of cysticercosis.

4.3.10.2.7 High population density due to limited water sources in Marang

The highest prevalence of bovine cysticercosis in Hukuntsi was recorded in Marang. Most boreholes in Hukuntsi are located in Marang because Marang has abundant underground water. This is the attraction point for both humans and animals leading to high human-cattle interaction and consequently high incidence of bovine cysticercosis.

There are few available boreholes that serve a large population of farmers and their animals. Contaminants from pit latrines can seep into underground water like borehole.

Currently, butchers and slaughter house owners avoid purchasing cattle from Marang in Hukuntsi because they are almost sure that cattle from Marang would be infested with bovine cysticercosis.

4.3.10.2.8 High population density due to communal settlement pattern

Another factor that favour high human-cattle interaction in other parts of Hukuntsi is that, the indigenes insist on living in communal settlement; that is, people live close to each other and with their animals. Living together helps locals to interact closely, as much as the high population serves as ready army to fight off predator animals. Therefore, population density is high within human occupied lands

4.3.10.3 RESULTS FROM CENTRAL AND KWENENG DISTRICTS

4.3.10.3.1 High population density due to communal settlement pattern

Although there is variegated civilization in central district, communal living persists. This leads to high population density and close proximity of man-animal interaction. In the central district, like in most other districts, there seem to be a correlation between high human population and incidence/prevalence of cysticercosis.

4.3.10.3.2 Lack of toilet facilities in farms and around boreholes

Few available boreholes serve a large population of cattle, herd boys and farmers. As these populations congregate, there are no toilet facilities around borehole points. The obvious reason being that digging pit latrines in a distance less than 800 meters from borehole will cause the feces to sip into ground water and back to the bore hole. On the contrary absence of toilet facilities around watering points leaves the farmers and herd boys no option but to defaecate indiscriminately around the watering points. These feces are eventually washed down into the watering point and consumed by cattle.

4.3.10.3.3 Unfenced sewage and drainage systems

Sewage and drainage systems are not fenced which allow cattle to have unhindered access to sewage waste and effluents. During the dry spells, herd boys take cattle to sewage dams to water their flock. Consequently, cattle are exposed to contamination and this forms a major risk factor for spread of cysticercosis. A typical example is the Glen Valley Sewage system. This is the country's largest and most populous sewage system. It is unfenced and during the dry seasons cattle has unhindered access to the sewage dump and water that drain from it. Cattle also drown in course of drinking from the dam.

4.3.10.3.4 Lack of basic amenities-scarcity and inadequacy of water:

Rural communities within the Central districts lack sufficient water supply. Farmers and cattle share water from bore holes. Due to this water scarcity, farmers are forced to water cattle from water parchments. Meanwhile, these rain-fed parchments, usually located in valleys, have been described as major focus for transmission of the taenia eggs.

4.3.10.3.5 Illiteracy and poor funding

As in most other districts of Botswana, cattle farming is designated for un-educated rural dwellers whereas the educated youth seek white collar jobs in the city. Most cattle farmers in the central districts are poor and subsistence. These poor farmers cannot afford the cost of constructing bore holes, farm machineries and deworming of herd boys. Access to credits is extremely difficult if not impossible because farmers lack collateral and other conditions to qualify for credits. Thus illiteracy and poverty seem to have close link with prevalence of cysticercosis.

4.3.10.4 RESULTS FROM NORTH EAST DISTRICT

4.3.10.4.1 The existence of enclosed water bodies and dams

Across other zones of Botswana cattle source water from boreholes and wells. At these boreholes the farmers draw water and serve the animals in containers, which are usually cleaned out before and after use; neither human beings nor animals faeces into the containers, so providing little or no focus for spread of bovine cysticercosis. However, in areas of zone 7, there exist water bodies, which simulate a full-fledged river or dam during the raining seasons and may reduce during the non-rainy seasons. At any of these seasons, these water bodies are rallying points for the nomadic herd boys and their cattle. It is common place for the herd boys and the general rural public to faeces in and around the water bodies. Cattle consequently pick up the worms passed out in the faeces and this methods perpetuates the cysticercosis cycle. This explains the relatively higher prevalence of cysticercosis in the zone 7 areas than in adjoining regions of Botswana.

4.3.10.4.2 The Mopane Worm Haunt

Mopane worm is arthropod insect larva. It is in the Kingdom of *Animalia*; Family of *Saturnitidae* and Species of *Gonimbrasia belina* (Siulapwal, et. al., 2014). Mopane worms are rich in protein and crude fats. It is said that dried mopane worms can provide up to 65% of a human's daily protein needs and many of the required vitamins and minerals (FAO, 2014). They contain significant amounts of phosphorus, iron and calcium. Upon hatching, it is green and gradually metamorphosis into a mature brown-colored worm. The adult fly lays its eggs within the Mopane trees where the eggs reside until maturing before hatching out as larva. This is how the worm acquired the name Mopane worms.

Mopane trees grow in the areas designated as disease control zone seven (7) areas and adjoining villages. Zone 7 areas refers to villages that are declared free from Foot and Mouth Disease. These villages are; Mmadinare and Bobirwa, Mopane trees do not grow in the other regions of Botswana. The worm is harvested and dried into a dark brown colored worm cast. Mopane worm has evolved into one of the most sought after delicacy; even much more preferred to the beef. It gained popularity for its high protein content and other positive religious and cultural myths and sentiments surrounding its consumption in Botswana.

Mopane worms are harvested during the months of December and April and these coincides with the rainy and flowering seasons of the years, which is also the lush periods for pasture. Mopane worms harvesters traverse the length and breadth of the zone 7 grasslands and farms in search of the worms. In course of which they defecate indiscriminately. During this same period of the year, Cattle herders allow their animals to graze freely across open fields and grass lands. Grazing cattle is exposed to these defecates, and this increases their probability of being infested with *Taenia saginata* cysticercosis. Records of seasonal index of bovine cysticercosis show high incidence during the months of June and February. You may recall that it takes 8-12 weeks for ingested *Taenia saginata* cysticercosis eggs to migrate from the GIT to the striated muscles, which is their predilection sites. In tracing back, it is most probable that the worms ingested in April and December manifested in the muscles in June and February respectively.

4.3.11 The seasonal collection of grass for thatch roofing

The Botswana traditional thatched roof house is made of grass. This grass is harvested around end of the year (October to December); just before the new rains. The process of gathering this grass causes widespread movement of humans across all regions of Botswana. In course of grass collection, humans defecate indiscriminately. Cattle graze across contaminated pasture fields and pick up Taenia eggs.

4.3.12 Politics of the EU beef market insisting on grass-fed cattle

The life cycle of *Taenia saginata* cysticercosis or *Taenia saginata* is primordial; it needs an obligate human host and cattle as intermediate host. Ordinarily, prevention and control should be easily achievable by breaking the chain at the human end by stopping indiscriminate defecation and/or at the cattle end by stopping the roaming of cattle; forcing all cattle permanently in ranches. However, stoppage of animal migration is not an option. Reason being that the European Union which is the major market for Botswana beef insist on grass fed cattle. It is known that grass fed cattle yield tender beef in addition to been healthier than cattle raised with genetically modified feed and feed additives. Botswana has to choose either to roam cattle in order to achieve tender beef and meet EU market or to

house their cattle and prevent cattle from eating grass contaminated human feces infested with *Taenia saginata* cysticercosis eggs. It does not appear that the latter option is foreseeable in the near future.

4.3.13 General Information

There are about fifty (50) Butcheries, three (3) low through-put abattoirs and one (1) district abattoir in North East District. Cattle population in Selibe-Phikwe, a major town in the North East District averages 85,000 cattle. The largest farms record about four hundred (400) cattle. Large herd owners are located in the communal areas, devoid of fenced farms thus animals graze freely. Records of prevalence derived solely from BMC located in only in Lobatse, Maun and Francistown, are not holistic and representative of the entire nation. Oral interview with farmers revealed that rural farmers know that the BMC meat inspectors are more strict and thorough and can detect more cysts from their carcasses; as such there's greater tendency for their animals either to be retained or condemned. Consequently, these farmers in trying to evade condemnation of their animal prefer to sell their animals to low through-put abattoirs, butcheries and slaughter houses where they believe meat inspection is less thorough.

Another reason for preference of lower low through-put abattoirs to BMC is that with the former, the farmers insist on collecting their money upfront, unlike in BMC where they get paid after slaughter and post-mortem inspection of animals. This trend therefore queries the representativeness of the prevalence from BMC as the country-wide prevalence.

4.3.14 DISTRICTS RECOMMENDATIONS BASED ON INTERVIEW AND OBSERVATION RESULTS

4.3.14.1 Recommendations for Ghanzi districts

1. **Mass Treatment:** Going by the high prevalence of cysticercosis in Ghanzi, particularly at Makunda, mass treatment for Taeniasis is recommended for farmers, herd boys and general public. This would reduce the worm burden circulating in the district.
2. **Mobile Toilets in Farms and construction sites :** It is feared that using pit latrines in farms and around bore holes can lead to contamination of underground water. Mobile toilets when installed in farms and construction sites will take care of this perceived pitfall of pit latrines. Installation of mobile toilets in farms, cattle post and construction sites should be legislated as compulsory prerequisite for operations.
3. **Mass Education:** Findings from this research should be disseminated to the public. Particular attention should be paid to the most important and peculiar risk factor for a particular extension area, as some risk factors do not apply to certain extension area.

4. **Legislation to Ban Defecation along Trans-Kalagadi Express Way:** The long stretch of Kalagadi Express way is equipped with resting points at intervals. These resting points are devoid of toilets. So, travelers who use the resting places resort to defecating in nearby bushes thus infected humans, pollute the environment. A legislation banning indiscriminate defecation along the trans-Kalagadi express way is highly recommended.
5. **Building of Mobile Toilets along Trans-Kalagadi Way:** Attendant to banning indiscriminate defecation along trans-Kalagadi express way, this legislature should be backed up by annexing mobile toilets to the resting places.
6. **Value Re-orientation for the Massires and Basarwa Tribes:** These tribes need to be educated on the use of toilets; its benefits for them, their animals and the entire Botswana.
7. **Obligate Rearing of Cattle in Farms:** Although this may be difficult, but the merits in the long run out weighs its demerits. Legislature must be put in place to

a. ban free roaming of cattle
b. ensure that all cattle post are fenced

8. **Provision of More Bore Holes:** Drilling of more bore holes is strongly recommended for extension areas reported to possess fewer than necessary bore holes. This will reduce population density around existing bore holes
9. **Treatment of enclosed water bodies:** immediately the rains begin, the feces in the uplands are washed into the enclosed water bodies in the low lands. Cattle in turn drink this water and pick up the infection. It is recommended that these water bodies must be treated to eliminate or reduce worm bodies before allow cattle to drink from them.
10. **Treatment of Grasses:** All grasses harvested for feeding of farm animals should be treated against Cysticercosis before use for feeding farm animals. This is another point at which the change can be broken. However, since most rural farmers allow their cattle to roam, this strategy may be more effective among fenced farms

4.3.14.2 RECOMMENDATIONS FOR KALAGADI DISTRICTS

1. **Construction of Mobile Toilets around Bore holes:** Mobile and or water system toilets should be constructed around borehole points as this will age indiscriminate defecation by herd boys and farmers around watering points
2. **Construction of More Slaughter facilities:** Kalagadi Districts needs more slaughter slabs and meat inspectors

3. **Formation of Farmers Cooperatives:** Since individual farmers do not meet requirements for credits, farmers should be encouraged to form co-operatives and these co-operative can approach banks for credits. The researcher advanced this idea to the farmers' Association in Hukuntsi
4. **Treatment of Water Bodies:** Following the early rains, water bodies should be treated with de-wormers and if possible first accumulated water should not be utilised to feed cattle since they have shown to be highly contaminated
5. **Construction of Toilets along Trans-Kalagadi Express way:** This would reduce indiscriminate defecation by travellers.
6. **Construction of Mobile Toilets at Construction Sites:** Construction sites must as a matter of pre-requisite for issuance of clearance construct toilet facilities within their construction sites.

4.3.14.3 GENERAL RECOMMENDATIONS FOR CENTRAL AND KWENENG DISTRICTS

1. Mobile and or water system toilets should be constructed around borehole points as this will discourage indiscriminate defecation by herd boys and farmers around watering points
2. Since individual farmers do not meet requirements for credits, farmers should be encouraged to form co-operatives and these co-operative can approach banks for credits.
3. Following the early rains, water bodies should be treated and if possible first accumulated water should not be utilised to feed cattle since they have shown to be highly contaminated with contaminates that accumulated over the previous dry seasons.

4.3.14.4 GENERAL RECOMMENDATIONS FOR NORTH EAST DISTRICT

1. **Licensing and Deworming of Mopane Worm Harvesters:** Mopane worm harvesters should be treated weeks prior to the emergence of the worms. This would ensure less environmental contamination with the infested human faeces
2. **Installation of Mobile toilets:** It is important to install mobile toilets around the bush where these worms are collected. This will reduce indiscriminate defecation in the bush.
3. **Barricade of Water Bodies and Control of Access:** Water bodies located in zone 7 open parchments are devoid of barricade thus access is unchecked. This leads to pollution by both humans and animals. Consequently, barricading these water bodies and controlling access will minimise pollution.
4. **Treatment of water bodies:** Following the early rains, water bodies should be treated with dewormers; and if possible first accumulated water should not be utilised to feed cattle since they have shown to be highly contaminated

5. **Treatment of Grasses:** All grasses harvested for feeding of farm animals should be treated against Cysticercosis before use for feeding farm animals. This is another point at which the change can be broken. However, since most rural farmers allow their cattle to roam, this strategy may be more effective among fenced farms

4.3.15 GENERAL RECOMMENDATION ON RISK FACTOR STUDIES

In addition to all the specific and peculiar recommendations made for the different districts, the following general recommendations are made;

1. All types of prevention and control strategies and their implementation should be specific for each district, which would be based on identified risk factor. Adopting 'one blanket' strategy has shown to be inefficient
2. Prevention and control Strategies should target to cause a paradigm shift and change in lifestyle of the locals in addition to addressing physical challenges
3. It is strongly recommended that the seasonal index of bovine cysticercosis should be studied as this can provide relevant information in time-targeted prevention and control of the infestation

4.3.16 OUTCOMES AND DELIVERABLES OF RISK FACTOR STUDY

1. **Uncovered and documented novel risk factors not yet available in literature:** In addition to studying documented risk factors of bovine cysticercosis and how they play out in Botswana, this work uncovered and documented about 13 novel risk factors associated with cysticercosis. These novel risk factors are: **i.** The Mopane worm haunt; **ii.** Seasonal Collection of grass for thatch roofing; **iii.** the politics of EU beef market insisting on grass-fed cattle; **iv.** cattle has unhindered access to unfenced enclosed water bodies and dams in the zone 7 & surrounding communities; **v.** cattle has access to unfenced sewage and drainage systems; **vi.** lack of toilet (mobile) facilities in farms, construction sites, community bore holes and along the Kalagadi Express way; **vii.** the Basarwa and Massires traditional preference and insistence on bush defecation instead of using toilets; **viii.** Improper and insufficient meat inspection personnel and facilities in rural areas and remote villages; **ix.** Farmers insistence on communal living (for security reasons) leading to high population density and close human-cattle interactions; **x.** feeding cattle in ranch and paddocks using harvested and untreated infested grass; **xi.** Conversion of former arable pasturelands to wildlife conservation leading to scarcity of grass for cattle and high population density; **xii.** Lack of basic social amenities in rural areas, e.g. lack of pipe borne water causes farmers to water cattle in contaminated water bodies and puddle; **xiii.** Illiteracy and poor funding

2. **Systematic (geographic) study of risk factor of bovine cysticercosis:** In addition to identifying and studying how literature documented risk factors of cysticercosis manifest nationally, this work studied the risk factors at districts level. Thus uncovering geographical peculiarities and lifestyle components of the risk factors. The benefit of this is that associating lifestyles and geography with risk factors saves time, energy and resources in planning and executing prevention and control strategies.

3. **Quantitative ranking of risk factors:** Using multinomial regression, the risk factors were ranked according to their individual contribution to the incidence and prevalence of bovine cysticercosis. It therefore becomes easy to target prevention/control efforts effectively.

4. **Adoption of Pareto Principle:** By adopting the Pareto Principle, which states that solving 20% of the most important causes of the problem will result in 80% of desired outcome, 20% most relevant risk factors were delimitated and it is hoped that by eliminating these top 20% important risk factors, the prevalence rate would drop by 80%.

4.3.17 CONNECTING RISK FACTOR STUDY TO SOCIO-ECONOMIC STUDY

Generally the type and magnitude of identified risk factors suggest type and magnitude of effects that bovine cysticercosis could have on the socioeconomy of the farmers. Animals grazing freely into open sewage treatment facility would pose a more risk with higher odds of bovine cysticercosis infestation than ranched animals that eat harvested grass once in a while. Consequently, the socioeconomic effect of bovine cysticercosis would be experienced more by farmers whose animals roam and graze freely on open sewage treatment facility than farmers whose animals are enclosed in ranches.

Majority of the farmers in the Kalagadi Districts (Hukuntsi) are subsistent farmers; they slaughter their animals so they bear the financial and socio-economic effects of beef measles. Conversely in Ghanzi, most farmers are large sale, they supply their animal to Botswana Meat Commission, Seen Foods, Low through-put abattoirs and emerging slaughter houses. Upon sales of animals, they collect upfront payments. Should the animal contains cyst and become detained or condemned, the farmer has no concerns. Thus investigation of socio-economic effects of cysticercosis focused more on farmers in the Kalagadi district but at the Ghanzi District the study focused more on abattoir owners and Meat market operators.

4.4 DETERMINATION OF THE EFFECTS OF BOVINE CYSTICERCOSIS ON THE SOCIO-ECONOMY OF CATTLE FARMERS IN BOTSWANA

4.4.1 Background of the Study

Beef from carcasses identified during meat inspection procedures as having beef measles cannot be exported to the European Union (Livestock and Meat Industries Act 2007). This resulted in annual loss of export earnings of about one million (P1M) (P1.00 = 0.5 USD) in 1978 (Grindle, 1978); five million pula (P5M), (P1.00 = 0.68 USD) in detained and/or condemned carcasses per year as at 1985 (Mosienyane, 1986) and about P35 million in 2008 (Aganga, 2009). Furthermore, beef worth about P100 million (P10 = 1USD) was lost in 2016. Statistics show that in 2009, 2010, and 2012, beef exports worth P99,645,780 (P10.56 = 1USD) (International Monetary Fund [IMF], n.d.), P100, 477, 260 (P6.33 = 1USD), and P83, 289, 960 (P7.78 = 1USD) respectively, could not be sold to the EU markets (Tshiamo, 2015).

In addition to the aforementioned macroeconomic costs, bovine cysticercosis bear financial and socioeconomic effect of the local farmer. Apart from a few qualitative and subjective assertions regarding the effectiveness of these intervention measures, there is no known work that has adopted objectively verifiable indicators (OVI) to assess the socioeconomic effects of the bovine cysticercosis. Such work would ascribe quantitative worth to the socio-economic and financial effects of the bovine cysticercosis and by extension query the validity of the current government intervention measures. In this study, the socio-economic effects of bovine cysticercosis were determined using 14 objectively verifiable indicators, in addition to attempting to estimate possible financial losses due to bovine cysticercosis. The results show that the highest scoring indicators, which showed significant effects ($p<0.05$) were "farmers' ability to save money" and "farmers' experience of emotional disturbance" Also affected were farmers ability to provide food, healthcare, education, rental for family. Equally, the advent of bovine cysticercosis caused farmers not to employ new workers; to diversify or abandon farming business. With reduced income came "farmers' inability to meet social, religious and family obligations", causing some to borrow money for upkeep of family and/or farming business. Severity of effects on farmers were dependent on the magnitude of the infestation, the scale of production of farmers and presence of absence of some survival strategies. Farmers' response to effects ranged from outright closure of farms, to reduction in farm capacity, to diversification of businesses. Some farmers experienced some psycho-social effects. Most effective government interventions were provision of re-stocking seed calves; payment for cold treatment of infested carcass and installation of socio amenities. All these played vital roles in cushioning the adverse effects of bovine cysticercosis on the livelihood pattern of the farmers.

4.4.2 MATERIALS AND METHODS

4.4.2.1 Data sources and data collection

The primary data was sourced through direct observation and face-to-face interview using questionnaires. Details of data source and data collection is available in section 3.9.1

4.4.2.2 Administration of questionnaire

The multistage sampling technique was used to enumerate the population because the population is stratified into region; districts and animal holdings (Statistics Botswana, 2015). Details of questionnaire administration as contained in 3.9.2

4.4.2.3 Sample Size

One hundred and forty nine (149) respondents were sampled from the entire population. Details of sample size determination is contained in section 3.4.2. Details of sample size according to districts are shown in table 2.

4.4.2.4 Data analysis

Descriptive Statistics was used to lay out the data. The Cross-referencing Method, a comparative approach was used to compared livelihood patterns. Comparisons: during and after the experience of bovine cysticercosis and again between affected and non-affected farms across these same periods of time was used to assess the socio-economic effects of bovine cysticercosis on the farmers. The binomial logistic regression, which uses binary variables was used to analyze the effects of the occurrence of bovine cysticercosis on the socio-economy of the farmers. Details of the formula for binomial logistic regression are discussed in section 3.9.3 and 3.9.4

4.4.3 RESULTS OF STUDY ON EFFECTS OF BOVINE CYSTICERCOSIS ON SOCIO-ECONOMY OF CATTLE FARMERS

4.4.3.1 Percentage distribution of negative effect of bovine cysticercosis on the social-economy of farmers

Table 28 Percentage distribution of negative effect of bovine cysticercosis on the social-economy of farmers

Response	Percentage negative effects of bovine cysticercosis on farmers responsibilities (%)													
	Food	HC	EDU	HR	SV	BMF	BMB	LW	ENW	SR	RR	EE	DVB	ABD
Yes	77.9	81.9	89.2	84.6	52.3	18.1	14.1	20.1	29.5	26.2	14.1	40.9	30.2	2.7
No	19.5	15.4	10.4	12.1	45.6	78.5	83.2	77.2	68.5	71.1	83.9	57.0	67.1	94.0
Missing	2.6	2.7	0.4	3.3	2.1	3.4	2.7	2.7	2.0	2.7	2.0	2.0	2.7	3.4
Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0

KEYS:

1. Food: ability to provide food for family
2. HC: ability or difficulty accessing Healthcare
3. EDU: ability to provide education for wards
4. HR: ability to afford house rent
5. SV: ability of difficulty to save money
6. BMF: Borrowed money for family upkeep
7. BMB: Borrowed money for business
8. LW: Lay off workers
9. ENW: Unable to employ new workers
10. SR: Unable to meet social responsibility
11. RR: Unable to meet religious responsibilities
12. EE: Suffered emotionally because of negative effect of bovine cysticercosis on farming business
13. DVB Diversify Business because of uncertainty of Cattle business
14. ABD: Abandon cattle farming because of bovine cysticercosis

4.4.3.2 Percentage distribution of respondents who experienced Negative effect of bovine cysticercosis across different farm capacity

Table 29 Number & percentage of negative effect of bovine cysticercosis across farm capacities

Farm Capacities	Percentage Negatives effects of bovine cysticercosis on Farmers Responsibilities N (%)						
(No of Cattle)	Food	HC	EDU	HR	SV	BMF	BMB
>500	4(13.8)	4(17.4)	2(9.1)	3(16.7)	16(23.5	5(18.5)	5(23.8)
100-499	5(17.2)	3(13.0)	3(13.6)	1(5.6)	16(23.5	4(14.8)	6(28.6)
50-99	12(41.4)	9(39.1)	8(36.6)	9(50)	22(32.4	12(44.4	7(33.3)
10-49	6(20.7)	5(21.7)	5(22.7)	4(22.2)	12(17.6	4(14.8)	1(4.7)
5-9	2(6.9)	2(8.6)	3(13.6)	1(5.6)	2(2.9)	2(7.4)	2(.9)
< 5	0(0)	0(0.0)	1(4.5)	0(0)	0(0)	0(0)	0(0)
Total Cases	29(100)	23(100)	22(100)	18(100)	68(100)	27(100)	21(100)

KEYS:

1. Food: ability to Provide Food for Family
2. HC: ability or Difficulty Accessing HealthCare
3. EDU: ability to provide Education for Wards
4. HR: ability to afford House Rent
5. SV: ability of difficulty to save money
6. BMF: Borrowed Money for Family Upkeep
7. BMB: Borrowed Money for Business

About 19.5% of the respondent were unable to provide food for their family due to the advent of the bovine cysticercosis, while 77.9% were un-affected (table 28). Table 29 shows that of the farmers who could not provide food for family, 13.8% and 7.2% had farm capacities of >500 and 100-499 cattle respectively. The most affected were farmers have capacities of 50-99 followed by capacities of 10-49; these contributed about 41.4% and 20.7% respectively.

About 15.4% of the farmers were unable to provide healthcare for their family whereas 81.9% of the respondents were not affected (table 29). Of these farmers who could not provide healthcare, the most affected are farmers with farm capacities of 50-99 with about 39.1%, followed by farmers with farm capacities of 10-49 with 21.7%. The least affected group are farmers with farm capacities of <5

At least 10.4% of the farmers claim that bovine cysticercosis caused them not to be able to provide education for their wards. Another 89.2% of the farmers were able to provide education for their wards. About 21.1% of the farmers claimed that the negative effect of bovine cyticercosis on their finances caused them not to be able to afford house rent. About 45.6% of the farmers could not save money.

As low as 18.1% of the farmers claimed they had to borrow money for family upkeep. Majority of the farmers that borrowed money for their family upkeep had farm capacities of 50-99 cattle. It seems that the farmers with capacities of >500 and 100-499 were affected at the same magnitude in their inability to save money. About 14.1% of the respondents borrowed money to revive their businesses. The farmers having farm capacities of <5 and 5-9 did not spend extra money to revive their farming business

4.4.3.3 Percentage distribution of respondents who experienced negative effect of bovine cysticercosis across different farm capacity (continued)

Table 30 Number & percentage of respondents who experienced negative effect of bovine cysticercosis across farm capacities

Farm Capacities	LW No(%)	ENW No(%)	SR No(%)	RR No(%)	EE No(%)	ABD No(%)	DVB No(%)
>500	7(23.3)	6(13.6)	7(17.9)	3(14.3)	16(26.2	0(0)	11(24.4
100-499	7(23.3)	9(20.5)	10(25.6	5(23.8)	13(21.3	1(25.0)	11(24.4
50-99	8(26.7)	13(29.5	11(28.2	9(42.8)	17(27.9	1(25.0)	11(24.4
10-49	6(20.0)	9(20.5)	9(23.1)	2(9.5)	13(21.3	1(25.0)	7(15.9)
5-9	2(6.7)	2(4.5)	2(5.1)	2(9.5)	2(3.3)	1(25.0)	3(6.7)
< 5	0(0)	3(6.8)	0(0)	0(0)	0(0)	0(25.0)	2(4.4)
Total Cases	30(100)	44(100)	39(100)	21(100)	61(100)	4(100)	45(100)

KEY:

1. LW: Lay off Workers
2. ENW: Unable to Employ New Workers
3. SR: Unable to meet Social Responsibility
4. RR: Unable to Meet Religious Responsibilities
5. EE: Suffered Emotionally because of Negative effect of BC on Farming Business
6. ABD: Abandon Cattle Farming because of BC
7. DVB Diversify Business because of Uncertainty of Cattle Business

About 20.1% of respondents laid off workers because of adverse effect of the bovine cysticercosis on their finance. Majority of the farmers that laid off workers had farm capacities of 50-99 heads of cattle followed by capacities of 100-499 and >500 both of which contributed 23.3% of the total unemployment. In addition to laying off workers, about 29.5% of the respondents were unable to employ new workers.

Due to the advent of the bovine cysticercosis meeting social responsibilities became a difficult or impossible task for 26.2% of the farmers. About 14.1% of the respondents had difficulty meeting religious responsibilities. Of the farmers who had difficulty meeting religious obligations, 42.8% and 23.8% were farmers whose farm capacities were 50-99 cattle and 100-499 cattle heads respectively.

About 40.9% of the respondents suffered emotional disturbance at various times because of the advent of bovine cysticercosis. Exactly 30.2 percent of the respondents diversified their business. About 2.7 percent of the farmers abandoned cattle farming entirely.

4.4.3.4 Percentage distribution of farmers according to types of effect of bovine cysticercosis on farming and socio-economy of farmers

Table 31 Percentage of farmers according to types of effect of bovine cysticercosis on farming and socio-economy of farmers

Socioeconomic Effects	Affected (%)	Not Affected (%)	No Answer (%)
Farmers who recorded bovine cysticercosis in their farms	42.3	57.7	0.0
Farmers who experienced negative effect on the farm capacity	17.4	79.3	3.9
Farmers who experienced negative financial effect due to cysticercosis	50.0	45.6	4.3
Farmers whose animals were detained	47.7	51.0	1.3
Farmers whose animals were condemned	38.9	59.7	1.3
Spent money treating carcass	44.7	54.0	1.3
Inability to provide food for family	19.5	77.9	2.6
Inability or difficulty accessing healthcare	15.4	81.9	2.7
Inability to provide education for wards	10.4	89.2	0.4
Inability to afford house rent	12.1	84.6	3.3
Inability of or difficulty to save money	45.6	52.3	2.1
Borrowed money for family upkeep	18.1	78.5	3.4
Borrowed money for business	14.1	83.2	2.7
Lay off workers	20.1	77.2	2.7
Unable to employ new workers	29.5	68.5	2.0
Unable to meet social responsibility	26.2	71.1	2.7
Unable to meet religious responsibilities	14.1	83.9	2.0
Suffered emotionally	40.9	57.0	2.0
Diversify business because of uncertainty of cattle business	30.2	67.1	2.7
Abandon cattle farming because of cysticercosis	2.7	94.0	3.4

Fig 5 Bar chat of percentage of farmers whose socio-economy were affected or not affected by bovine cysticercosis

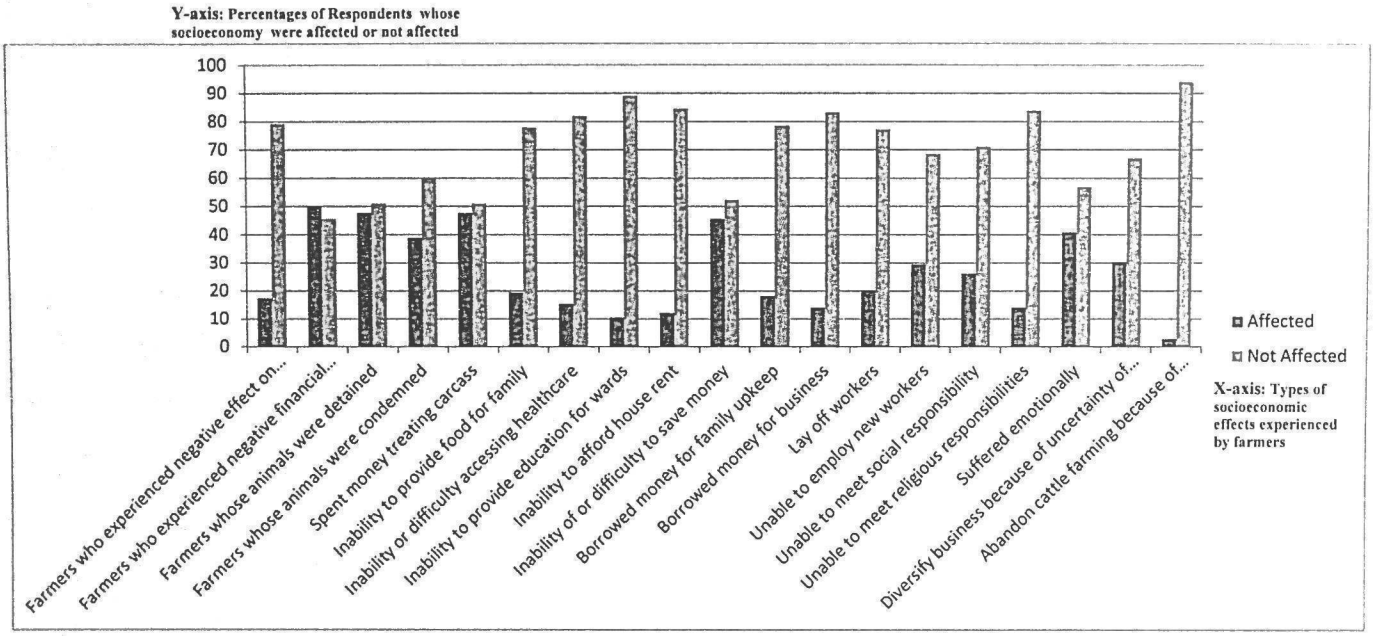


Figure 5: a bar chat, showing percentages of farmers whose socioeconomy were effected by bovine cysticercosis; marked as blue bars and percentages of farmers whose socioeconomy were NOT affected by bovine cysticercosis; marked as orange bars. This chat shows highest effect was on finances on farmers while lowest effect was adornment of farming business.

4.4.3.5 Socioeconomic effect of *C bovis* in order of relevance

Table 32 Socioeconomic effect of *C bovis* in order of magnitude

	N	Percentage of Cases
Inability to save money	48	45.6
Experienced emotional disturbance due to bovine cysticercosis	48	40.9
Diversified business because of bovine cysticercosis	32	30.2
Inability to employ new workers	32	29.5
Difficulty to meet social responsibility	31	26.2
Laid off workers	25	20.1
Inability to provide food for family	26	19.5
Borrowed money for family upkeep	22	18.1
Inability to provide healthcare for family	21	15.4
Difficulty to meet religious responsibility	19	14.1
Borrowed money for business upkeep	15	14.1
Inability to afford house rent	15	12.1
Inability to provide education	21	10.4
Abandoned business because of bovine cysticercosis	2	2.7

Table 6 shows percentage ranking of magnitude of effects of bovine cysticercosis on the socio-economy of farmers. The most affected indicator is "ability to save money" (45.6%). This is followed by "experienced emotional disturbance due to bovine cysticercosis" (40.9%); followed by diversification of business (30.2%); "ability to employ new workers (29.5) etc. The least affect was abandonment of farming business which was 3.6%

Figure 6 bar chat of numbers of farmers who experienced negative effect of cysticercosis according to farm capacities

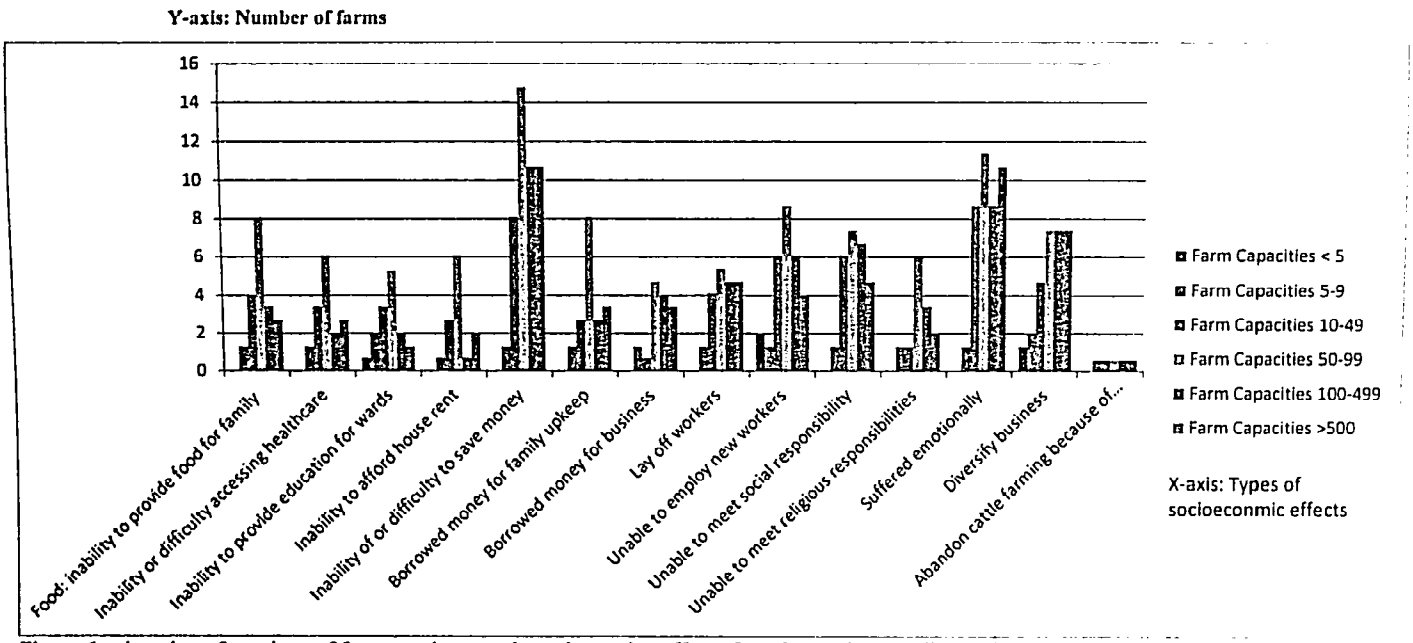


Figure 6: a bar chat of numbers of farmers who experienced negative effect of cysticercosis according to farm capacities. Farm size less than five (<5) marked as blue bars; five size 5-9 cattle marked as orange bars; farm capacities of 10-49 marked as grey; farm capacities 50-99 marked as yellow; farm capacities 100-499 marked as sky blue; farm capacities above five hundred (>500) marked as green. Most affected socioeconomic factor was ability to 'save money' for farmers with capacity 50-99; least affected socioeconomic factor was abandonment of farm for farm capacities above 500 cattle

4.4.3.6 Socio-economic effects of bovine cysticercosis on farmers across gender and ages of farmers

Table 33 a socio-economic effects of bovine cysticercosis across gender and ages of farmers

Socio-economic Effects	Category	Gender		Age of farmer						
		Male (%)	Female (%)	16-25 (%)	26-35 (%)	36-45 (%)	46-55 (%)	56-65 (%)	66-75 (%)	>75 (%)
Ability to provide food for family	Affected	23.2	13	80	29.2	12.2	12.5	27.8	40	0
	Not affected	76.8	87	20	70.8	87.8	87.5	72.2	60	100
Ability to provide healthcare for family	Affected	18.2	10.9	60	25	9.8	6.3	33.3	20	0
	Not affected	81.8	89.1	40	75	90.2	93.8	66.7	80	100
Ability to provide education	Affected	17.3	13	40	25	7.3	10.4	27.8	25	25
	Not affected	82.7	87	60	75	92.7	89.6	72.2	75	75
Ability to afford house rent	Affected	11.2	15.2	20	25	9.8	4.2	22.2	25	0
	Not affected	88.8	84.8	80	75	90.2	95.8	77.8	75	100
Ability to save money	Affected	48	43.5	60	44	43.9	37.5	66.7	100	25
	Not affected	52	56.5	40	56	56.1	62.5	33.3	0	75
Borrow money for family upkeep	Affected	23	9.1	40	20	15	14.9	27.8	40	0
	Not affected	77	90.9	60	80	85	85.1	72.2	60	100
Borrow money for business upkeep	Affected	16	11.1	20	16	10	16.7	16.7	20	0
	Not affected	84	88.9	80	84	90	83.3	83.3	80	100
Laid off workers	Affected	23	15.6	40	28	12.5	18.8	22.2	60	0
	Not affected	77	84.4	60	72	87.5	81.3	77.8	40	100
Ability to employ new workers	Affected	31	28.3	40	24	19.5	31.3	50	80	0
	Not affected	69	71.7	60	76	80.5	68.8	50	20	100
Difficulty to meet social responsibility	Affected	25.3	30.4	60	37.5	22	20.8	27.8	60	0
	Not affected	74.7	69.6	40	62.5	78	79.2	72.2	40	100
Difficulty to meet religious responsibility	Affected	18	6.5	60	20	9.8	6.3	22.2	40	0
	Not affected	82	93.5	40	80	90.2	93.8	77.8	60	100
Experienced emotional disturbance	Affected	40	45.7	60	36	34.1	37.5	66.7	60	50
	Not affected	60	54.3	40	64	65.9	62.5	33.3	40	50
Diversified business because of bovine cysticercosis	Affected	27.3	39.1	20	36	31.7	21.3	50	60	0
	Not affected	72.7	60.9	80	64	68.3	78.7	50	40	100
Abandoned business because of bovine cysticercosis	Affected	3.1	2.2	0	4.2	2.5	4.2	0	0	0
	Not affected	96.9	97.8	100	95.8	97.5	95.8	100	100	100

Generally across board and except in issues relating to experiencing emotional disturbances, meeting social obligations, ability to pay rent, diversifying business and ability to provide family upkeep, where females were more affected, males were more affected than females in the other ten (10) socioeconomic parameters. More men (23%) than 13% of women could not provide food for the family. Of all the age ranges, the most affected age is age range 66-75 years. This was closely followed by age range of 16-25 years. Age range of 65-75 years with 40% unable to provide food for the family were the most affected; followed by age range 26-35 years. Males more than females had difficulty providing/accessing healthcare for the family.

4.4.3.7 Logistic regression of effect of bovine cysticercosis on the socio-economy of the farmers

Table 34 the Binomial logistic regression of effect of bovine cysticercosis on the socio-economy of the farmers

	Equations (Dependent Variables)	B	S.E.	Wald	df	Sig.	Exp.(B)
1	Ability to provide food for family(1)	2.115	1.275	2.749	1	0.097**	8.288
2	Ability to provide healthcare for family(1)	23.101	7903.886	0.000	1	0.998	1.08E+10
3	Ability to provide education(1)	-2.983	1.904	2.454	1	0.117	0.051
4	Ability to afford house rent(1)	-1.956	1.605	1.484	1	0.223	0.141
5	Ability to save money(1)	2.399	0.889	7.279	1	0.007*	11.015
6	Borrowed money for family upkeep(1)	0.170	1.298	0.017	1	0.896	1.185
7	Borrowed money for business upkeep(1)	-0.325	1.118	0.085	1	0.771	0.723
8	Laid off workers(1)	1.497	1.120	1.785	1	0.181	4.468
9	Ability to employ new workers(1)	-1.218	1.122	1.179	1	0.278	0.296
10	Difficulty to meet social responsibility(1)	-1.003	0.924	1.178	1	0.278	0.367
11	Difficulty to meet religious responsibility(1)	-0.139	1.727	0.006	1	0.936	0.870
12	Experienced emotional disturbance due to (1)	3.411	0.866	15.519	1	0.000*	30.299
13	Diversify business because of BC (1)	0.050	1.081	0.002	1	0.963	1.052
14	Abandon business because of BC (1)	0.260	1.498	0.030	1	0.862	1.297
	Constant	-21.772	7903.886	0.000	1	0.998	0.000

Key: BC: bovine cysticercosis; *Significance at 5% ; ** Significance at 10%

Generally, occurrence of bovine cysticercosis had negative socioeconomic effects on all the affected farmers. The variables significantly affected by the occurrence of bovine cysticercosis ($P<0.05$) are "ability to save money" and "experience of emotional disturbance". The 'ability to provide food for family' was also equally significantly affected ($P<0.10$). The effect of bovine cysticercosis on farmers' healthcare, education, rental for family; farmers' ability to meet social and religious obligation were not statistically significant. Equally, some other socio-economy effects on farmers that were not statistically significant were 'ability to employ new workers', 'diversification or abandonment of farming business', borrowing money to keep up with family and business needs'. In using SPSS for analysis the odd is obtained by subtracting 1from Exponential B [Odds equal to Exponential B – 1]. For example, for farmers who could not save money, B (odd) = Exp.B -1 = 11.015 -1 = 10.015. The farmers who experienced bovine cysticercosis in their farms where 10.015 times more like not to save money compared to farmers who did not experience bovine cysticercosis in their farms. Equally, farmers who experienced bovine cysticercosis in their farms where 29.2990 times (Exp.B = 30.299) and 7.288 times (Exp.B = 8.288) more likely to experience emotional disturbance and inability to provide food for their family are respectively (table 34).

4.4.3.8 Financial effects of bovine cysticercosis on farmers in 2017

Table 35 financial effects of bovine cysticercosis on farmers in 2017

Amount (pula)	Midpoint Amount in (pula)	Cold treatment of carcass		Devaluation of carcass		Condemnation of carcass	
		Frequency	Amount Spent (P)	Frequency	Amount Lost (P)	Frequency	Amount Lost (P)
1,000-3,000	2,000	21	42,000	38	76,000	0	0.0
3,001-5,000	4,000.5	12	48,006	25	100,012.5	0	0.0
5,001-7,000	6,500.5	4	26,002	23	149,511.5	18	117,009
7,001-9,000	8,500.5	4	34,002	11	76,504.5	11	93,505.5
9,001-11,000	10,500.5	3	31,501.5	9	94,504.5	7	73,503.5
60,000-70,000	65,000.5	0	0.0	3	19,501.5	9	585,004.5
70,001-80,000	75,000.5	0	0.0	1	75,000.5	5	375,002.5
80,001-90,000	85,000.5	0	0.0	0	0.0	7	595,003.5
90,001-100,000	95,000.5	0	0.0	0	0.0	3	285,001.5
100,001-110,000	105,000.5	0	0.0	0	0.0	2	210.001
Total		**44**	**181,511.50**	**110**	**522,635.00**	**62**	**2,124,240.00**

1. Total amount of money spent by forty four (44) respondents in cold treatment of infected carcasses in 2017 was one hundred and eighty one thousand five hundred and eleven thousand pula, fifty thebe (181,511.50). This brings it to an average of four thousand, one hundred and twenty five pula, twenty six thebe (P4, 125.26) spent per farmer in the cold treatment of infested carcass in 2017 alone (table 35).
2. Total amount of money lost by one hundred and ten (110) respondents due to devaluation of carcasses infested with cysticercosis in 2017 was five hundred and twenty two thousand, six hundred and thirty five pula. This brings it to an average of four thousand, seven hundred and fifty one pula, twenty seven thebe (P4, 751.27) lost per farmer due to devaluation of carcasses in 2017 alone (table 35)
3. Total amount of money lost by sixty two (62) respondents due to condemnation of carcasses in 2017 was two million, one hundred and twenty four thousand, two hundred and forty pula (2,124,240.00). This brings it to an average of thirty four thousand, two hundred and sixty one pula, ninety three thebe (P34, 261.93) lost per farmer due to condemnation of carcasses in 2017 alone (table 35)

4.4.4 DISCUSSIONS OF SOCIO-ECONOMIC EFFECTS OF BOVINE CYSTICERCOSIS ON CATTLE FARMERS IN BOTSWANA

Upon identification of more than 10 cysts in a carcass in BMC, the carcass is detained. Detained carcasses are treated by chilling at temperature below 4°C for about 10 days before passing for public consumption. Treated carcasses do not qualify for export; they are sold locally at a reduced price. At BMC, the farmers receive 75 percent of the actual value of the detained carcass, whereas at Senn Foods®, a major meat company in Botswana, farmers receive 85 percent of the original value of the carcass (Aganga, 2017). This low compensation from BMC has equally made farmers to resort to other buyers than BMC.

Identified issues leading to losses in detained carcass include, among others; detaining carcass in refrigerator leads to loss of meat water, which leads to loss in carcass weight. This agrees with the findings of Muela *et. al.*, (2010) who claimed that the chilling of a carcass leads to weight loss after 90 hours of chilling; carcass owners spend extra money chilling the animal; when an animal is not sold in time, extra money is spent keeping a carcass fresh; some meat sellers agree to treating the meat to enhance its presentation; detained carcass loses quality; detained carcass is sold at a reduced price to customers and the price drops with time; customer confidence and trust on meat operators may be either reduced or lost entirely; some carcass may be turned to by-products, which are sold at a reduced price; the retention of meat and the subsequent low sales distorts the business investment and market plan; sometimes extra meat is mixed with the detained meat to make customers purchase them; because of incessant retention of carcass at BMC, farmers have lost confidence in BMC (Olupot, personal communication, April 16, 2017); both BMC and Senn Foods® and other large scale consumers of beef have partially blacklisted certain extension areas and have reduced the purchase of animals from this areas.

Forty seven point seven percent (47.7%) of farmers' animals were devalued; this is higher than the prevalence of bovine cysticercosis, which is 42.3 (table 31). Both figures should be equal or the prevalence should be higher than the percentage devalued, since it is assumed that all the farmers who experienced bovine cysticercosis must have had their animals either condemned (if cysts exceed 10) or treated (if cysts is less than 10). However, the figure of percentage devalued is higher than prevalence because whereas, the prevalence calculates for one year, percentage devalued calculates for five years (2012-2017).

The percentage of farmers who treated carcasses was less than the percentage of farmers whose animals were detained. The local government paid for the treatment of some detained carcasses instead of the farmers (Olupot, personal communication, April 16, 2017).

In an event that number of cysts presents in the carcass exceeds 10, the carcass is totally condemned and it does not pass for treatment (Livestock and Meat Industries Act, 2007). The farmers' would receive a paltry sum of sixty pula (P60) or the equivalent of transport money spent to convey the animals to the abattoir.

The most affected socioeconomic factor of farmers was their 'ability to save money' which was 0.007 ($P<0.05$).

Type and magnitude of the effects of the farmers socioeconomy include; the total inability to save money for respondents whose carcasses were totally condemned and destroyed with little or no compensation; inability to save money commiserate to investment for respondents whose carcasses harboured few cysts and were devalued after treatment; delay in saving money later than expected for respondents whose monies were paid at a much later date after the buyer had sold the beef.

In particular, in the villages, among the small scale farmers, some respondents reported that they borrowed money for their family's upkeep. Similarly, a majority of the farmers that borrowed money for their upkeep were small scale farmers (farm capacity of 50-99 cattle). This is understandable because the liquidity of small scale farmers is meagre thus any financial shock would affect them. It seems that the large scale and medium scale farmers were affected at the same magnitude; which is much less than the prevalence rate of the bovine cysticercosis borrowed money to revive their businesses. This may be as a result of the fact that although 42.2 percent of the respondents experienced the bovine cysticercosis, not all of them experienced serious financial shock that could cause them to require a loan in order to revive their businesses.

Farmers observed that due to the loss of money from devalued or condemned carcasses some of them experienced difficulties paying salaries when they were due. Consequently, some of the farmers and beef industry operators were laid off workers. In addition to laying off workers some respondents stated that due to the advent of the bovine cysticercosis, they were unable to employ new works into the business. Majority of the cattle industry operators who were not affected were low scale operators who did not need extra hands in their business due to low scale of operations. Also unaffected across the board were operators whose financial loss was minimal such that the effect of bovine cysticercosis on their business was negligible.

The farmers' "experience of emotional disturbance" was significantly affected at 0.000. The 'ability to provide food for family'. The farmers ability to provide food was significantly affected at 0.097 ($P<0.10$); causing some to borrow money for upkeep of family and/or farming business. Ability to provide healthcare, education, rental for family were affected. Equally, bovine cysticercosis caused farmers not to employ new workers; to diversify or abandon farming business. With reduced income came a "farmers' inability to meet social, religious and family obligations". Severity of effects on farmers were dependent on the magnitude of the infestation, the scale of production of farmers and presence of absence of some survival strategies. Farmers' response to effects ranged from outright closure of farms, to reduction in farm capacity, to diversification of businesses. Some farmers experienced some psycho-social effects.

There were gender or age difference of the effects of bovine cysticercosis, which were not statistically significant ($P< 0.05$). The farmers who experienced bovine cysticercosis in their farms were 10.015 times more likely not to save money compared to farmers who did not experience bovine cysticercosis in their farms. Equally, farmers who experienced bovine cysticercosis in their farms were 29.2990 times and 7.288 times more likely to experience emotional disturbance and inability to provide food for their family respectively. This is so because in addition to the financial loses, cattle is a source of pride and some farmers have shown emotional attachment to their cattle (Gender Researcher 2012 in Andrea, 2016).

Affected farmers also cut down on socializing due to a shortage of funds. Summarily, the bulk of the respondents who could not meet their social obligations were small scale farmers. The large and medium scale farmers were less affected by the bovine cysticercosis. Some farmers skipped flamboyant celebration of Christmas, Independence day the popular "expo" (festival). The losses caused some farmers to experience emotional and psychological negative effects. The percentage of farmers who suffered emotional effects ties closely with the prevalence of the bovine cysticercosis. Some farmers who did not experience bovine cysticercosis in their farms confessed to being emotionally affected in anticipation that in the near future their animals may equally be affected. In the heat of the uncertainty some farmers diversified their businesses within and outside the farming industry. The reason for the diversification includes the following; the drop in finance realized from the beef industry affected farmers ability to meet needs thus they sought extra sources of income; the uncertainty associated with the possibility of diagnosing bovine

cysticercosis in carcass caused some farmers to resume other farming or artisan business as an alternative source of income.

The percentage of farmers who suffered emotional disturbance is less than the prevalence rate; it is probable that some farmers who experienced bovine cysticercosis did not suffer emotional maybe because the impact was minimal to ignorable. Beyond diversification of business some farmers abandoned cattle farming because of the advent of the bovine cysticercosis. This group consist mainly of migrant farmers who could no longer sustain their livelihood through cattle farming.

The farmers who experienced bovine cysticercosis in their farms where by far more likely not to save money and more likely to be unable to provide food for their family compared to farmers who did not experience bovine cysticercosis in their farms. This is because the occurrence of bovine cysticercosis causes both direct and indirect financial losses, which also distorts the planning and saving process of the farmers thus making saving more difficult.

On the average each farmer spent about (P4, 125.26) four thousand, one hundred and twenty five pula, twenty six thebe in the cold treatment of infested carcass in 2017 alone. Also each farmer lost about (P4, 751.27) four thousand, seven hundred and fifty one pula, twenty seven thebe due to devaluation of carcasses in 2017 alone. Total amount of money lost by 62 respondents due to condemnation of carcasses in 2017 was (2,124,240.00) two million, one hundred and twenty four thousand, two hundred and forty pula. This brings it to an average of (P34, 261.93) thirty four thousand, two hundred and sixty one pula, ninety three thebe lost per farmer due to condemnation of carcasses in 2017 alone.

About 17.4 percent of the farmers claimed that bovine cysticercosis affected the capacity of their farms negatively (table 31). Although bovine cysticercosis is known to cause zero mortality (Murrell, *et. al.*, 2005), the condemnation of animals and devaluation of carcasses caused some farmers to deliberately reduce stocking capacity. Under this condition, bovine cysticercosis has an indirect negative effect on the capacity of the farm. Less than 10 percent of the rural farmers in the North East district complained that following the high incidence of bovine cysticercosis, the farmers delayed farm expansion for cattle. About 30 percent of the farmers resorted to other business alongside diversifying into goat, sheep and crop farming. This agrees with the findings of Uchendu, *et. al.*, 2015 who recorded that adverse losses to farmers can cause farmers to resort to diversification of business or outright abandonment of farming business.

The percentage of farmers whose finances and livelihood pattern were affected by bovine cysticercosis is less than the prevalence (table 31), although the former should have been equal to or more than the latter. The explanation is that some of the farmers who experienced the advent of bovine cysticercosis are part time farmers, or large scale farmers or farmers having other livestock who do not depend solely on cattle farming for their livelihood. Consequently, the advent of bovine cysticercosis did not have a negative effect on their livelihood pattern. Majority of the farmers whose livelihood patterns were affected by bovine cysticercosis are the farmers who own 50-99 cattle and the farmers who own 10-49 cattle and are operating at full time basis. Across all the indicators of socioeconomic effect, the small scale farmers who are full time farmers are the most negatively affected. This pattern is expected because this category of farmers who own < 10 cattle (small scale) who are also full time are the most vulnerable since they do not have other sources of income yet their business is not big enough to cushion the negative financial effect of the bovine cysticercosis.

Important features for determining vulnerability of farmers to the advent of the bovine cysticercosis included the following: the scale of production (amount of animal in farm or post); the level of involvement (part time or full time); the magnitude of dependents on farmers vis-à-vis income level (family size versus scale of production versus level of involvement); the magnitude of effect of bovine cysticercosis on farming business (amount lost by detention, devaluation and destruction of animals and the number of animals destroyed), and the type, level and promptness of government intervention to farmers following devaluation or destruction of affected animals.

The least affected group is the farmers who have less than 5 cattle and are part time farmers followed by the farmers who have less than 10 cattle and are part time farmers. This category are mainly starters or retiring farmers or part time farmers who do not depend fully on the cattle farming business as the major source of income as such the negative effect of the bovine cysticercosis on their finance had minimal effect on their ability to meet their socio-economic and sundry needs.

The farmers who have less than 10 cattle and operate full accounted for the majority who could not provide food for their family. Percentage of farmers who could not feed their family is much less than the prevalence because some of the farmers who experienced the advent of bovine cysticercosis are part time farmers, or large scale farmers or farmers having other livestock thus do not depend solely on cattle farming for feeding their family. Consequently, the advent of bovine

cysticercosis did not prevent them from being able to feed their family. A cross tabulation of the effects of bovine cysticercosis on farmers according to their scale of production shows that the small scale full time farmers were more affected by the bovine cysticercosis than the large scale and or part time farmers.

About 15 percent of the respondents experienced inability to provide healthcare for themselves and their family. The National Health Policy of Botswana bears the slogan 'Towards a Healthier Botswana', implying that the provision of health services is not just merely curing the sick but also promoting healthy lifestyles in order to prevent diseases/ill-conditions for all people living in Botswana. The Policy covers all the six building blocks of health systems, with specific direction for each of them. It also provides the platform for well-coordinated planning, financing, monitoring and evaluation of health systems (National Health Policy, 2012 pg 5). The government of Botswana through the National Medical Aid has provided free and accessible health-care for all her citizens. Thus, the negative effect of bovine cysticercosis on the finances of the farmers may not easily affect their ability to provide health care for their families.

Despite the negative effect of bovine cysticercosis on the farmers' finances, the percentage of children who quit school on account of parents' inability to pay school fees is negligible. Education in Botswana is free and almost compulsory; increasing education funding ranks third most important priority areas for the ruling party. Botswana well acknowledges that knowledge, and not capital per se, is the most important resource and ingredient for socio-economic development (BFTU, 2007). The nations ruling party claims that the best economic policy is, in actual fact, education (BFTU, 2007) thus every citizen is afforded opportunity to earn free education up to post-graduate level. Consequently low income earning parents can afford to send their wards to school. It therefore follows that the negative effect of the bovine cysticercosis on the income of farmers did not have gross impact on the ability of farmers to provide education for their children.

Upon interview and analysis of questionnaire, the researcher realized that majority of the unaffected farmers were locals who lived in their own houses within the rural areas proximal to their farms and cattle posts thus did not need to pay house rent. However some of the migrants or farmers who lived in the cities far from their farms and cattle posts complained that the negative effects of the bovine cysticercosis on their finances made them to forfeit the extra rented houses and lodges in the villages where their farms where located; normally they would live in these house when they visit their farms from the city.

Some of the intervention strategies the government provided are re-stocking seed calves to severely affected farmers, social amenities like pipe borne water to the farms proximal to the urban areas and bore holes to farms and cattle post removed from the urban areas. The government also organized regular campaign programs on prevention and control of bovine cysticercosis.

4.4.5 CONCLUSIONS

This study has shown that bovine cysticercosis had negative effect on the socio-economy of the cattle farmers in Botswana. Generally, devaluation or condemnation of carcasses arising from identification of cysts in carcass, had financial implications. The advent of bovine cysticercosis had significant effects on farmers' ability to save money and farmers emotional and psychological health. Also affected were farmers ability to provide food, healthcare, education, rental for family, farmers' ability to employ new workers, diversification or abandonment of farming business. With reduced income came the farmers' inability to meet social, religious and family obligations, causing some to borrow money for upkeep of family and/or farming business. Severity of effects on farmers were dependent on the magnitude of the infestation, the scale production of farmers and presence or absence of some survival strategies of affected farmers. On the average each farmer spent about four thousand, one hundred and twenty five pula, twenty six thebe (P4, 125.26) in the cold treatment of infested carcass in 2017 alone. Also each farmer lost about four thousand, seven hundred and fifty one pula, twenty seven thebe (P4, 751.27) and thirty four thousand, two hundred and sixty one pula, ninety three thebe (P34, 261.93) due to devaluation and condemnation of carcasses respectively in 2017 alone.

Farmers' response to effects ranged from outright closure of farms for severely affected to reduction in farm capacity, particularly for farmers who own large number of cattle and were not severely affected; to diversification of business for mostly low scale farmers. In addition to the socioeconomic effects, farmers experienced some emotional and psychosocial effects. Of the existing government intervention measures, the most effective were provision of re-stocking seed calves, payment for chilling of infested carcass, installation of socio amenities, which also played vital role in cushioning the adverse effects of bovine cysticercosis on the livelihood pattern of the farmers. Additionally, since the binomial logistic regression, determined the most affected aspects of the socio-economy to be ability to save money; emotional and psychological disturbances and

ability to provide food for the family, these aspects should be the primary focus of government intervention measures.

4.4.6 OUTCOMES AND DELIVERABLES OF SOCIOECONOMIC STUDY

1. This study determined the effects of bovine cysticercosis on livelihood pattern of cattle farmers.
2. Evaluated the effectiveness of the current intervention measure.
3. Determine financial worth to the loss due to bovine cysticercosis.
4. Informed effective and sustainable socio-economic interventions from government e.g. amenities.

4.5 ELECTROPHORETIC PROFILING OF BOVINE CYSTICERCOSIS PROTEINS AND IDENTIFICATION OF THOSE THAT ARE IMMUNOGENIC

4.5.1 INTRODUCTION

Taenia saginata cysticercosis is the larval stage of the human tapeworm, *Tania saginata*. It is found in beef as cysts in skeletal muscles with economic and public health implications (Urquhart *et. al.*, 1996). The life cycle of *T. saginata* is dependent on the link between humans and cattle (Urquhart *et. al.*, 1996 Harrison *et. al*, 1989). Any interruption of this link can result in the elimination of the parasite. Whereas this appears to imply that prevention and control of taeniasis and cysticercosis should be straightforward and practical, the reality is that many traditional prevention and control methods have not sufficed. This is because of the highly successful dissemination and reproductive features of the parasite and because of well-entrenched cultural factors of the human hosts (Murrell *et. al.*, 2005).

Effective prevention and control of bovine cysticercosis could be achieved by vaccination. The objective of the study was to identify vaccine candidates in the form of immuno-genic (dominant) protein epitopes that could be utilized for the production of bovine cysticercosis vaccines. Following an electrophoretic teasing out of the constituent proteins, bovine polyclonal antibody and Rabbit anti-Bovine IgG (H+L) Secondary Antibody conjugated with Horse Radish Peroxidase (HRP) were used to challenge antigens derived from cysts fluid and whole cysts of bovine cysticercosis isolated from beef slaughtered at Abattoir in Gaborone Botswana. One-dimensional electrophoresis using Sodium dodecyl sulphate Polyacrylamide electrophoresis (SD-PAGE) of bovine cysticercosis identified proteins with molecular weights of 4, 6, 14, 17, 22, 25, 28, 32, 38, 44, 50, 67, 75, 100, 115, 135, 150, 190 and 245kDa. Immunoblotting under reducing conditions, identified immunogenic proteins at 14, 22, 25, 50, 98, 135, 190 and 245kDa. These proteins were further confirmed to be immunogenic proteins specific for *Taenia saginata* cysticercosis by treating transblotted membrane with rabbit serum, containing antibodies specific for *Taenia saginata* cysticercosis and Goat anti-Rabbit IgG (H+L) Secondary Antibody Horse Radish Peroxidase (HRP). The identified immunogenic proteins had molecular weights at 14, 22, 25, 50, 98, 135, 190 and 245kDa. Chilling of samples did not cause significant difference ($P<0.05$) in quantity of proteins contained in samples however, immunogenic proteins were lost after 7 days. Protein sequencing is required to identify epitopes and to go a step closer to vaccine development

4.5.2 Background of the Study

The prevalence (rate) of bovine cysticercosis in Botswana is high (Mosienyane, 1986; Tshiamo, 2015); and consistently increasing at rates from 12% in 1974 to 15% in 1983 (Mosienyane, 1986); 10% to 12% around 2006 and 18% to 20% between 2006 and 2014 (Modisa, 2014). Beef from carcasses identified during meat inspection procedures as having beef measles cannot be exported to the European Union (Livestock and Meat Industries Act 2007); resulting in annual loss of export earnings of about one million (P1M) (P1.00 = 0.5 USD) in 1978 (Grindle, 1978); five million pula (P5M), (P1.00 = 0.68 USD) in detained and/or condemned carcasses per year as at 1985 (Mosienyane, 1986) and about P35 million in 2008 (Aganga, 2009). In 2009, 2010, and 2012, beef exports worth P99, 645, 780, P100, 477, 260, and P83, 289, 960 respectively, could not be sold to the EU markets (Tshiamo, 2015).

In its determination to control this public health hazard and economic loss, the government of Botswana has practiced mass literacy on proper disposal of human effluent and general hygiene (which is considered the major risk factor); adequate and efficient meat inspection among several other prevention, control and intervention measures. Going forward, the government has proposed mass human treatment with the use of anthelmintic. Strategist project that non-compliance of stake holders and\or non-adherence of individuals to treatment regimen may render this exercise futile. Left with no better alternative and being that vaccines and vaccination have shown to be effective in the prevention and control of bovine cysticercosis (Lightowlers, *et. al.*, 1996), the need to produce a vaccine against bovine cysticercosis became imperative. The objective of this research is to identify immunogenic proteins and designate immuno-dominant ones that can be utilized for the production of vaccines for bovine cysticercosis.

Polyclonal primary antibody of bovine origin and Rabbit anti-Bovine IgG (H+L) Secondary Antibody Horse Radish Peroxidase (HRP) were used to challenge antigens derived from cysts fluid and whole cysts of *Taenia saginata* cysticercosis isolated from beef slaughtered at the Multi Specie Abattoir Botswana, Gaborone. The antigens that showed affinity were further purified and analyzed by immunoblotting under reducing conditions. Using streptavidin-HRP mixture, proteins that showed affinity for the antibody were recognized as 25kDa and 50kDa. However, increasing the concentration of the primary and secondary antibodies and using SIGMAFASTä BCIPÒ /NBT (5-bromo-4-chloro-3-indolyl phosphate/nitro blue tetrazolium) tablets as a coloration elicited immunogenic proteins with weights of 14kDa, 25kDa, 50kDa, 98kDa and 135kDa. Identified

proteins were confirmed specific for bovine cysticercosis by treating transblotted membrane with rabbit serum, containing antibodies specific for bovine cysticercosis and Goat anti-Rabbit IgG (H-L) Secondary Antibody Horse Radish Peroxidase (HRP). The identified immunogenic proteins had molecular weights at 14, 22, 25, 50, 98, 135, 190 and 245kDa.

This research proposes these 8 proteins as possible vaccine candidates for the production of vaccine against bovine cysticercosis; while recommending for further purification of protein of interest and possible preparation of monoclonal antibodies, specific for bovine cysticercosis which may be used in an ante-mortem test kit.

4.5.3 MATERIALS AND METHODS

4.5.3.1 Sample collection

Multi Specie Abattoir Botswana (MSAB) Gaborone, was chosen for the collection of bovine cysticercosis cysts and meat samples because of its proximity to the research laboratory at the Botswana Vaccine Institute (BVI); and because of adequate meat inspection practices at MSAB under the supervision of the Official Veterinary Surgeon (OVS). Meat samples from the massetter muscle, heart, tongue and triceps brachii with evidence of cysticercus cysts were collected from bovine carcasses during post mortem examination following the provisions of the Livestock and Meat Industries Act (2007).

4.5.3.2 Sample Preparation

Cysts were teased out of the beef using rat-toothed forceps and scalpel blade; ensuring that cysts are free of beef particles. The cysts were washed with 9% saline solution. Cysts fluid was aspirated using syringe and needle. Cysts were washed twice in fresh PBS solution using a rocking platform. Cysts shell/walls and meat samples were crushed using mortar and pestle. Equal volume of PBS (V/M) (e.g. 60µl of PBS to 60µg of cyst) was added to crushed samples. Both the cyst fluid and cyst shell samples were serially diluted to 50%, 25% and 10% using PBS. Resultant samples were meat sample; whole cyst samples; cysts fluid samples, each at concentrations of 100%, 50%, 25% and 10%. All mixtures were centrifuged at 10,000 revolutions per minute at 4°C for 3 minutes. The supernatants were separated from the sediments and stored in separate Nunc® Cryotube at temperature of -15°C for 7, 14, and 21 days; depending on when they were needed. Detailed procedure available at operations 3, 4, & 5; APPENDIX II.

Quantities of proteins in samples were determined using the Qubit® 3 Fluorimeter, following instruction of Thermofisher Scientific Inc. (2014) as modified by (Manchester, 1996) Detailed procedure available at operations 8; APPENDIX II.

4.5.3.3. Experiment 1A: Electrophoretic profiling of *C bovis* proteins using 10% gel concentration and samples of different dilution factors across days of chilling

Reagent Preparation: Following manufacturers' instructions, fresh electrophoresis reagents were prepared; these reagents are: 10X MES (4-Morpholine Ethane Sulfonic Acid) SDS-PAGE Running Buffer (School of Chemistry and Biochemistry [SCB], 2001); 10X Tris-glycine SDS Buffer; 1.5M Tris-HCl buffer at pH 8.8 (SCB, 2001); 0.5M Tris-HCl buffer at pH 6.8 (SCB, 2001; Laemmli, 2003); 2x sample loading buffer (non-reducing) and 2x sample loading buffer (reducing) and 20% (w/v) Sodium Dodecyl Sulfate (SDS) (SCB, 2001); 10% (w/v) Ammonium Persulfate APS; 4X Electrophoresis Buffer; Staining and De-staining Solutions, Fixing solution; Coomassie Blue stock solution; Buffers for western blotting, 10x Transfer buffer; 1x Transfer buffer (SCB, 2001) Detailed procedure shown in operation 10, APPENDIX II, pages 201-204.

Sample Preparation: Ten (10) to twenty (20)µg of protein contained in different volumes of sample was loaded into separate Nunc® Cryotube. The protein sample was made up to a volume of 20-25µl using SDS-PAGE sample loading buffer and the mixture homogenized by voltexing then incubated for 5 minutes at 88^0C.

Gel preparation: Different concentrations of gel are recommended for different weights of protein; these are: 8% gel concentration for proteins of 80-200kDa; 10% for proteins of 35-100kDa; 12% for proteins of 25-60kDa; 15% for proteins of 20-40kDa (Proteintech, 2019). By optimization, 10% concentrations of SDS-PAGE gels proved most suitable for the protein of interest. Details of procedure available in operation 11; APPENDIX II.

Table 36 protocol for preparation of 10% concentration of SDS-PAGE gel

S/N	Reagent	Resolving Gel	Stacking Gel
1	Tris Buffer HCl	4mL 1.5M Tris pH 8.8	2.5 ml of 0.5M tris pH 6.8
2	Distilled Water (ddH$_2$O)	7.7ml ddH$_2$O	6.3 ddH$_2$O
3	Acrylamide	4ml 40% Acrylamide	1ml 40% Acrylamide
4	SDS	160µL of 10% SDS	100µL of 10% SDS
5	APS	160µL of 10% APS	100µL of 10% APS
6	TEMED	16µL TEMED	10µL TEMED

KEYS AND EXPALNATIONS:

SDS = Sodium Dodecyl Sulfate (SDS) - a strong detergent with a hydrophobic tail and a negatively charged head. (Please refer to literature for more details)

APS = **Ammonium Persulfate (APS)** is an oxidizing agent that is used with TEMED to catalyze the polymerization of acrylamide and bisacrylamide to prepare polyacrylamide gels for **electrophoresis.**
TEMED: Tetramethylethylenediamine (**TEMED**) is an essential catalyst for polyacrylamide gel polymerization. **TEMED** is used with **ammonium persulfate** (APS) to catalyze acrylamide polymerization when preparing gels for **electrophoresis.**
Note that 16ml of the gel was prepared for 2 gel; one gel for staining and the second gel for electro-blotting.

Gel pouring and casting: Resolving gel prepared at 10% concentration was poured before the stacking gel. Gel was poured using pipette. Bubbles were removed by overlaying gel with isopropanol. The stacking gel prepared at 10% was poured using a pipette up to the point of the short glass plate, then comb inserted.

Loading samples and buffers: By optimization, loading 25µL of mixture of protein and buffer produced a good gel picture (Appendix II). Sample and loading buffer quantities and volumes were worked (table 37; page 138). Loading buffer was added at the two last wells; that is well 9 and 10 (table 37; page 138 and figure 6b; page 140).

Running the gels: Following manufacturer's manual, gels well submerged by pouring 1X Tris-glycine SDS running buffer into the chamber. Three gels marked G1A, G1B and G1C were run. G1A was stained for protein profiling while G1B and 1GC were used western blotting (figure 6b, 7 & 8) .The gel was run at 100V until samples descended below the stacking gel before increasing to 120V. At the end of the electrophoretic process, gel G1A was stained with Commassie blue fixed and de-stained. The stained gel was photographed using a still camera (figure 6b; page 140).

4.5.3.4. Experiment 1B: Transblot of protein onto a nitrocellulose membrane.

Transblot: Following manufacturer's manual; BIORAD (2005), the proteins in gels G1B and G1C were transblotted to two nitrocellulose membranes marked M1B (figure 7; page 141) and M1C (figure 8; page 142). In western blot, M1B challenged using serum from cattle positive for *Bovine cysticercosis* while M1C was challenged with serum from cattle negative for *C bovis.* Transblot machine was set at Protocol setting: Mixed MW; Mw, Kilo Dalton: 5-150; Time 7 minutes; 2.5 A; 25 Volts.

Serologic processing of the Membrane: The 2 membranes M1B & M1C were washed twice in fresh Phosphate Buffer saline Tween (PBST) solution for duration of 5 Minutes each time. PBST solution, a washing detergent, was combined with milk and used as blocker to prevent non-specific proteins from binding with membranes. The membranes were blocked by immersing in PBST-

Milk and gently agitated using a rocking platform for 1 hour 30 minutes. Membrane 1B was incubated with serum from bovine cysticercosis positive cattle for 10 hours. Membrane 1C was incubated with serum from bovine cysticercosis negative cattle for 10 hours. Both positive and negative sera were prepared at dilution factor of 1 part of serum (primary antibody) in 5000 parts of PBST-Milk. The 2 membranes were incubated for 2 hours in Rabbit anti-Bovine IgG (H+L) secondary antibody conjugated to HRP at approximate protein concentrations (IgG) of 1.0 mg/ml. Secondary antibody dilution factor was 1 part of secondary antibody to 5000 parts of PBST-Milk. Amplified Opti-4CN substrate provided by BIORAD was used to amplify the serologic result and to provide colour to the antigen-antibody result. To achieve this, the membranes were washed twice in a solution of 20% DMSO-PBST for a duration of 5 minutes each wash. Gentle agitation was provided using a rocking platform at room temperature. At a dilution factor of 1 part of Streptavidin-HRP with 1000 parts of 1% of PBST-Milk, this mixture was used for immune-detection of biotinylated proteins by shaking for 30 minutes with gentle agitation provided using a rocking platform at room temperature. Coloration was achieved by shaking the membranes separately in a mixture of 1 part of Opti-4CN diluent concentration added to 9 part of double distilled water; then 0.2µL of Opti-4CN substrate was added. The colorimeter mixture was agitated until the desired colour change was observed. Both positive and negative membranes were washed with PBST, dried and stored (7 and 8; pages 141 and 142 respectively).

4.5.3.5. Experiment 2: Electrophoretic profiling of bovine cysticercosis proteins using Dithiothreitol (DTT) loading buffer and 1X MES SDS-PAGE running buffer

Following experiments 1A and 1B; (page 133 & 134), clarity of gel was increased by replacing Sodium dodecyl sulfate polyacrylamide sample buffer with Dithiothreitol (DTT) and replacing 1X Tris-glycine SDS running buffer with 1X MES SDS-PAGE running buffer.

Sample preparation: Samples were collected and prepared as described in materials and methods (section 4.5.3.1 and 4.5.3.2; page 132). Volumes of each sample containing 10µg of sample were made up to 20µl using Dithiothreitol (DTT) sample loading buffer. This mixture was homogenized by voltexing and incubated for 5 minutes at 88^0C.

Gel pouring and casting: Both the stacking and resolving gels were prepared at 12% and poured as described in experiment 1A (section 4.5.3.1)

Loading samples and dye: Based on concentrations of each sample, quantities and volumes of samples and loading buffers were worked (table 37, page 138). Sample loading buffer was added

to wells 9 and 10 (table 37; page 138 and Figure 9; page 143). Tobacco Mosaic Virus with a known weight of 17kDa was loaded into well 8.

Running the gels: Gels were submerged in 1X MES SDS-PAGE running buffer. Gel electrophoresis ran as described in experiment 1A, page 133. At the end of the electrophoretic process the gel was stained, fixed and de-stained as recommended by Thermo Scientific Invitrogen (2015) as described in section operation 15, appendix II, page 207. The gel was photographed using a still camera (figure 9; page 143).

4.5.3.6. Experiment 3A: Gel electrophoresis using MES running buffer and identification of immunogenic proteins through transblot using Polyvinylidene fluoride (PVDF) membrane and APS coloration Tablet

Experiments 1A (page 133) and experiment 1B (page 134), produced blurred gels. Clarity of gel background was enhanced by replacing nitrocellulose membrane with PVDF membrane. Distinctiveness of immunogenic proteins bands in the membrane was enhanced by replacing Amplified Opti-4CN substrate coloration with SIGMAFASTä BCIPÒ/NBT (5-bromo-4-chloro-3-indolyl phosphate/nitro blue tetrazolium) tablets.

Gel Electrophoresis: The gel electrophoresis was carried out as described in experiments 1 and 2; page 133 & 134 respectively. Quantities and volumes of samples and loading buffer were worked out (table 37; page 138).

Transblot: Two Polyvinylidene fluoride (PVDF) membranes marked as M3B and M3C were used instead of the nitrocellulose membrane previously used in experiment 1B page 132. PVDF membrane retains target protein very strongly during protein transfer, and reduces nonspecific protein binding that can obscure high-sensitivity detection (BIO-RAD, 2000). Thus, PVDF reduces background noise.

PVDF membrane is hydrophobic (Tisch Scientific, 2018); it does not dampen in aqueous fluid. Soaking membrane in 85% methanol for 10 minutes at room temperature causes them to dampen in aqueous fluid and enhance its absorption of proteins from the gel.

Following manufacturer's manual BIO-RAD (2000), the proteins from the 2 gels were transblotted to the PVDF membrane. Transblot method has been described in section 4.5.4, experiment 1B, page 134.

Experiment 3B. Serologic processing of the Membrane: The 2 membranes were washed twice in fresh PBST solution for a duration of 5 minutes each time. Blocking was achieved by immersing

membranes in PBST-Milk and agitated for 1 hour 30 minutes. Membrane 3B was incubated for 2 hours in serum from bovine cysticercosis positive cattle. Membrane 3C was incubated in serum from bovine cysticercosis negative cattle. Positive serum was prepared at a dilution factor of 1 part of primary antibody in 200 parts of PBST-Milk. Negative serum was prepared at a dilution factor of 1 part of serum in 300 parts of PBST-Milk. The membranes were incubated for 2 hours using HRP conjugated Rabbit anti-Bovine IgG (H+L) Secondary Antibody with approximate protein concentrations (IgG) of 1.0 mg/ml. Secondary antibody was diluted at a factor of 1 part of secondary antibody to 3000 parts of PBST-Milk.

SIGMAFAST™ BCIPO©/NBT (5-bromo-4-chloro-3-indolylphosphate/nitro blue tetrazolium) (APS) Tablets was used for colometric reaction. One Tablet, was dissolved in 10 ml of water to provide 10 ml of BCIP (0.15 mg/ml), NBT (0.30 mg/ml), Tris buffer (100 mM), and MgCl2 (5 mM), pH 9.25–9.75. This was poured over the membranes and agitated until a desired colour change was observed. The membranes were washed with PBST, dried and stored (figures 11, membrane 3B, page 145 and figure 12, membrane 3C, page 144).

Table 37: Quantities and volumes of samples and sample loading buffers loaded in experiments 1, 2 and 3

S/N	Type of Sample	Protein quantity (ng/µL)	Volume of Sample to achieve 10µg (µl)	Volume of Buffer to make up 20µl	Well Position
Quantities and volumes of samples and sample loading buffer for experiment 1					
1.	Protein Marker	----	7	---	Well 1
2.	100% Cyst fluid (Day 0)	Too high	4.0 (arbitrary but low)	16.0	Well 2
3.	30% Cyst fluid + 70% PBS (Day 0)	1124	8.9	11.1	Well 3
4.	30% Cyst shell + 70% PBS (Day 21)	2280	4.4	15.6	Well 4
5.	30% Cyst fluid + 40% Beef crush + 30% PBS (Day 0)	2200	4.5	15.5	Well 5
6.	50% Cyst fluid + 50% PBS (7 days)	2000	5.0	15.0	Well 6
7.	40% Cyst fluid + 60% PBS (14 days)	2320	4.3	15.7	Well 7
8.	50% cyst shell + 50% PBS	2220	4.5	15.5	Well 8
9.	Sample Loading Buffer	---	0.0	20.0	Well 9
10.	Sample Loading Buffer	---	0.0	20.0	Well 10
Quantities and volumes of samples and sample loading buffer for experiment 2					
1.	Protein Marker	----	7	---	Well 1
2.	Cyst fluid + 75% Water	4024	0.0	15	Well 2
3.	Cyst Only + 50% Water	2022	7.0	--	Well 3
4.	Cyst +Beef + 50% Water	NA	3.73	11.27	Well 4
5.	Cyst +Beef	1833	8.20	6.80	Well 5
6.	Beef Only + 50% Water	2184	6.88	8.12	Well 6
7.	Standard TMV	NA	7.43	7.57	Well 7
8.	Beef Only + 75% Water	NA	5.00	10.00	Well 8
9.	Sample Loading Buffer	---	0.0	15.00	Well 9
10.	Sample Loading Buffer	---	0.0	15.00	Well 10
Quantities and volumes of samples and sample loading buffer for experiment 3 (equal V/V: Sample/DTT)					
1.	Protein Marker	----	7	---	Well 1
2.	Calcified cyst +DTT (Day 21)	8000	18.75	18	Well 2
3.	Cyst Shell +DTT (Day 14)	8000	18.75	18	Well 3
4.	Crushed beef free from Cyst +DTT (Day 7)	7720	19.40	19	Well 4
5.	Crushed beef from Cyst Site +DTT (Day 7)	7760	19.40	19	Well 5
6.	Supernatant of cyst fluid + shell + PBS (Day 1)	7560	20.00	20	Well 6
7.	Supernatant of cyst +PBS (Day 7)	7760	19.40	19	Well 7
8.	Sediment of cyst shell + PBS (Day 1)	7720	19.40	19	Well 8
9.	Sediment of cyst +PBS (Day 1)	7640	19.30	19	Well 9
10.	Sediment of Beef + Beef +PBS (Day 1)	7720	19.40	19	Well 10

4.5.4 Results

4.5.4.1 Protein Quantification

Table 38 showing quantity of protein in cysts across days of freezing

S/N	Sample Number	Sample Description	Quantity of Protein ng/µL
1.	Sample 1	Supernatant Day 0	2250
2.	Sample 2	Supernatant Day 7	2184
3.	Sample 3	Supernatant Day 14	2180
4.	Sample 4	Supernatant Day 21	2140
5.	Sample 5	Supernatant Day 28	2000
6.	Sample 6	Sediments Day 0	2320
7.	Sample 7	Sediments Day 7	2200
8.	Sample 8	Sediments Day 14	2312
9.	Sample 9	Sediments Day 21	2250
10.	Sample 10	Sediments Day 28	2219

Freezing samples did not result in significant change in protein content of the samples. Highes protein quantity was observed in sample sediment, day 0; lowest protein quantity was noticed from sample supernatant frozen for 28 days at temperature -15°C.

4.5.4.2 Results of Experiment 1. Electrophoretic profiling of *C bovis* proteins

Electrophoresis of cysts fluid and cysts shell produced bands at 6kDa, 13kDa; 14kDa; 17kDa; 22kDa, 25kDa, 28kDa, 32kDa, 38kDa, 44kDa, 50kDa, 75kDa; 115kDa, 135kDa and 190kDa (table 39; page 147).

Western blot analysis of the proteins identified immunogenic proteins at 25 and 50kDa (table 39; page 147 Figure 7; page 141). Membrane treated with serum bovine cysticercosis negative cattle did not have protein bands (figure 8; page 142).

4.5.4.3 Result of Experiment 2

Across all wells, from all samples, protein brands were produced at 14, 17, 25, 32, 44, 50, 75, 100, 115 and 135kDa (figure 9; page 143). Tobacco Mosaic Virus (TMV) of a known weight of 17kDa was loaded into well 8. It served as a positive control; observing TMV at 17kDa mark validated appropriateness of protein markers and identified bands. TMV characteristically formed a dimer at 34kDa (figure 9; page 143).

4.5.4.4 Results of Experiment 3

Identified proteins bands following experiment 3 had weights of 6, 14, 25, 28, 30, 35, 44, 50, 62, 67, 75, 100, 115, 198kDa (table 39; page 147, figure 10; page 144). PDVF membrane M3B was treated with serum collected from cattle positive for cysticercosis. Identified immunogenic

proteins band formed at 14, 25, 50, 100 and 135kD (figure 11; page 145). PDVF membrane M3C which was treated with serum collected from cattle negative for cysticercosis showed no immunogenic proteins (figure 12; page 146).

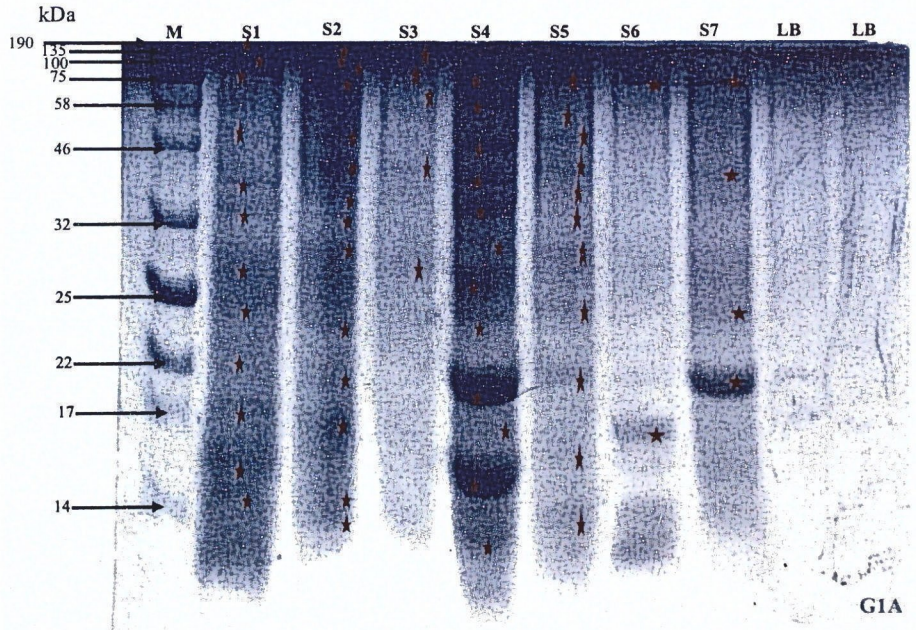
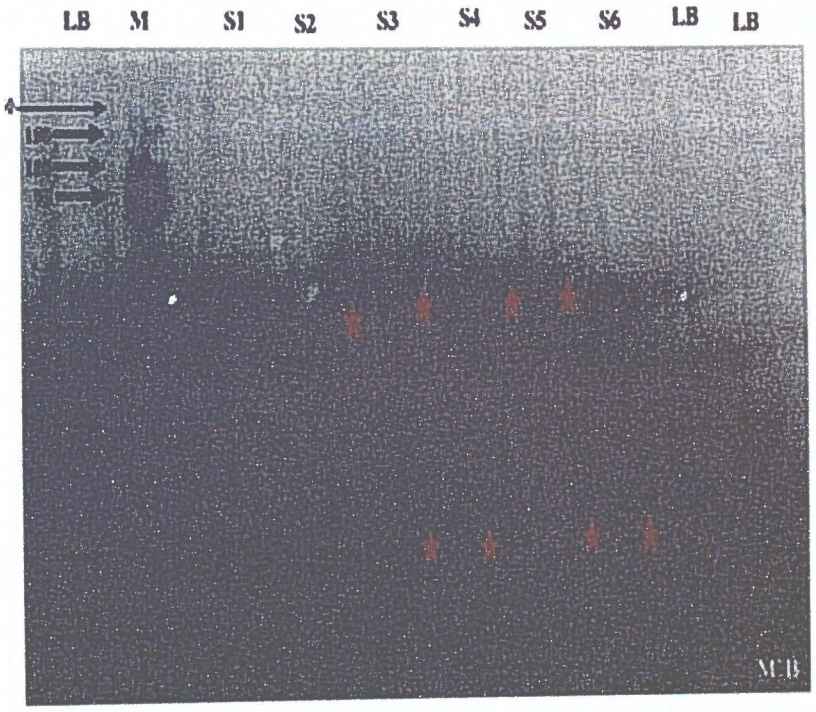


Figure 6b: bovine cysticercosis protein profile determined using one dimensional SDS-PAGE as in **experiment 1A**. *Taenia saginata* cysticercosis cysts from beef were prepared for electrophoresis and loaded in gel wells. *Taenia saginata* cysticercosis proteins were proteins identified at 6kDa, 13kDa; 14kDa; 17kDa; 22kDa, 25kDa, 28kDa, 32kDa, 38kDa, 44kDa, 50kDa, 75kDa; 115kDa, 135kDa and 190kDa in the different wells highlighted with the red star symbols

Key:
S1 = 100% Cyst fluid (Day 0)
S2 = 30% Cyst fluid + 70% PBS (Day 0)
S3 = 30% Cyst shell + 70% PBS (Day 21)
S4 = 30% Cyst fluid + 40% Beef crush + 30% PBS (Day 0)
S5 = 50% Cyst fluid + 50% PBS (Day 7)
S6 = 40% Cyst fluid + 60% PBS (Day 14)
S7 = 50% Cyst Shell Crush + 50% PBS
LB = Loading Buffer
LB = Loading Buffer



ure 7: Identification of immunogenic *Cysticercosis bovis* proteins by western blot as per experiment 1B. *Taenia ginata* cysticercosis proteins resolved by SDS-PAGE was transferred by transblot into PDVF membrane. The mbrane was probed with polyclonal serum from cattle positive for *Taenia saginata* cysticercosis. The membrane s incubated in Rabbit anti-Bovine lgG (H+L) secondary antibody conjugated to horse radish peroxidase. e Amplified Opti-4CN substrate colometric imaging was used. Immunogenic proteins are identified with red star nbol at 25kDa and 50kDa

y:
ell 2 = 100% Cyst fluid (Day 0)
ell 3 = 30% Cyst fluid + 70% PBS (Day 0)
ell 4 = 30% Cyst shell + 70% PBS (Day 21)
ell 5 = 30% Cyst fluid + 40% Beef crush + 30% PBS (Day 0)
ell 6 = 50% Cyst fluid + 50% PBS (Day 7)
ell 7 = 40% Cyst fluid + 60% PBS (Day 14)
ell 8 = 50% Cyst Shell Crush + 50% PBS

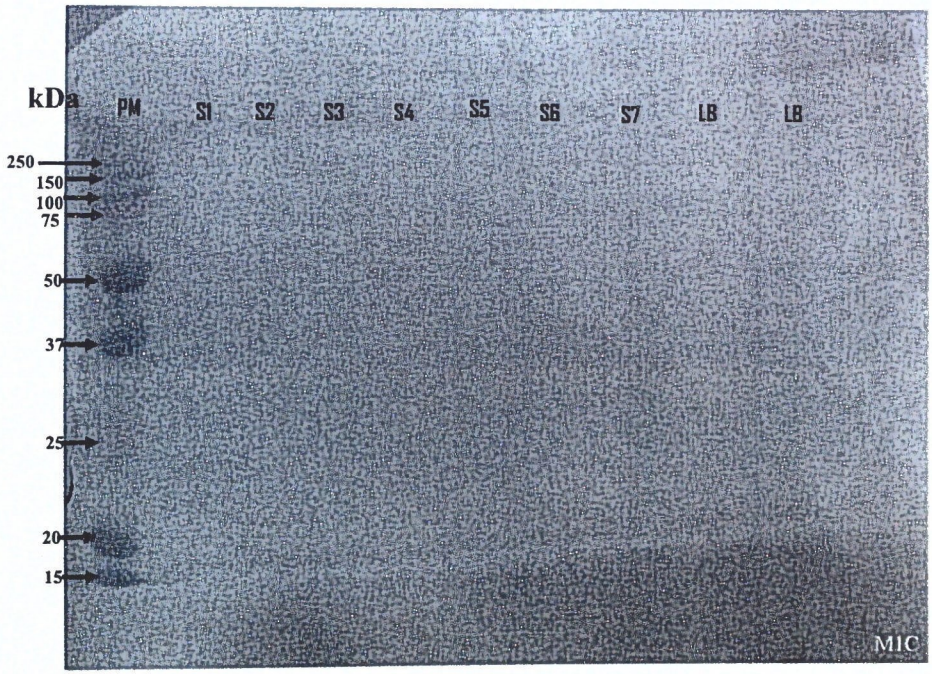


Figure 8: Identification of immunogenic *Taenia saginata* cysticercosis proteins by western blot as in experiment 1B. *Taenia saginata* cysticercosis proteins resolved by SDS-PAGE was transferred by transblot into PDVF membrane. The membrane was probed with polyclonal serum from cattle **negative** for *Taenia saginata* cysticercosis. The membrane was incubated in Rabbit anti-Bovine IgG (H+L) secondary antibody conjugated to horse radish peroxidase. **No immunogenic proteins observed**

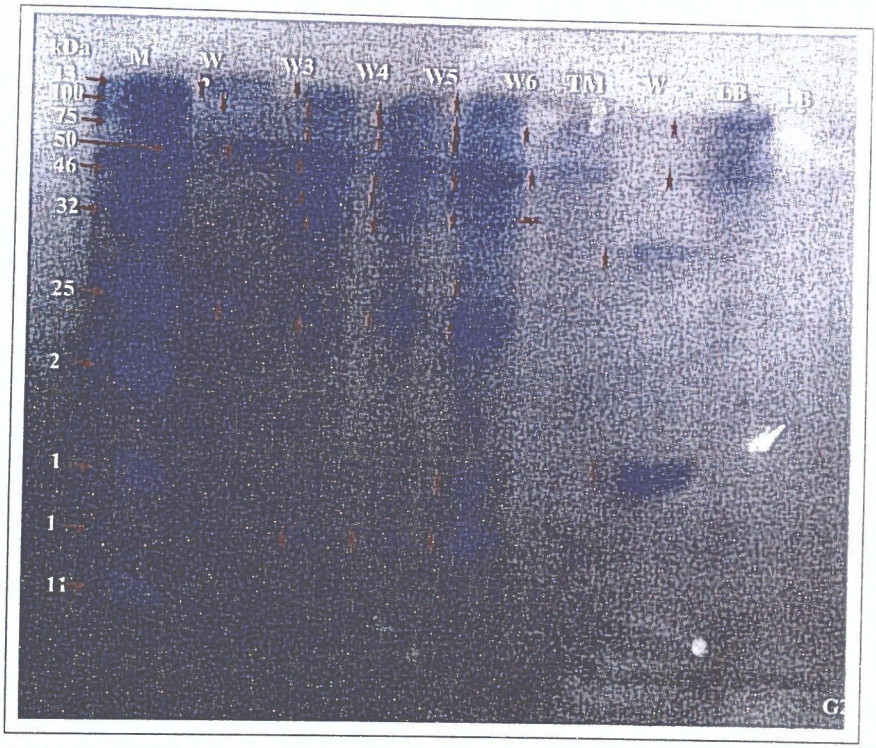


Figure 9: *Taenia saginata* cysticercosis protein profile determined using one-dimensional SDS-PAGE. *Taenia saginata* cysticercosis cysts from beef were prepared for electrophoresis and loaded in gel wells as in experiment 2. This image shows that replacing Tris-glycine SDS running buffer with MES running buffer produced gels with less noisy background. For *Taenia saginata* cysticercosis proteins were proteins identified at weights 14, 17, 25, 32, 44, 50, 75, 100, 115 and 135kDa. These are highlighted with red star symbols. Tobacco Mosaic Virus with a known weight of 17kDa was introduced in well to serve as a positive marker.

KEY:
W2: Cyst fluid +75% water
W3: cysts only +50% water
W4: Cyst + Beef + 50% water
W5: Beef +Cyst = full profile of protein
W6: Beef Only + 50%Water = faint P50; P25, P14
W7: TVM standard (P17) at forms dimer at P34
W8: Beef Only + 75% water
W9 and W10: Sample Loading Buffer

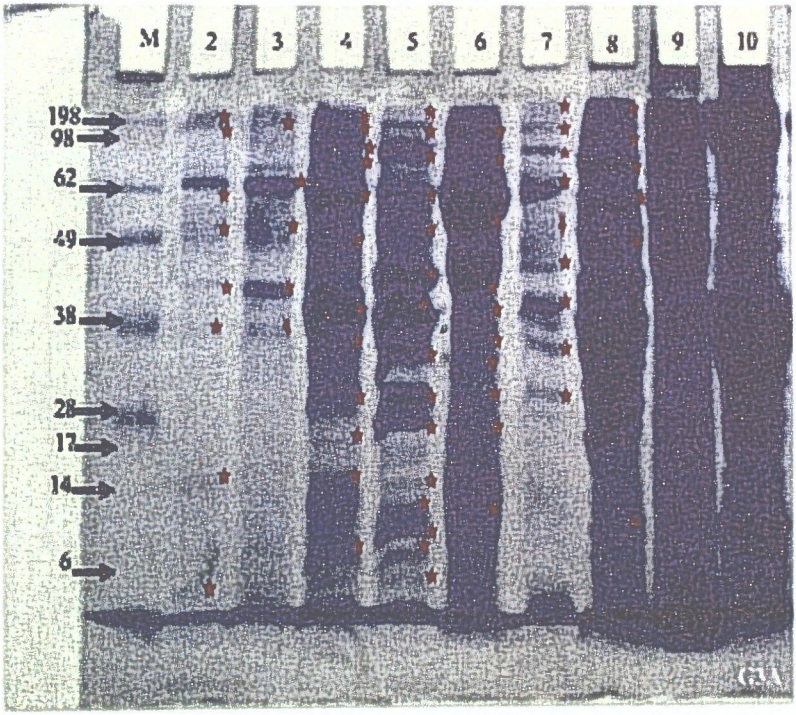


Figure 10: *Taenia saginata* cysticercosis protein profile determined using one dimensional SDS-PAGE using MES running buffer. *Taenia saginata* cysticercosis cysts from beef, which were prepared for electrophoresis and loaded in gel wells as demonstrated in experiment 3A
Taenia saginata cysticercosis proteins were proteins identified at weights 6, 14, 25, 28, 30, 35, 44, 50, 62, 67, 75, 100, 115, 198kDa. These proteins are highlighted with red star symbols.

KEY:

W2 = Calcified cyst +DTT (Day 21)
W3 = Cyst Shell +DTT (Day 14)
W4 = Crushed beef free from Cyst +DTT (Day 7)
W5 = Crushed beef from Cyst Site +DTT (Day 7)
W6 = Supernatant of Cyst fluid + shell + PBS (Day 1)
W7 = Supernatant of Cyst +PBS (Day 7) *
W8 = Sediment of Cyst shell + PBS (Day 1) **
W9 = Sediment of Cyst +PBS (Day 1) **
W10 = Sediment of Beef +PBS (Day 1)

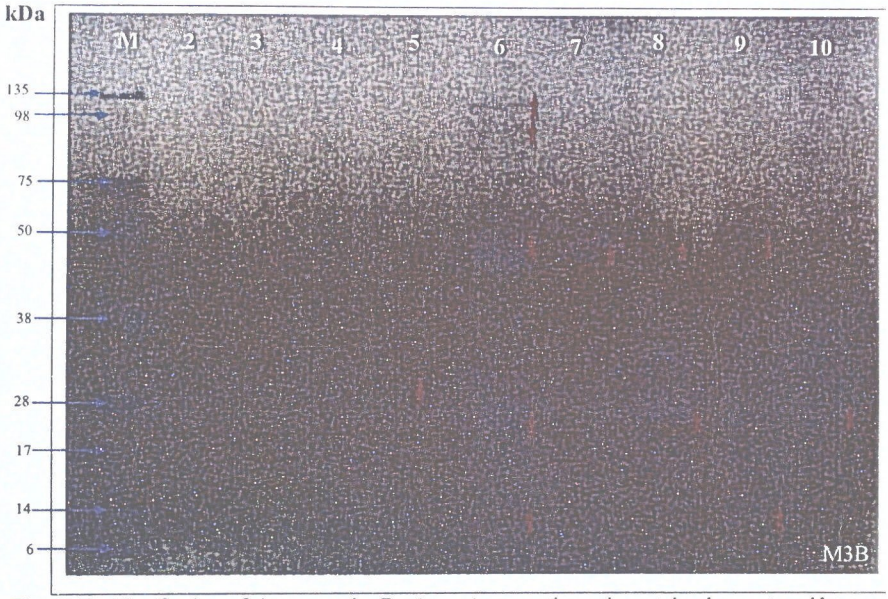


Figure 11: Identification of immunogenic *Taenia saginata* cysticercosis proteins by western blot as demonstrated in experiment 3B. *Taenia saginata* cysticercosis proteins resolved by SDS-PAGE was transferred by transblot into PDVF membrane. The membrane was probed with polyclonal serum from cattle positive for *Taenia saginata* cysticercosis. The membrane was incubated in Rabbit anti-Bovine IgG (H+L) secondary antibody conjugated to horse radish peroxidase. APS colometric tablet, was used. This showed higher sensitivity to immune-complexes than Amplified Opti-4CN substrate colometric imaging. Immunogenic proteins identified are 14kDa, 25kDa, 50kDa, 100kDa and 135kDa and highlighted with red star symbols. No immunogenic proteins were identified in well 2, 3 & 4 containing cyst chilled for 14 and 21days respectively and well 5 containing meat sample

KEY:

No Immunogenic protein were seen in wells

W2 = Calcified cyst +DTT (Day 21)

W3 = Cyst Shell +DTT (Day 14)

W4 = Crushed beef free from Cyst +DTT (Day 7)

W5 = Crushed beef from Cyst Site +DTT (Day 7)

Immunogenic proteins were found in

W6 = Supernatant of Cyst fluid + shell + PBS (Day 1)

W7 = Supernatant of Cyst +PBS (Day 7) *

W8 = Sediment of Cyst shell + PBS (Day 1) **

W9 = Sediment of Cyst +PBS (Day 1) **

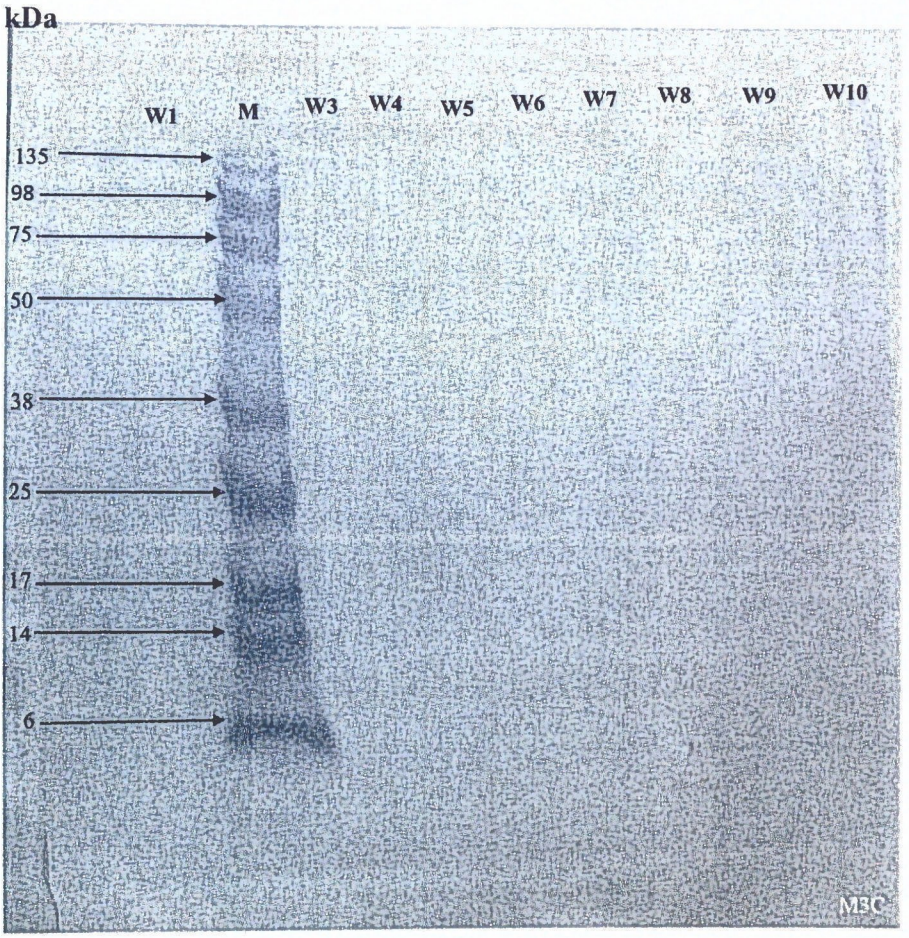


Figure 12: Identification of immunogenic *Taenia saginata* cysticercosis proteins by western blot as in experiment 3. *Taenia saginata* cysticercosis proteins resolved by SDS-PAGE was transferred by transblot into PDVF membrane. The membrane was probed with polyclonal serum from cattle **negative** for bovine cysticercosis. The membrane was incubated in Rabbit anti-Bovine IgG (H+L) secondary antibody conjugated to horse radish peroxidase. **No immunogenic proteins were observed**

Table 39 summary of protein profile available in gels arising from Experiment 1, 2 and 3

S/N	Type of Sample	Protein quantity (ng/μL)	Well No	Proteins available								
GEL NUMBER 1 Figure 1, 2 & 3												
1.	100% Cyst fluid (Day 0)	Too high	Well 2	135	100-115	98	75	34	32	25	17	14	
2.	30% Cyst fluid + 70% PBS (Day 0)	1124	Well 3	135	100 -115	75	50	44	38	28	22	17	14
3.	30% Cyst shell + 70% PBS (14 days)	2280	Well 4	100	75	50	25						
4.	30% Cyst fluid + 40% Beef crush + 30% PBS (Day 0)	2200	Well 5	75	50	44	32	28	25	23	17	14	6
5.	50% Cyst fluid + 50% PBS (7 days)	2000	Well 6	75	50	44	32	28	25	23	17	14	
6.	40% Cyst fluid + 60% PBS (21 days)	2320	Well 7	75	14								
7.	50% cyst shell + 50% PBS	2220	Well 8	75	50	25	22						
GEL NUMBER 2 Figure 4													
1.	Cyst fluid + 75 Water	4024	Well 2	135	110-115	75	50	25					
2.	Cyst Only + 50 Water	2022	Well 3	135	100-115	75	50	44	32	25	14		
3.	Cyst +Beef + 50 Water	NA	Well 4	115	75	50	44	32	25	14			
4.	Cyst +Beef	1833	Well 5	135	100-115	75	50	32	25	17	14		
5.	Beef Only + 50 Water	2184	Well 6	100	50	44							
6.	Standard TMV	NA	Well 7	32	17								
7.	Beef Only + 75 Water	NA	Well 8	115	50								
GEL NUMBER 3 Figure 5, 6 & 7													
1.	Calcified cyst +DTT (Day 21)	8000	Well 2	198	135	100	75	67	50	44	38	14	6
2.	Cyst Shell +DTT (Day 14)	8000	Well 3	135	115	67	50	44	38				
3.	Crushed beef from Cyst Site +DTT (Day 7)	7720	Well 4	198	115	100	62	50	38	35	28	14	6
4.	Crushed beef free from Cyst +DTT (Day 7)	7760	Well 5	198	115	98	62	50	35	28			
5.	Supernatant of cyst fluid + shell +PBS (Day 1)	7560	Well 6	100	75	50	44	25	17	14			
6.	Supernatant of cyst + PBS (Day 7)	7760	Well 7	198	100	75	67	50	44	38	35	30	14
7.	Supernatant of Bee + PBS (Day 7)	7720	Well 8	198	100	75	67	50	44	38	35	30	14
8.	Sediment of Cyst +PBS (Day 1)	7640	Well 9	198	100	75	67	50	44	38	35	30	14
9.	Sediment of Beef +PBS (Day 1)	7720	Well 10	198	100	75	67	50	44	38	35	30	14

4.5.5 Experiment 4: Confirmation of observed immunogenic complexes as specific for *Taenia saginata* cysticercosis antigen-antibody reaction through western blot using serum from Rabbit inoculated with bovine cysticercosis immunogens

4.5.5.1 Background

In conventional *Taenia saginata* cysticercosis western blot, a transblotted membrane is treated with bovine serum positive for cysticercosis. This serves as a source of primary antibody. The membrane is further treated with Rabbit anti-Bovine IgG (H+L) Secondary Antibody HRP. However, bovine serum could contain non-cysticercus primary antibodies which are capable of forming misleading non-specific immune-complex with the *Taenia saginata* cysticercosis antigen. This false positive can be eliminated by treating membrane with serum containing antibodies specific for bovine cysticercosis.

When an emulsion of immunogens made from bovine cysticercosis cyst is introduced into sterile rabbit, the rabbit develops antibodies specific for the bovine cysticercosis antigen after 14 days (Leennars and Hendriksen, 2005).. Carrying out a western blot using this serum as source of primary antibody and using Goat anti-Rabbit IgG (H+L) Secondary Antibody HRP would produce antibody-antigen complex specific for *Taenia saginata* cysticercosis

The objective of this study was to form immune-complexes using rabbit serum treated with Goat anti-Rabbit IgG (H+L) Secondary Antibody HRP and comparing same with immune-complexes formed using bovine serum.

4.5.5.2 Materials and Methods

Adjuvant mixtures were injected into rabbit to induce immune response. Adjuvant (antigen) mixtures were produced by mixing adjuvant with either crushed *Taenia saginata* cysticercosis whole cyst or cyst fluid. Following Toth, *et. al.*, (1989) protocol first dose of immunogens emulsion was produced by mixing **complete adjuvant** with crushed *Taenia saginata* cysticercosis whole cyst or cyst fluid. Second (boost) dose adjuvant was produced by mixing **incomplete adjuvants** with crushed *Taenia saginata* cysticercosis whole cyst or cyst fluid.

4.5.5.1.1 Laboratory animal

Five sterile rabbits, 6 months old were used. One rabbit, untreated with immunogens was held as negative control and the other four received different concentrations of the adjuvants. Different concentrations of adjuvants were administered to the rabbits in order to determine relationship between concentration of adjuvants and time of antibodies production.

4.5.5.1.2 Preparation of antigen mixture

4.5.5.1.2.1 **Cysts Fluid:** Cyst fluid was collected by puncturing the cysts and the fluid along with the protoscolices drained into a Nunc® Cryotube. Fluid was centrifuged at speed of 10,000rpm at 4°C for 3 minutes. The supernatant was separated from the sediments into different Nunc® Cryotube.

4.5.5.1.2.2 **Whole Cysts:** Whole cyst samples were prepared by crushing cysts under pressure using mortar and pestle. Crushed cyst was mixed using phosphate buffered saline (PBS). The mixture was centrifuged at speed of 10,000 rpm at 4°C for 3 minutes. The supernatant was separated from the sediments into separate Nunc® Cryotube. All samples were used on day (1) of preparation

4.5.5.1.3 Volumes and administration of Adjuvant-Antigen

4.5.5.1.3.1 Complete Freund Adjuvant-Antigen Emulsion

Mixing ration: 1ml of complete adjuvant was mixed with 1ml of antigen mixture according to protocol recommended by Jackson & Fox (1995) and Petrovsky (2015).

Volume administered: 0.25ml of the adjuvant-antigen emulsion was administered to each rabbit via the subcutaneous route.

4.5.5.1.3.2 **Booster/Incomplete Freund Adjuvant**

Mixing ration: 1ml of incomplete adjuvant was mixed with 1ml of antigen mixture according to protocol recommended by Jackson & Fox (1995) and Petrovsky (2015).

Volume administered: 0.25ml of the adjuvant-antigen emulsion was administered to each rabbit via the subcutaneous route according to protocol recommended by Broderson (1998). Booster doses were administered at intervals of 2 weeks according to protocol recommended by Grumpstrup-Scott & Greenhouse (1988) and Stills & Bailey (1991).

4.5.5.1.4 Collection of Blood and production of Serum

Negative Control Serum: serum was collected from negative control rabbits that did not receive immunogen mixture.

Positive Serum: The complete adjuvant was administered on day 1. Booster doses of adjuvant prepared using incomplete adjuvants were administered day 14 and day 28. Blood was collected weekly from the rabbits using direct cardiac puncture. Serum was collected from blood by allowing blood to stand under room temperature. The collected serum was further centrifuged at revolutions of 10,000 rpm at 4°C for 5 minutes. Clear serum sample was collected and aliquots stored at room temperature until when used. Serum from Rabbit was used as source of primary antibody for serological test of membranes

4.5.5.3 *Experiment 4A: Gel Electrophoresis*

The gel electrophoresis was carried out as described in experiments 1 (section 4.5.4) and 2 (4.5.5). Quantities and volumes of samples and loading buffer were worked out as shown in Table 40.

4.5.5.4 Transblot of membrane

Transblot process was carried out as described in experiment 3 (4.5.6) using two PVDF membranes marked as M4B (figure 14, page 153) and M4C (figure 15, page 154).

4.5.5.5 Experiment 4B. Serologic processing of the Membrane:

The 2 membranes designated as 4A and 4B were washed twice in fresh PBST solution for a duration of 5 minutes each time. Blocking was achieved by immersing membranes in PBST-Milk, gently agitated using a rocking platform and incubated for 1 hour 30 minutes. Membrane M4B was incubated for 2 hours in primary antibody contained in serum collected from *Taenia saginata* cysticercosis treated rabbit. Membrane M4C was incubated in serum collected from rabbit not treated with immunogen emulsion, thus *Taenia saginata* cysticercosis negative. Primary antibody was prepared at a dilution factor of 1 part of primary antibody in 100 parts of PBST-Milk for positive serum and a dilution factor of 1 part of serum in 200 parts of PBST-Milk for serum negative of *Taenia saginata* cysticercosis. The membranes were incubated for 2 hours using the Goat anti-Rabbit IgG (H+L) Secondary Antibody HRP at approximate Protein concentrations (IgG) of 1.0 mg/ml at a dilution factor of 1 part of secondary antibody to 3000 parts of PBST-Milk.

The SIGMAFAST™ BCIPO©/NBT (5-bromo-4-chloro-3-indolylphosphate/nitro blue tetrazolium) (APS) tablet was used for colorimetric reaction. One quarter of a tablet was dissolved in 10 ml of water. This mixture was poured over membranes and agitated until desired colour change was obvious. Membranes was washed with PBST, dried and photographed (M4B [figure 14, page 153] and M4C [figure 15, page 154]).

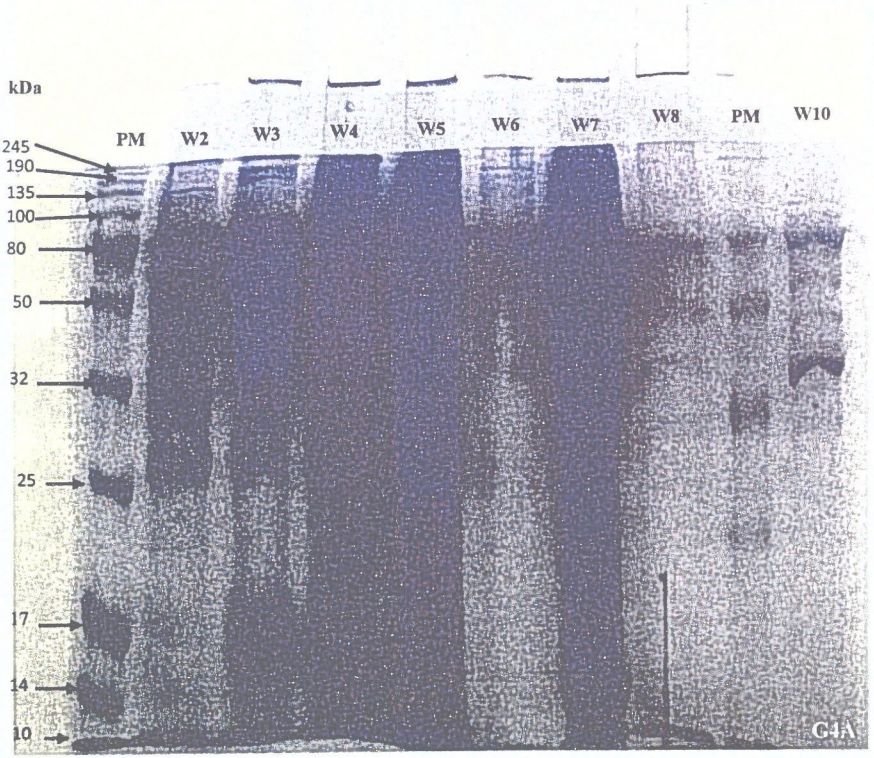


Figure 13: *Taenia saginata* cysticercosis protein profile determined using one dimensional SDS-PAGE. *Taenia saginata* cysticercosis cysts from beef, which were prepared for electrophoresis and loaded in gel wells as demonstrated in experiment 4A above. The MES running buffer was changed to Glycine running Buffer

KEY:
W2: Cyst filtrate Day 7
W3: Cyst Sediment Day 21
W4: Beef Filtrate
W5: Whole Cyst Sediment
W6: Cyst Fluid Day 0
W7: Beef Sediment Day 0
W8: Whole Cyst Filtrate
W9: Protein Marker 2
W10: Beef

Table 40: Quantities and volumes of samples and sample loading buffer for experiment 4

Type of Sample	Protein quantity (µg/µL)	Well No	Proteins weights in gel											
Cyst filtrate Day 7	769	Well 2	245	190	150	135	115	100	80	75	50	25	22	
Cyst Sediment Day 21	804	Well 3	245	190	150	135	115	100	75	50	32	28	25	17
Beef Filtrate	800	Well 4	150	115	100	75	32							
Whole Cyst Sediment	748	Well 5	245	190	150	135	115	100	80	75	50	25	17	14
Cyst Filtrate Day 0	804	Well 6	245	190	150	135	115	100	32					
Beef Sediment Day 7	808	Well 7	245	190	150	100	80		75	17				
Whole Cyst Filtrate	789	Well 8	245	190	150	135	115	100	80	75	50	32		
Marker	–	Well 9	Marker											
Beef	800	Well 10	245	190	150	115	100	80			32			

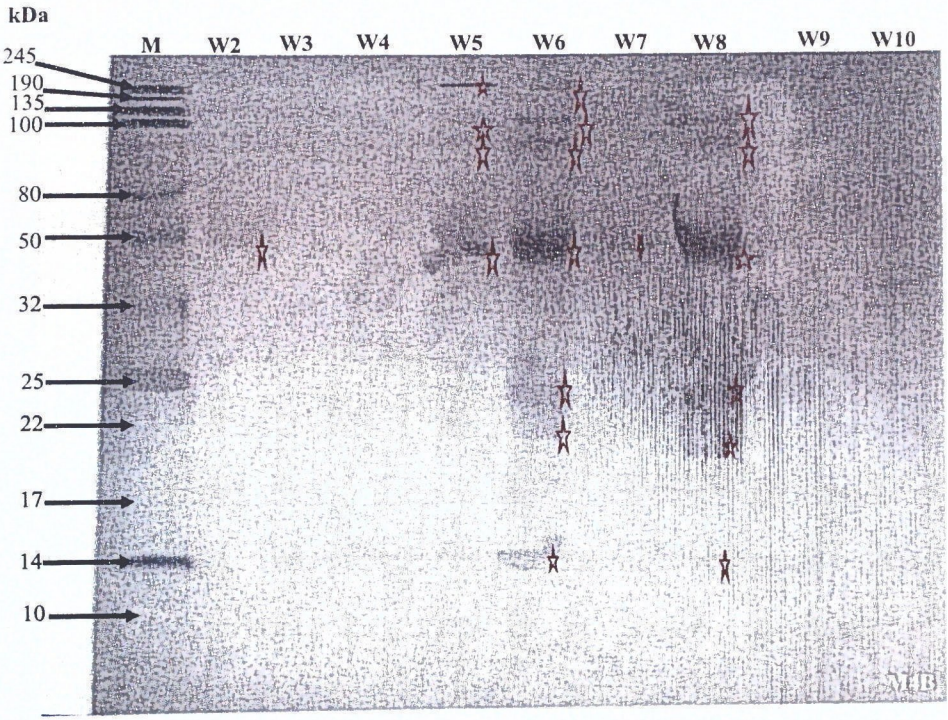


Figure 14. Western blot identifying *Taenia saginata* cysticercosis immunogenic proteins. *Taenia saginata* cysticercosis proteins resolved by SDS-PAGE was transferred by transblot into PDVF membrane. The membrane was probed with polyclonal serum from rabbit inoculated with *Taenia saginata* cysticercosis protein emulsified in Complete Freund's Adjuvant. The membrane M4B, was incubated in Goat anti-Rabbit IgG (H+L) secondary antibody conjugated to horse radish peroxidase. APS colometric tablet was used. Experiment 4B above.

Identified immunogenic proteins have weights of 14, 22, 25, 50, 100, 135, 190 and 245kDa. These are highlighted with the red star symbol. No immunogenic proteins were identified in well 3 containing cyst after 21 days and well 4 containing meat sample

KEY:

Immunogenic proteins found in:
W2: Cyst filtrate Day 7
W5: Whole Cyst Sediment
W6: Cyst Fluid Day 0
W8: Whole Cyst Filtrate

Immunogenic proteins not found in:
W3: Cyst Sediment Day 21
W4: Beef Filtrate
W7: Beef Sediment Day 0
W9: Protein Marker 2
W10: Beef

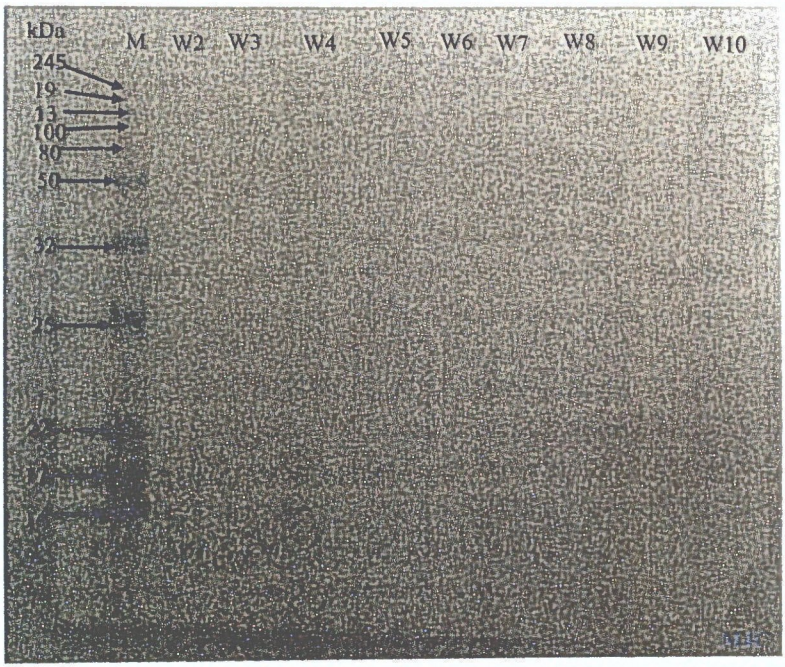


Figure15: Identification of immunogenic *Taenia saginata* cysticercosis proteins by western blot. *Taenia saginata* cysticercosis proteins resolved by SDS-PAGE was transferred by transblot into PDVF membrane. The membrane M4C, was probed with serum collected from rabbit at day 0 before treatment with antigen of *Taenia saginata* cysticercosis. The membrane was incubated in Goat anti-Rabbit IgG (H+L) secondary antibody conjugated to horse radish peroxidase. APS colometric tablet was used.
No Immunogenic proteins were found in all the wells in PVDF membrane treated

4.5.6 Discussion on profiling of *Taenia saginata* cysticercosis proteins and identification of immunogenic ones

All cysts and beef samples utilized in this research were collected from carcasses of naturally infested cattle slaughtered at the Multi Specie Abattoir (MSAB) Gaborone, Botswana. Samples were collected from MSAB, which was purposively selected because of its close proximity to the research laboratory at the Botswana Vaccine Institute (BVI). Secondly, this abattoir practices adequate meat inspection under the supervision of the Official Veterinary Surgeon (OVS) in keeping with provisions of Livestock and Meat Industries Act (2007).

Most available protein markers measure eleven (11) distinct protein weights thus, running an electrophoresis using one markers allows profiling of 11 to 13 proteins (Dharmawan *et. al.*, 2013). This work profiled nineteen (19) proteins by using four protein markers cumulatively covering weights from 4kDa to 250kDa. Identified proteins have molecular weights of 4, 6, 14, 17, 22, 25, 28, 32, 38, 44, 50, 67, 75, 100, 115, 135, 150, 190 and 245kDa. There are similarities in molecular weights of identified proteins with the work of Dharmawan *et. al.*, (2013) who detected proteins with the molecular weights ranging from 14.86 kDa to 122.40 kDa. Dharmawan *et. al.*, (2013) utilized one protein marker with detection range of 10-130 kDa.

At recommended dilution factor of 1:100 for primary antibody and 1:1000 for secondary antibody (Iqbal & Ahmad, 2017), two (2) immunogenic proteins were detected. These proteins are 25kDa and 50kDa. Higher serologic sensitivity was achieved by increasing dilution factor of primary antibody from 1:100 to 1:500 and dilution factor of secondary antibody from 1:1000 to 1:3000 as well as replacing nitrocellulose membrane with PVDF membrane and replacing Amplified Opti-4CN substrate coloration with SIGMAFASTä BCIPÒ /NBT (5-bromo-4-chloro-3-indolyl phosphate/nitro blue tetrazolium) tablets. Five (5) immunogenic proteins were identified. These are 14kDa, 25kDa, 50kDa, 100kDa and 135kDa.

Serum of infected cattle was used as source of primary antibody because the cysts are known to elicit both humoral and cell-mediated antibody reaction in the animal. However, this serum could contain non-specific IgG antibodies elicited by for non-*Taenia saginata* cysticercosis antigen from the same species. To avert error due to non-specific immunogenic complexes, arising from using serum of naturally infested cattle, rabbit sera containing primary antibody specific for *Taenia saginata* cysticercosis was treated with Goat anti-Rabbit IgG (H+L) Secondary Antibody HRP to

produce antibody-antigen complex specific for *Taenia saginata* cysticercosis. This experiment yielded specific antibody-antigen complexes, and identified three (3) more immunogenic proteins. The eight (8) identified immunogenic proteins were 14, 22, 25, 50, 100, 135, 190 and 245kDa. These findings and the weights of the immunogenic proteins agree with the findings of Guimaraes *et. al.*, (2018), who recorded TSA16, TSA18, TSA25 and TsP36 as immunogenic proteins of bovine cysticercosis, with TSA25 as most immunogenic fit for serological diagnosis of cysticercosis. Similarly, Abuseir *et. al.*, (2013) recorded 260, 150, 130, 67, 60, 55, 50, and 23 kDa as immunoreactive with known positive sera of *Taenia saginata* cysticercosis-infested cattle. Two immunogenic proteins identified in this work were also identified by Abuseir *et. al.*, (2013); these are 23kDa and 50kDa. Dharmawan *et. al.*, (2013) also detected 7 immunogenic proteins with the molecular weights of 16.81 kDa; 19.22 kDa; 20.98 kDa; 27.41 kDa; 34.02 kDa; 38.31 kDa; and 54.94 kDa.

All three authors (Abuseir, et. al., 2013; Dharmawan *et. al.*, 2013; Guimaraes *et. al.*, 2018), identified P25 and P50 as immunogenic proteins of *Taenia saginata* cysticercosis. This study identified P25 and P50 in all 3 membranes of immunoblotting experiment. Abuseir *et. al.*, (2013) identified 23kDa and Dharmawan *et. al.*, (2013) identified 27.41kDa instead of 25kDa. The slight differences in weights between these studies may be due to differences in the markers used. But it is obvious that the identified proteins fall within range of 23-27kDa.

Membranes treated with serum collected from *Taenia saginata* cysticercosis negative cattle and incubated with Rabbit anti-Bovine IgG (H+L) secondary antibody conjugated to horse radish peroxidase did not show antibody-antigen complex. Similarly, membrane treated with serum collected from rabbits injected with adjuvants alone and incubated with Goat anti-Rabbit IgG (H+L) secondary antibody showed no antibody-antigen complex specific for *Taenia saginata* cysticercosis. These results indicate absence of primary antibody in sera collected from *Taenia saginata* cysticercosis negative cattle and rabbit.

Nitrocellulose membrane with a pore size of 0.45μm was selected for western blot because the pore size is suited for most western blotting and because it produces less background noise

PBS was more ideal for washing off non-specific proteins from nitrocellulose membrane. But Tris-Buffered Saline (TBS) was preferred for the PVDF membrane, particularly when studying protein phosphorylation as 'P' in PBS may affect the results.

Non-Fat Dry Milk (NFDM) was preferred to Bovine Serum Albumin (BSA) because it is cheaper and faster to prepare. NFDM reduces background noise because it has less binding, but this property can prevent antigen recognition. Sahu, (2017) argues that casein present in the NFDM (milk) may react with antibody when working with phosphor-antibodies thus, debarring the actual intended signals. NFDM is contraindicated when working with Streptavidin-biotin-based systems. Koschembahr (2015) combined NFDM and BSA to get good signals and recommends same.

Freezing caused no significant drop in quantities of proteins in meat and cyst samples frozen at -15^{0}C across days, but these samples did not demonstrate immunogenic proteins after 7 days of freezing. This finding suggests loss of vitality of cysts between 7 to 14 days. This finding agrees with Ranson (1914) and Georgsson *et, al.*, (2006), who states that *Taenia saginata* cysticercosis loss of vitality does not result from actual depletion of proteins rather may be due to attenuation, denaturing and/or decomposition of these proteins. It is probable that freezing the parasite beyond 7 days re-assorts the genome makeup of the protein thus interfering with the parasite's ability to infest or elicit immune response from its host. This vital validates the recommended carcass treatment by the Meat and Livestock industries Act, (2007).

Meat crush supernatants and sediments were included as negative controls in the electrophoresis; for example, in experiment 2; section 4.5.5 (figure 9, well 5, 6 & 7; page 143) and experiment 3; section 4.5.6 (figure 10, well 8 and 10; page 144). This helped in preliminary differentiation between the cyst protein and beef protein and to corroborate with the standard protein marker. Sreedevi, *et. al.*, (2011) studied sero-diagnosis of porcine cysticercosis and showed differences in specificity and sensitivity between the cyst fluid antigens (CFA) and whole cyst antigens (WCA) of *Taenia solium* metacestode. This work showed similarity in protein profiles in bovine cysticercosis cyst fluid and whole cyst. Cysts fluid and whole cyst shared some proteins in common with the beef sample however, some of the proteins in the beef were missing in the cyst samples and vice versa. Importantly, immunogenic proteins found in the cysts were not present in beef samples. P25 was detected in meat samples collected proximal to cyst infested sites, as shown in experiment 3, well 5 figure 9. Presence of P25 in meat sample may be a contamination from the cyst.

4.5.7 CONCLUSION

One-dimensional electrophoresis of *Taenia saginata* cysticercosis using Sodium dodecyl sulphate Polyacrylamide electrophoresis (SD-PAGE) identified proteins with molecular weights of 4, 6, 14, 17, 22, 25, 28, 32, 38, 44, 50, 67, 75, 100, 115, 135, 150, 190 and 245kDa.

Immunoblotting under reducing conditions used bovine polyclonal antibody and Rabbit anti-Bovine IgG (H+L) Secondary Antibody conjugated with Horse Radish Peroxidase (HRP) to challenge antigens derived from cysts fluid and whole cysts of *Taenia saginata* cysticercosis. Identified immunogenic proteins were 14, 22, 25, 50, 98, 135, 190 and 245kDa. These proteins were further confirmed to be immunogenic specific for *Taenia saginata* cysticercosis by treating transblotted membrane with rabbit serum, containing antibodies specific for *Taenia saginata* cysticercosis and Goat anti-Rabbit IgG (H+L) Secondary Antibody Horse Radish Peroxidase (HRP). Identified immunogenic proteins were 14, 22, 25, 50, 98, 135, 190 and 245kDa. Freezing samples did not cause significant difference ($P<0.05$) in proteins quantity contained in samples however, immunogenic proteins were lost after 7 days. Protein sequencing is required to identify immune-dominant epitopes and to go a step closer to vaccine development

4.5.8 RECOMMENDATIONS

This research recommends further purification and characterization of these identified eight 8 proteins as, these may be possible vaccine candidates for the preparation of vaccine against bovine cysticercosis. A ponceau-stained nitrocellulose gel can be run to compare proteins with Nitrocellulose.

About P500 million (10.56 pula = 1USD) is lost annually in Botswana due to detention and/or condemnation of infested cattle. Ability to detect infested cattle at ante-mortem inspection and non-slaughter of infested cattle will avert this financial loss. This study recommends further purification of proteins of interest and possibly, preparation of monoclonal antibodies specific for bovine cysticercosis which may serve as reagents in an ante-mortem test kit.

CHAPTER FIVE

1.1 GENERAL CONCLUSIONS

Most government data have attributed the decline in Botswana's cattle population to unthrifty climatic conditions, unavailability of feed, poor funding of the agricultural sector due to change of government policies favouring non-agricultural sectors of the economy etc. However, the study on demography of cattle and cattle farmers in Botswana in this research has shown that politics, sociocultural, and gender related issues have more of an effect on cattle ownership and population than the aforementioned. Cattle ownership is a demonstration of "male power"; thus, although women and young adults provide majority of the manpower in the cattle industry, there are more male cattle owners than female cattle owners. Male farmers owned larger farms and stayed longer in the farming business than their female counterparts. The Land Act of 1968 that encouraged male dominance in land tenure was amended in 1993 and finally abolished in 2004 through the Abolition of Marital Power Act. A consequence of this legal reform is that women can legally own (or use) land and cattle. However, this study shows that poor implementation of this legal reform leaves room for gender inequality and discrimination against women in the land tenure system, cattle ownership and cattle inheritance. It is implied that since a number of rich white Afrikaans women own large cattle farms and run viable businesses in Ghanzi,, the lack of local female contributors to the cattle industry illustrates how gender discrimination is affecting the cattle industry. This is a deficit in achieving the fifth (5th) sustainable development goals (SDG), which is to achieve gender equality and empower all women and girls. Most populous breeds of cattle in their order of popularity are Tswana, Brahman, then Simmental etc. Although Botswana is the highest beef exporter to the EU, the country imports most of its consumed milk due to the fact that less than 5% of farmers are involved in dairy farming. The high temperatures and low rainfall do not support thriftiness of the dairy animals and feed for dairy cattle. Beef cattle (both exotic and local) are more adapted than dairy cattle. Advancing the dairy industry as an alternative source of household food and income facilitates achievement of 2nd SDG, which is to end hunger, achieve food security and improved nutrition and promote sustainable agriculture. All farmers owned cattle along with other farm animals (livestock). Ghanzi district in the Western region owned the highest cattle population and the highest largescale farms while the North East district in Central region owned the least population and the least number of largescale farms. Despite government efforts to

encourage more citizen participation in cattle farming, cattle farming has dropped over the years, particularly among young people and retirees. Encouraging more retirees and youth participation in cattle farming will help revive the cattle industry as well as to achieve SDG 8, which is to promote sustained, inclusive and sustainable economic growth, full and productive employment and decent work for all. Nonetheless, citizen participation in cattle farming is higher in Botswana than in most other African countries because it seemingly bestows a sense of pride among citizens. Due to a high literacy level, the adoption of cutting edge technology and access to credit is relatively easy and the effects of this would be increased and sustained productivity. This makes for achievement of SDG 12, which is to ensure sustainable consumption and production patterns.

Available published prevalence statistics of bovine cysticercosis in Botswana emanate mainly from records available at the Botswana Meat Commission (BMC), the country's national export abattoir. Although BMC slaughters 44% of Botswana's annual cattle slaughter, prevalence data arising from BMC does not reflect prevalence from lower throughput abattoirs and potential hotspots. Thus, reporting national prevalence rate using solely BMC statistics may not be very informative and reflective of the bigger picture. This study probed prevalence of bovine cysticercosis using a cross-sectional study through passive abattoir inspection; covering a wider scope (more regions) and some lower throughput abattoirs previously not accounted for. Furthermore, non-participatory interview using structured questionnaires was employed to elicit prevalence information directly from the farmers. Prevalence arising from the survey was used to compare and query results from the statutory (traditional) passive abattoir method. An analysis of the results showed an abattoir prevalence of 17.17% and the questionnaire method resulted in 42.35%; both of which are higher than the published prevalence of 13.5% and at 0.025 ($P<0.05$) are significantly different from each other.

Unlike the abattoir meat inspection, which is passive and investigates only the major and government abattoirs, the survey method pro-actively investigates across boards. Additionally, the survey methods investigated private abattoirs; slaughter houses and the individual farmers. Thus, although both data originate from the same population, the questionnaire (survey) method is more holistic than the passive abattoir method. At a significant score of 0.000 (P 0.05), there is a significant difference in the prevalence (means) among the sampled abattoirs. Thus, the proportion of cattle with bovine cysticercosis in Botswana is significant. It also shows that there is a significant difference between the BMC and published prevalence and the prevalence obtained

through this study. This disparity holds and equally calls for a holistic prevalence study since it is apparent that relying on BMC results alone is not representative of cysticercosis prevalence in Botswana. Although some meat premises showed higher prevalence than both the BMC and national prevalence, only the BMC prevalence is significantly different from the published prevalence ($P < 0.05$); the reason being that most meat premises having high prevalence posseses small sample sizes. The hotspots of cysticercosis are homogenously distributed across districts and regions, as both high and low prevalence scores were obtained from abattoirs within the same district or region. Across the board an identifiable pattern was that the prevalence is generally higher in the rural and poorer communities than in the urban and elite communities even within the same district. This work showed that categorizing a district as a hotspot based solely on the prevalence records obtained at the abattoir may be erroneous. Cattle slaughtered in an abattoir may be reared and purchased from another district or region, hence bovine cysticercosis could be imported. Thus, in the assessment of cysticercosis hotspots, an efficient trace back mechanism is important to trace the carcass back to its original source. Consequently, this study determined the prevalence of bovine cysticercosis as well as demonstrated its hotspots in Botswana. This study provided data that can guide location-targeted prevention and control efforts to achieve reduced bovine cysticercosis in animal and *T. saginata* taeniansis in man and ensure food security. These contributes to achieving SDG, which is to end hunger, achieve food security and improved nutrition and promote sustainable agriculture, and SDG 3, which is to ensure healthy lives and promote well-being for all at all ages. Although the time series analysis of prevalence of cysticercosis does not show a consistent pattern, it does appear that there is a correlation between annual temperature and prevalence of cysticercosis. Highest cysticercosis prevalence was noticed between 2012 and 2014 with prevalence of 18% and 20% respectively. The probable explanation for the spike in prevalence is that Botswana experienced drought between 2010-2012. Drought leads to hunger and starvation, both of which cause immunosuppression, germ recrudescence; that in turn allows worm burden to establish easily. Scavenging arising from scarcity of pasture increases contact exposure of cattle to oocysts of *Taenia saginata* cysticercosis contained in human feces.

Prevalence results obtained from the questionnaire investigation are higher than abattoir prevalence. There is significant difference between prevalence in Kalagadi and the other three

districts but not between Ghanzi and North East or North East and Central. The results emanating from this methodological approach have provided a more all-inclusive and reliable prevalence rate. Despite several prevention, control and intervention measures practiced by the government of Botswana, the prevalence of bovine cysticercosis increases persistently. Similarly, the differences in prevalence within and among districts informed the need to study the lifestyles of the indigenes as possible risk factors for cysticercosis. It was apparent that none of these strategies had sufficed in reducing incidences of bovine cysticercosis. It is probable that the main underlying risk factors of bovine cysticercosis in Botswana had been misdiagnosed or that the adopted control and prevention methods have not been effective in reducing the prevalence. Although these risk factors are behavioural, systemic and progressive, there is no known work that associates these risk factors with the lifestyle of residents of Botswana and climatic changes as a means corelating life style with persistent prevalence of bovine cysticercosis and also to uncover novel risk factors of bovine cysticercosis in Botswana. This work, in addition to studying patterns of literature-documented risk factors in Botswana, identified novel risk factors of bovine cysticercosis. Identified novel risk factors are political, behavioural, systemic and sociocultural. Of the eighteen (18) tested risk factors, 14 showed to contribute significantly ($P<0.05$) to the prevalence of bovine cysticercosis., with 'access to contaminated feed being the single most important risk factor'. However, there is strong agreement (high consensus) among respondents that the factors of, 'failure to deworm herd boys'; 'access to contaminated feed'; 'access to contaminated water'; 'proximity to uncontrolled human defecation'; 'access to contaminated pasture'; 'grazing of animal'; 'absence and or distanced pit latrine in the farm' are major contributors to the prevalence of bovine cysticercosis. A/ The Seasonal index study revealed some novel risk factors; among them include: close association between increased bovine cysticercosis incidences and **outset of rainfall in December; harvesting of Mopane worms in April and cutting of grass for roof thatching in October, perpetual cattle grazing, cattle access to open water bodies in the Zone 7 areas,** which serve as common watering point for man and animal thus, become a focus for spread of bovine cysticercosis. The EU, Botswana's most viable beef market insists on grass-fed cattle, which produces more tender beef than beef from paddock reared cattle. Roaming exposes cattle to *Taenia saginata*-contaminated human feces. A vital policy decision that Botswana must make is whether to stop cattle from roaming and lose the EU market, which would be inimical to the survival of the industry or to allow cattle to graze freely and evolve a sustainable cure for bovine

cysticercosis. One sample *t*-test, was used to determine quantitative relevance of each risk factor in contributing to the prevalence of bovine cysticercosis. Out of the 18 tested risk factors, fourteen (14) showed statistically significant contributions to the prevalence of bovine cysticercosis . Of these risk factors, the top 20% contributors are 'absence and or distanced pit latrine in the farm,' 'proximity to uncontrolled human defecation', 'access to contaminated pasture' and 'failure to deworm herd boys'. In applying the Pareto principle, eliminating these top 20% risk factors can lead to 80% drop in prevalence of bovine cysticercosis in Botswana. This study has shown that interpreting the risk factors of this zoonosis in relation to Batswana's lifestyle provides adequate knowledge that can enhance its effective control and prevention. It is therefore strongly recommended that control and prevention strategies must emphasize provision of latrines at designate points prone to contamination with human feces; stoppage of cattle access to contaminated water bodies and contaminated pastures, failure to deworm herd boys, since these risk factors have shown to contribute about 80% of the prevalence of bovine cysticercosis in Botswana.

Beef from *Taenia saginata* cysticercosis infested carcasses are not exported to the European Union. On account of this, the government loses export earnings and rural farmers lose a household. The government of Botswana practiced several intervention measures to cushion the effects of bovine cysticercosis on socio-economy of affected farmers. However, apart from a few qualitative and subjective reports regarding the effectiveness of these intervention, there is no known work that has adopted objectively verifiable indicators (OVI) to assess the socioeconomic and psychosocial effects of the bovine cysticercosis on cattle farmers in Botswana. This work determined quantitatively the socio-economic and financial effects of the *Bovine cysticercosis* and by extension, queried the effectiveness of the existing government intervention measures. The 'ability to save money' was the most significantly affected OVI; followed by 'experience of emotional disturbance', and 'ability to provide food for family'. Also affected, were farmers' ability to provide food, healthcare, education, rental for family. Equally, bovine cysticercosis caused farmers not to employ new workers; to diversify or abandon farming business. With reduced income came "farmers'' inability to meet social, religious and family obligations", causing some to borrow money for upkeep of family and/or farming business. Severity of effects on farmers were dependent on the magnitude of the infestation, the scale of production of farmers and presence of absence of some survival strategies. Farmers' response to effects ranged from

outright closure of farms, reduction in farm capacity and to diversification of businesses. Some farmers experienced some psycho-social effects. Most effective government interventions were provision of re-stocking seed calves; payment for cold treatment of infested carcass and installation of socio amenities. All these played vital roles in cushioning the adverse effects of bovine cysticercosis on the livelihood pattern of the farmers. Farmers coping ability depended on the scale of production (amount of animal in farm or post); level of involvement (part time or full time), magnitude of dependents on farmers vis-à-vis income level (family size versus scale of production versus level of involvement); the magnitude and the effects of bovine cysticercosis on the farming business (amount lost by detention, devaluation and destruction of animals and the number of animals destroyed); the type, level and promptness of government intervention to farmers following devaluation or destruction of affected animals. Majority of the farmers whose livelihood patterns were affected by bovine cysticercosis are the farmers who own 50-99 cattle and the farmers who own 10-49 cattle and are operating at full time basis. The least affected group is the farmers who have less than 5 cattle and are part time followed by the farmers who have less than 10 cattle and are part time.

The farmers who experienced bovine cysticercosis in their farms where 10.015 times more like not to save money compared to farmers who did not experience bovine cysticercosis in their farms. Equally, farmers who experienced bovine cysticercosis in their farms where 29.2990 times and 7.288 times more likely to experience emotional disturbance and the inability to provide food for their family respectively. The results of this study provided information that is relevant for development of effective interventions & policy advocacy for improved meat production in Botswana. This answers to 2nd SDG.

On average each farmer spent about (P4, 125.26) four thousand, one hundred and twenty five Pula, twenty six thebe in the cold treatment of infested carcass in 2017 alone. Also, each farmer lost about (P4, 751.27) four thousand, seven hundred and fifty one pula, twenty seven thebe due to devaluation of carcasses in 2017 alone. Total amount of money lost by 62 respondents due to condemnation of carcasses in 2017 was (2,124,240.00) two million, one hundred and twenty four thousand, two hundred and forty pula. This brings it to an average of (P34, 261.93) thirty four thousand, two hundred and sixty one Pula, ninety three thebe lost per farmer due to condemnation of carcasses in 2017 alone.

Since all traditional control and prevention methods have failed to reduce incidence of bovine cysticercosis, and since the government of Botswana cannot abolish free grazing of cattle, an alternative and sustainable remedy must be advanced. Experimentally, vaccines have been used to immunize calf against bovine cysticercosis. This research profiled bovine cysticercosis proteins and identified some immunogenic proteins.

One-dimensional electrophoresis of *Taenia saginata* cysticercosis using Sodium dodecyl sulphate Polyacrylamide electrophoresis (SD-PAGE) identified proteins with molecular weights of 4, 6, 14, 17, 22, 25, 28, 32, 38, 44, 50, 67, 75, 100, 115, 135, 150, 190 and 245kDa.

Immunoblotting under reducing conditions used bovine polyclonal antibody and Rabbit anti-Bovine IgG (H+L) Secondary Antibody conjugated with Horse Radish Peroxidase (HRP) to challenge antigens derived from cysts fluid and whole cysts of *Taenia saginata* cysticercosis. Identified immunogenic proteins were 14, 22, 25, 50, 98, 135, 190 and 245kDa. These proteins were further confirmed to be immunogenic specific for *Taenia saginata* cysticercosis by treating transblotted membrane with rabbit serum, containing antibodies specific for *Taenia saginata* cysticercosis and Goat anti-Rabbit IgG (H+L) Secondary Antibody Horse Radish Peroxidase (HRP). Identified immunogenic proteins were 14, 22, 25, 50, 98, 135, 190 and 245kDa.

Increasing concentration of the primary from (1:100 to 1:300) and secondary antibodies from (1:1000 to 1: 3000) and replacing streptavidin-HRP mixture with SIGMAFASTä BCIPÒ /NBT (5-bromo-4-chloro-3-indolyl phosphate/nitro blue tetrazolium) Tablets as a coloration mixture caused detection of more immunogenic proteins

Membranes treated with serum collected from *Taenia saginata* cysticercosis negative cattle and incubated with Rabbit anti-Bovine IgG (H+L) secondary antibody conjugated to horse radish peroxidase did not show antibody-antigen complex. Similarly membrane treated with serum collected from *Taenia saginata* cysticercosis-negative rabbit and incubated with Goat anti-Rabbit IgG (H+L) secondary antibody showed no antibody-antigen complex specific for *Taenia saginata* cysticercosis. These result indicate absence of primary antibody in sera collected from *Taenia saginata* cysticercosis negative cattle and rabbit.

Freezing samples did not cause significant difference ($P<0.05$) in proteins quantity contained in samples however, immunogenic proteins were lost after 7 days. Protein sequencing is required to identify immune-dominant epitopes and to go a step closer to vaccine development

1.2 GENERAL RECOMMENDATIONS

At the end of each study recommendations were made. In addition to the aforementioned, based on the findings of this research, the following general recommendations are made; that:

1. the government of Botswana should provide better incentives and enabling environment for more youths and women to get involved in local fodder production; as strategy to reduce the cost of cattle feeding, especially for the dairy cattle industry. This will create employment, increase farmers income and foreign earning, reduce rural poverty and ensure sustainable consumption vis-a-vis production; ultimatley achieving SDGs 1, 2, 8, 9, 12
2. the government should create intervention programs to ensure proper compensation of affected farmers, whose carcases are either destroyed or detained. This will discourage the underground trafficking of livestock by local farmers. Intervention will help quick recovery of affected farmers and farms; ensure sustainable consumption and production patterns. Disease tracking and management will ensure healthy lives and promote well-being for man and animal. These will enable achievement of SDG 1, 2, 3 8 and 12
3. an implementation task force should be created to ensure efficient of the Abolition of Marital Power Act. This will achieve gender equality and empower women to legally own (and/ or use) land and cattle. Woman will contribute their quota in food production, hunger and poverty reduction (eradication) and provide employment and foreign earning. These shall ensure achievement of SDG 1, 2, 3, 5 and 8
4. prevalence studies should derive holistic data; involving all levels of abattoir and all districts of Botswana instead of relying on data from BMC only. This ensures efficient disease tracking and management leading to healthy living and promote well-being for man and animal. This shall ensure achievement of SDG 3 and 12
5. the institutional prevalence study should employ the active survey method alongside traditional passive abattoir investigations as a way of validating results from a passive abattoir post mortem examination.

6. Bovine cysticercosis control and prevention advocacies must be specific and strategically target peculiar risk factor and challenges within localities rather than making blanket statements at a national level. Examples are listed below:
    i. Procurement of spoilt meat from unlicensed abattoir must be checked as this was the only risk factor that **individually contributed** significantly to prevalence of bovine cysticercosis
    ii. prevention and control measures should target a paradigm shift in the socio-cultural lifestyle of Batswana **rather than** just pointing out literature-documented determinants of bovine cysticercosis
    iii. mobile toilets should be built along Trans-Kalahari Way and other long stretching high ways in Botswana, which should be backed up with enforceable legislation to ban and prosecute defecation along Trans-Kalahari Express Way **rather than** asking travellers not to defecate indiscriminately
    iv. there should be an effective value re-orientation programs for Massires and Basarwa Tribes (Bush people) in Botswana, which would help them to become agreeable to civilization
    v. enclosed water bodies in Zone 7 areas of Botswana should be treated for worm burden at the start of raining seasons before cattle gains access to the water
    vi. mobile toilets and a means of ablation should be provided in farms and bushes during the harvest of grasses and mopane worms and monetary fine must be instituted for defaulters.
7. graduate and undergraduate BUAN students should conduct comprehensive studies of the seasonal index of bovine cysticercosis. This will provide relevant data necessary for time-targeted prevention and control of bovine cysticercosis. This ensure achievement of SDG 4
8. operations of BMC should be liberalized to allow private sector into beef export industry. This will allow construction of abattoir in Ghanzi district, which although is the highest beef cattle producer do not have an abattoir. Licensing and building abattoir in Ghanzi will create employment, drive farm capacities up, ensure adequate meat inspection and earn foreign exchange. By these the government would achieve SDGs 1, 2, 3, 8, 10, 12.
9. while achieving a paradigm shift in the lifestyle of Batswana may be far-fetched, the Botswana government and relevant stake holders should support production of vaccines using the eight

(8) identified immunogenic proteins. Support could be in the form of enabling research environment, finance (grants, scholarships funding). Vaccine production would be the best prevention and control approach of bovine cysticercosis on the long run

0. antemortem diagnostic kits using monoclonal antibodies and silver dyes as markers should be developed. This will enable antemortem detection of *Taenia saginata* cysticercosis thus, reduce financial and cattle heads lost when infested cattle is slaughtered

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APPENDIX I

STUDENT'S QUESTIONNAIRE

Dear respondent,

The purpose of this study is to assess the socio-economic effects of bovine cysticercosis on cattle farmers in Botswana. The contents of this questionnaire shall help elicit information for answering questions raised by the study. The information that you shall supply shall be held in confidence and used for only the above stated purpose.

Please, respond with a tick (√) in the appropriate space

SECTION A: Demographics

1. √Gender Male (….); Female (….)
2. √Age 16-20 (….); 21-25 (….) 26-30 (….) 31-35 (….)
   36-40 (….)41-45 (….) 46-50 (….) 51-55 (….)
   56-60 (….)61-65 (….) 66-70 (….) 71-75 (….)
3. √What is your marital status? 1. Unmarried (…..) 2. Married (…..) 3. Divorced (…..)
4. √Education: what is your highest educational level
   Junior School (….) Senior School (….) Diploma (….) First Degree (….) Masters (….) PhD (….) Others Specify (……)
5. √Please describe your work as a cattle farmer 1. Full time (…..) 2. Part time (…..)
6. √If you are not a fulltime cattle farm owner, can you state other occupation(s)?

1. Trading (…..); 2. Civil Service (…..) 3. Professional (specify) (……)
4 Others (……)

7. √Family size (immediate children born to you). Please tick appropriately

1. No Child (……) 2. 1-5 (…..) 3. 6-10 (…..) 4. above 10 (…..)

SECTION B: Description of Farm(s)

i. Name and Address of Farm: ……
ii. √District and Region……
iii. √Age of Farm: (Please tick appropriately) 1. Less than 1 yr (…..) 2. 1-5 yrs (…..)
   3. 6-10 yrs (…..) 4. above 10 yrs
iv. √Other Livestock in your farm (please list)

a. (……) b. (……) c. (……)

SEECTION C: Farm Capacity and Farm Practice (Cattle Only)

1. √Farm Size

a. (>500 cattle (…..);
b. (100-499 cattle) (…..)
c. (50-99cattle) (…..)
d. (10- 49 cattle) (…..)
e. (5-9 cattle) (…..)
f. (un-housed cattle< 5) (…..)

2. √Types of cattle farming practice

a. Beef only (…..) b. Diary only (…..) c. Both (…..)

3. √Available Breeds of Cattle in Farm. Please, tick appropriately

Tswana (.....)Tuli (.....) Afrikaaner (.....) the Brahman(.....) Musi (.....): Hereford (.....) Simmental (.....) Aberdeen Angus (.....) Charolais (.....) Limousin (.....) Red Sussex (.....) Red Poll (.....) Brown Swiss (.....) Murray Grey (.....)Pinzgauer (.....) Holstein (.....) Friesland (.....) Ayrshire (.....) Jersey (.....) Guernsey (.....)

SECTION D: Incidence of *Bovine cysticercosis* in the Farm

1. √Do you know about bovine cysticercosis *(beef measles)?*

a. Yes (.....), b. No (.....)

2. √Have you ever recorded bovine cysticercosis in cattle from your farm at the abattoir

a. Yes (.....), b. No (.....)

SECTION E: DIRECT IMPACTS

Production Capacity

1. Can you estimate your farm capacities (Nos of cattle heads) across times?
   2009.....2011.....2013.....2015.....2017.....
2. √Can you say that the bovine cysticercosis affected the growth rate of your cattle?
   a. Yes (.....) b. No (.....)
3. √Can you say that the occurrence of bovine cysticercosis in your farm affected your farm capacity? a. Yes (.....) b. No (.....)
4. √In what ways did the occurrence of bovine cysticercosis affect your farm capacity?

a. Death of cattle (.....); Please estimate number (.....)
b. Unwillingness to invest on more cattle heads (.....)
c. Culling of sick cattle; Please estimate number (.....)

5. √Does the occurrence of bovine cysticercosis have financial effect on your farming business?

a. Yes (.....) b. No (.....)

6. √Animal totally condemned because of presence of cysts? A. Yes (.....) b. No (.....)
   √6b. Estimate total value of loss in Pula (.....)
7. √Animal devalued because of presence of cysts? Yes (.....) b. No (.....)
   √7b. Estimate level of devaluation in Pula (.....)
8. √Extra money spent treating infected carcass? Yes (.....) b. No (.....)
   √8b. Estimate cost of treatment in Pula (.....)
9. Can you estimate the drop in number of cattle heads in your farm following the occurrence of the bovine cysticercosis (P.....)

SECTION F: SOCIOECONOMIC IMPACTS:

Please tick where applicable the types of effects that the outbreak of bovine cysticercosis caused to your socio-economy

1. √Can you say that the occurrence of bovine cysticercosis affected you socio-economically?

a. Yes (.....) b. No (.....)

2. √Inability to provide food for your family? a. Yes (.....) b. No (.....)
3. √Inability to gain access/pay for health care facility? a. Yes (.....) b. No (.....)
4. √Inability to provide education to ward & family members? a. Yes (.....) b. No (.....)
5. √Inability to afford house rent? a. Yes (.....) b. No (.....)
6. √Inability to save money a. Yes (.....) b. No (.....)

7. √Did you borrow money in order to meet basic family needs? a. Yes (……) b. No (……)
8. √Did you borrow money to revive farming business? a. Yes (……) b. No (……)
9. √Did you lay off workers because of inability to pay salary? a. Yes (……) b. No (……)
10. √Were you unable to employ new workers? a. Yes (……) b. No (……)
11. √Did you have difficulty meeting up with social responsibilities a. Yes (……) b. No (……)
12. √Did you have difficulty meeting up with religious responsibilities
    a. Yes (……) b. No (……)

SECTION G: PSYCHOSOCIAL EFFECTS

1. √Did the outbreak of bovine cysticercosis and the destruction, condemnation or devaluation of your cattle affect you emotionally? a. Yes (……) b. (……)
2. √Did you invest in order kinds of business apart from cattle because of losses in cattle business? a. Yes (……) b. (……)
3. √Did you abandon cattle farming because of your experience with bovine cysticercosis?

a. Yes (……) b. (……)

FORM B: INFORMATION ABOUT RISK FACTOR OF *Cysticercus bovis*

1. DATE OF VISIT:.....

DESCRIPTION OF FARM/CATTLE POST

2. NAME OF FARM:..... 3. LOCATION:.....

4. GPS (CORDINATES):..... 5. TYPE OF ABATTOIR:..... 6. CAPACITY:.....

7. OPERATORS OF FARM:.....

Hereunder are listed risk factors of bovine cysticercosis as identified in literature; the intent of this questionnaire is to ascertain strength of association between the Risk Factors and the occurrence of the Cysticercus bovis.

Which of these practices do you agree leads to spread of *Cysticercus bovis*. Please tick appropriately.

	S/DA	DISAGREE	NEUTRAL	AGREE	S/A
1. Preference for rare to well done meat (colour of deep portion of cooked meat changes to greyish white)					
2. Absence/distanced pit latrine in farm					
3. Grazing (Hoofing) of animal					
4. Access to contaminated pasture					
5. Sharing machinery or hiring contractors					
6. Having visitors on farm.					
7. Organic farming (effectiveness of sewage treatment)					
8. Proximity to uncontrolled human defecation					
9. Access to contaminated water					
10. Access to contaminated feed (issue with feedlot)					
11. Dairy animals/ Being Female (being kept longer than beef)					
12. Beef sold at non-licensed shambles					
13. Lack of routine meat inspections at licensed abattoirs					
14. Butcheries procure infested meat because it is cheaper					
15. Consumers prefer to purchase cheaper meat without questioning its fitness					
16. Failure to de-worm herd boys and cattle keepers					
17. poor awareness campaign to farmers and stake holders					
18. Non-adherence to fencing Policy,					

S/A= STRONGLY AGREE; S/DA= STRONGLY DISAGREE,

APPENDIX II

METHODOLOGIES FOR EXPERIMENTS ON IDENTIFICATION OF IMMUNOGENIC PROTEIN

SECTION A: CYSTS COLLECTION, PREPARATION AND ACTIVATION

Operation 1: Collection of cysts from the abattoir

Literature recommends several methods of examination for cysts and sites for collection of the cyst of *Cysticercus bovis*. Some of those locations include: the esophagus; stomach and intestine, general surfaces of the carcass; muscles of the shoulder behind the elbow, the chuck, muscular diaphragm and the fillet (Livestock and Meat Act, 2007). However, not all of these methods and sites were utilized in this research. Table1 below lists sites and methods used for collection of samples. Examination was carried out by meat inspectors at the Multi Specie Abattoir Botswana (MSAB), Gaborone from where all cysts samples were collected Cysts were collected. Samples were collected in petri dishes, stored in cold packs and transported to freezers at the laboratory

Table1 Methods and sites of collection of bovine cysticercosis cysts

Site of examination	Procedure and method of examination
Cheek muscles	Two deep linear incisions were made parallel to the mandible from its upper muscular insertion; incision sites were parted and by visual search cysts were identified and collected
The tongue	Upon palpation along the long axis of the tongue with hands and scrapping with knife, we felt the cyst and by incising lengthwise on the lower surface from base to root, we identified the cyst.
The heart	The heart was split from base to the apex and further incisions made into the musculature to probe for cysts.
Triceps brachii	Three deep, adjacent and parallel transverse incisions were made above the point of the elbow.

Operation 2: Preparation of Saline (9% Saline solution) 4.5gm of NaCl was mixed with 50ml of distilled water. Mixture was homogenized by voltexing

Operation 3: Decontamination of Samples Using normal Saline The samples were washed in saline. Cysts were sorted with spatula and dissected out of tissue. Saline was drained from cysts

Operation 4: Stabilizing the Cyst in PBS The decontaminated cysts were washed in cold phosphate buffered saline (PBS) by rocking the petri dish gently. This washing was repeated 2

times with fresh PBS each washing. Cysts was transferred from the petri dish unto clean tissue papers using a non-toothed forceps to avoid bursting the friable shell. In this paper the shells were allowed to drain off the PBS.

Operation 5: Preparation of Samples

Cysts Fluid: Cyst fluid was collected by puncturing the cysts and the fluid along with the protoscolices was drained into Nunc® Cryotube. Fluid was centrifuged at speed of 10,000rpm at 4^0C for 3 minutes. The supernatant was separated from the sediments into separate Nunc® Cryotube.

Whole Cysts: whole cyst samples were prepared by crushing the cysts with fluid under pressure using mortar and pestle. The crushed cyst was mixed using PBS. The mixture was centrifuged at speed of 10,000rpm at 4^0C for 3 minutes. The supernatant was separated from the sediments into separate Nunc® Cryotube

Meat Crush: Portion of the meat was crush under pressure using the mortar and pestle. The crushed meat was mixed using PBS. The mixture was centrifuged at speed of 10,000rpm at 4^0C for 3 minutes. The supernatant was separated from the sediments into separate Nunc® Cryotube

Operation 6: Storage of Sample: All Samples were stored in Nunc® cryotube at -15◦C until when needed.

Operation 7: Thawing of Sample

Sample was removed from the freezers where they had been stored and thawed at room temperature. After samples have been thawed, they are homogenized by voltexing

Operation 8: Quantification of Protein using the Qubit® 3.0 Fluorometer

There are many laboratory methods of quantifying protein. The nanodrop and the Qubit method is described underneath.

The Nanodrop: A Nanodrop is a common lab method that reads a single 2µl drop on a pedestal. Although the Nanodrop does an excellent job at measuring across a wide spectrum that spans ultra violet (UV) and visible lights, it can't automatically determine that the sample on the pedestal is DNA, RNA or protein. The sample type (protein, DNA or RNA) must first be determined by the user before beginning measurements so it can report an accurate concentration. The apparent pitfall of Nanodrop is that it may read impurities as sample. Again, in using Nanodrop, the sample must be within the limits of your nucleic acid extractions measurable range of the Nanodrop, which is (2ng – 15µg) per µl.

The Qubit fluorometer uses fluorescent dyes to determine the concentration of either nucleic acids or proteins in a sample. The UV-absorbance method uses a spectrophotometer to measure the natural absorbance of light at 260 nm (for DNA and RNA) or 280 nm (for proteins) (Manchester, 1996). The more DNA, RNA or protein in the sample, the more light is absorbed at this wavelength. The absorbance is a natural property of DNA, RNA, free nucleotides, proteins and some amino acids and many other compounds as well. Because so many molecules absorb light at 260 nm, this measurement is subject to inaccuracy due to potential contamination of the sample with these other molecules (Glasel, 1995). In addition, using the absorbance method, it is not possible to distinguish between DNA, RNA, protein or free nucleotides or amino acids in the sample, leading to potentially highly inaccurate measurements (Huberman, 1995; Manchester, 1915)

Qubit contrasted with the Nanodrop: Across measurements collected by multiple laboratories, there is notable variability between DNA quantified spectrophotometrically by Nanodrop versus fluorometrically by Qubit. Whereas Spectroscopic methods apply UV absorbance measurements (measuring absorbance at 260 nm and 260 nm/280 nm ratio), the Qubit uses Fluorescence-based dyes that bind specifically to DNA, RNA, or protein. Qubit accurately quantifies DNA samples with concentrations as low as 10 pg/µl, but Nanodrop is not recommended for concentrations under 2 ng/µl; variation for sample concentrations <10 ng/µl is often high. However, Nanodrop can be used to detect presence of contaminants, which Qubit cannot do.

For this research the **Qubit® 3 Fluorometer Method** was adopted. This method uses a reagent that is an organic dye provided as a solution in 1,2-propanediol.

Qubit® 3 Protein Assay Kits- Preparing samples and standards: This protocol resumes with preparation of standards for calibrating the Qubit®3 Fluorometer except when using the last calibration performed on the instrument

8.1 Setting up the required number of 0.5-ml tubes for standards. The Qubit® Protein Assay requires 3 standards. Only thin-wall, clear, 0.5-mL PCR Qubit® assay tubes (Cat. no. Q32856) tubes were used. Number of tubes needed for quantification was number of samples plus three.

8.2 Labelling the tube lids. Tubes were labeled correctly only on the tops and not on the sides since labeling on the sides could interfere with the sample read. Calibration of the Qubit® Fluorometer requires the standards to be inserted into the instrument in the right order (1-3)

8.3 Preparation of the Qubit® working solution. The Qubit® working solution was prepared by diluting the Qubit® Protein Reagent 1:200 in Qubit® Protein Buffer. A clean plastic tube (not a glass container) was used to prepare Qubit® working solution ensuring the final volume in each tube was 200µl.

The total number of samples and standards were multiplied by 200, which gave the volume of buffer measured. Since the protein reagent is purchased in a concentration of 200X, it was diluted to 1X solution by mixing 199 parts of the protein buffer with 1 part of protein reagent.

So, for 7 samples and 3 standards, for examples, the total working solution was 200µl per tube multiplied by 10 tubes which is 2000µl (2ml) of working solution. To achieve this, 10µl of Qubit® reagent was mixed with 1990µl of Qubit® buffer.

8.4 Adding Qubit® Working solution: Exactly 190µl of Qubit® working solution was added to each standard tube, while 199µl of Qubit® working solution were added to each sample tube.

8.5 Adding Qubit® Standards: Exactly10µl of Qubit® standard was added to each of the appropriate tube. The tubes were mixed by voltexing for 2–3 seconds; carefully avoiding bubbles or staining the tube by clamping the last finger on the edge while was vortexed and the vibration transfers to the tubes.

Note: Careful pipetting was critical to ensure that exact quantity of Qubit® protein buffers, Qubit® protein reagents, Qubit® Standards and samples were pipetted.

However, due to pipetting error some extra volumes may be prepared but all increases in volume were proportional

8.7 Incubation of tubes: All tubes were allowed to incubate at room temperature for 5 minutes.

8.8 Inserting and Reading standards; The Qubit® 3.0 Fluorimeter machine was used for reading the standards. On the home screen of the Qubit® 3.0 Fluorimeter, "protein" was selected as sample type to be read. The "Read standards" screen was displayed. To proceed, the "Read Standards" was selected. The unit of measurement was set at ng/µl

Tube containing Standard #1 was inserted into the sample chamber, then lid closed and Read standard button was then pressed. It takes 3 seconds to read the tubes. After reading, the tube containing Standard #1 was removed. Standard #2 and Standard #3 are read just as Standard #1. When the reading is complete, the machine displayed the results on the Read standard screen. All three reading were recorded correctly.

NOTE: The top value (in large font) is the concentration of the original sample. The bottom value is the dilution concentration.

Operation 9: Alternative method of calibration of Machine using three uniform samples:

The Qubit® 3.0 Fluorimeter machine is calibrated before each use. This process can be cumbersome, money and time consuming. As an alternative to continuous calibration of machine using standards, the researcher developed a means of ascertaining calibrating the machine.

9.1 Preparation of Sample: One cyst was prepared following methods of operations 3 and 4 and 5.

9.2 The crushed material was weighed and the weight was 6.4mg. This material was added into a Nunc® Cryotube. 64µl of Phosphate Buffer Saline (PBS) was added to the tube and the mixture was homogenized by voltexing.

9.3 Equal amount of 10µl of the sample was pipetted each into 3 different 0.5-ml PCR Qubit® assay tubes (Cat. no. Q32856)

9.4 Since the protein reagent is purchased in a concentration of 200X, it was diluted to 1X solution by mixing 199 parts of the protein buffer with 1 part of protein reagent.

So, for the 3 samples the total working solution was 200 µl per tube in 3 tubes which is 600 µl of working solution. To achieve this, we added 3µl of Qubit® reagent plus 597µl of Qubit® buffer.

9.5 Adding Qubit® working solution: Exactly 190µl of Qubit® working solution was added to each SAMPLE tube.

9.6 Adding Samples: Exactly 10 µl of each sample was added to the assay tubes containing the correct volume of Qubit® working solution, then mixed by vortexing 2–3 seconds. The final volume in each tube was 200µl.

Note: Careful pipetting was critical to ensure that exact quantity of Qubit® protein buffers, Qubit® protein reagents, Qubit® Standards and samples were pipetted.

9.7 Incubation of tubes: All tubes were allowed to incubate at room temperature for 15 minutes

9.7 Reading of Samples: The procedure of protein quantification as carried out in operations 8.7 was repeated and the quantity of proteins in each tube was read out. However, instead of selecting "Read Standards", "Read Samples" was selected.

Results of protein concentration from three similar sample

S/N	Description of Samples	Quantity of Protein (ng/μL)
1	Sample tube 1	741
2	Sample tube 2	742
3	Sample tube 3	742

Following the above results, the machine can be said to be well calibrated as it could measure the three samples from the same stock, three different successive times and give the same results under the same condition. The continuous use of standard kits to calibrate the fluorimeter is costly in addition to being cumbersome. There have been times when one of the standards may be unavailable. At such a time and/or for the reasons of saving cost the researcher has found this method of calibrating the machine useful.

Operation 10: PREPARATION OF REAGENTS FOR ELECTROPHEROSIS

General Precaution: For the preparation of the following reagents, the researcher wore safety kits; like nose mask transparent plastic eye cover, safety hand gloves and thick lab coats and safety boast.

Preparation of 1.5M Tris-HCl buffer at pH 8.8

By calculation 181.65g of Tris base (MW = 121.14 g/mol) was required to make 1L of 1.5M Tris base solution (Laemmli, 2003). This amount of Tris base was poured into 1L bottle containing 800 mL of distilled water. The pH was measured and with each successive addition of drops of concentrated HCl, adjusted to 8.8 according to SCB (2001). The solution was allowed to cool down to room temperature before making the final pH adjustment. The volume was made up to one (1) liter using distilled water, and mixture homogenized by voltexing and stored in labelled container.

Preparation of 0.5M Tris-HCl buffer at pH 6.8

By calculation 181.65 g Tris base (MW = 121.14 g/mol) was required to make 1L of 1.5M Tris base solution (Laemmli, 2003). This amount of Tris base was poured into 1L bottle containing 800 mL of distilled water. The pH was measured and with each successive addition of drops of concentrated HCl, the pH was adjusted to 6.8 according to SCB (2001). The solution was allowed to cool down to room temperature before making the final pH adjustment. The volume was made up to 1L using distilled water.

Results of protein concentration from three similar sample

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2	Sample tube 2	742
3	Sample tube 3	742

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Preparation of 2x sample loading buffer (non-reducing): For 100ml

5ml of 1M Tris, pH 7 was mixed with 25ml of 20% SDS and mixed with of 20ml glycerol and 2mg of bromophenol blue. This mixture was made up to the volume to 100ml with distilled H2O

Preparation of 2x sample loading buffer (reducing): For 1ml

From the non-reducing 2x sample loading buffer prepared in section 10.3 above, 950µl of the mixture was mixed with 50µl of β-mercaptoethanol. This is the reducing sample loading buffer

Preparation of 2X Dithiothreitol (DTT) 10ml Sample Buffer

1. 4mL of 10% SDS
2. 1.2mL of 1M Tris-Cl pH 6.8
3. 200µL of 1% Bromophenol Blue
4. 2.6mL of Water
5. 2mL of Fresh DTT (1M) fresh stock

Preparation of 5ml of 20% (w/v) Sodium Dodecyl Sulfate (SDS)

For a 20% of SDS solution, 1g of SDS was dissolved in distilled water and the final volume was made up to 5mL. This reagent was stored at room temperature as recommended by SCB (2001).

Preparation of 10% (w/v) Ammonium Persulfate APS (Prepare fresh) Exactly 0.1g of ammonium persulfate was weighed out in an analytical balance and poured directly into a 1.5 ml micro-centrifuge tube. To this 1ml (1000µl) of distilled water was added using a P1000 pipettor (SCB, 2001).

Preparation of 4X Electrophoresis Buffer

12g of Tris free base and 57.6g of glycine, and 4g of SDS were dissolved in distilled water and the volume was made up 1L.

At the time of use, this 4X Electrophoresis buffer was diluted to 1X Electrophoresis buffer by adding 250 mL of 4X electrophoresis buffer to 750 mL of distilled water (SCB, 2001).

Preparation of 10X MES SDS 200Ml Electrophoresis Buffer

1. 39.04g MES (4-Morpholine Ethane Sulfonic Acid)
2. 24.24g Tris Base
3. 4g SDS (2% SDS) (4g in 200mL of (distilled Water) v/w
4. 1.2g EDTA
5. Make up to 200Ml using distilled Water

Preparation of 1X MES SDS Buffer

50mL of 10X MES SDS Buffer is made up to 1L using distilled water

Preparation of Staining and De-staining Solutions 250ml

De-staining: The combination of 125 ml of 65% methanol and 25 ml of (10%) glacial acetic acid, and 100 ml of distilled water gave 250 ml of de-staining solution.

Staining: To 50ml of the de-stain solution, 0.1g of Coomassie Brilliant Blue R-250 was added and stirred to dissolve. This gives 50 ml of Coomassie staining solution (SCB, 2001).

Preparation of Fixing solution: For 1L

600 ml of absolute ethanol was added with 75 ml of glacial acetic acid and the entire mixture was brought up to 1 L with distilled water (ddH2O)

Preparation of Coomassie Blue stock solution: For 100 mL

Exactly 250mg of Coomassie Brilliant Blue G-250 (not R-250) was dissolved in 100 mL of fixing solution prepared in section 10.9 above.

Preparation of Buffers for western blotting10x Transfer buffer: For 4 L

We mixed 121.1g of Tris base with 576g of glycine and the mixture was made up to a volume of 4L with double distilled water (ddH2O)

Preparation of 1x Transfer buffer: For 1 L

700 mL of cold double distilled water (ddH2O) was added to 100mL of 10x Transfer buffer (as prepared in 10.11 above); to this mixture was added 200mL of methanol

General Note: All reagents were labeled after preparation. Labelling included, reagent name, concentration, measured pH, name of researcher and the date of preparation. Reagents were kept stored at room temperature until when needed.

Preparation of Stacking & Resolving Gels: 16ml of 10% SDS-polyacrylamide Gel Mixture

Literature recommends the use of different concentration of gel for different weight of protein. These are; 8% gel concentration for proteins of 80-200kDa; 10% for proteins of 35-100kDa; 12% for proteins of 25-60kDa; 15% for proteins of 20-40kDa (Proteintech, 2019). At the start of this research, weights of the protein(s) of interest were unknown thus, it was difficult to select the appropriate gel concentration. So, the electrophoretic process was conducted many times. During

these series of experimentation, the concentrations of the gel, the voltage and duration of running the gel were optimized.

Higher concentrations of gel resulted to slow movement of samples thus longer gel running time. Similarly, very low concentration of gel reduced gel running time but produced poor gel picture. Gel concentration was optimized at 10%. Optimization of gel concentration is discussed in APPENDIX III.

Preparation of 10% SDS-polyacrylamide Gel: 2 gels.

S/N	Reagent	Resolving Gel	Stacking Gel
1	Tris Buffer HCl	4mL 1.5M Tris pH 8.8	2.5 ml of 0.5M tris pH 6.8
2	Distilled Water (ddH_2O)	7.7ml ddH_2O	6.3 ddH_2O
3	Acrylamide	4ml 40% Acrylamide	1ml 40% Acrylamide
4	SDS	160µL of 10% SDS	100µL of 10% SDS
5	APS	160µL of 10% APS	100µL of 10% APS
6	TEMED	16µL TEMED	10µL TEMED

KEYS AND EXPALNATIONS:

SDS = **Sodium Dodecyl Sulfate (SDS)** - a strong detergent with a hydrophobic tail and a negatively charged head. (Please refer to literature for more details)

APS = **Ammonium Persulfate (APS)** is an oxidizing agent that is used with TEMED to catalyze the polymerization of acrylamide and bisacrylamide to prepare polyacrylamide gels for **electrophoresis.**

TEMED: Tetramethylethylenediamine (**TEMED**) is an essential catalyst for polyacrylamide gel polymerization. **TEMED** is used with **ammonium persulfate** (APS) to catalyze acrylamide polymerization when preparing gels for **electrophoresis.**

Note that 16ml of the gel was prepared for 2 gel; one gel for staining and the second gel for electro-blotting.

Operation 11: Pouring and Casting of gels. The electrophoresis chamber was rinsed under running water and left to dry. Two glass plate; the tall and short plates were placed together, with the short plate in front of the tall plate; making contact with the elevated side ridges of the tall place. The glass plates were inserted into the gel casting stand and clamped firmly. The plates were tested for leakage by pouring water into it and checking for leakages. With no leakages, the water is drained by inserting a tissue paper. After setting up the chamber; a mark was made on the tall glass plate about one (1) inch from top of the glass. This is the limit mark of the resolving gel. The

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3	Acrylamide	4ml 40% Acrylamide	1ml 40% Acrylamide
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resolving gel was poured first using a pipette. Bubbles were removed by overlaying the resolving gel with isopropanol. The gel is allowed to polymerize by standing for 5 minutes at room temperature. After the resolving gel had aligned horizontally, the isopropanol was drained out by inserting a piece of drain paper. The stacking gel was poured using a pipette up to the point of the short glass plate. Then comb is placed in between the glasses. The stacking gel was allowed to cast by standing for a period of 5 minutes at room temperature. The gels (stacking and resolving) is 1mm thick, between the glass plates. When the combs were removed, the top of the gel had a castle-like top formed by the well. Samples are loaded into these wells.

The glass plates containing the gels were dismantled from the stand and placed inside the electrophoresis stand carrying the electrodes. The shorter glass was made to face inward allowing for easy injection of the samples.

11.2 Mounting the Electrophoretic System: The gel stands were transferred into the electrophoretic chamber, which is a vertical chamber. Running buffer was poured into the chamber; up to the correct mark as recommended by the manufacturers; either for 2 gels or four gels; ensuring to submerge the gels.

Operation 12: Preparation of Samples

Following methods used in operation 5, supernatants and sediments of whole cyst, cysts fluid and meat crush were prepared. These gave rise to the first 3 samples. The supernatants were separated from the sediments by aspirating only the supernatants using pipette. The samples of the whole cyst and cyst fluid were diluted with equal volumes of PBS. Samples used for electrophoresis were: Supernatant of cyst fluid; Sediment of cyst fluid; 50% diluted cyst fluid supernatant; Supernatant of whole cysts; Sediments of whole cysts; 50% diluted whole cyst supernatant ; Supernatant of meat crush. Furthermore, each of the 7 samples were separated into 7 different tubes and labelled accordingly.

Following the methods described in operation 8 the quantity of protein contained in each sample was determined and recorded.

Calculation of volumes of sample to add into each well: Preliminary study optimized 25µg of samples to be loaded into each well. By dividing this optimal quantity with the protein quantity of each sample, the volume of each sample to be loaded was calculated.

Calculation of Volume of Sample Loading Dye: For optimum electrophoresis and best gel picture the wells should be loaded between 25µl of samples. Volume of loading dye was worked out by subtracting volume of sample from 25µl. Please find table below. Six (6) Nunc® Cryotube were labelled on top from 1 to 6. After calculation the volumes of samples and the corresponding sample loading dyes these amounts were added to the tubes as listed in the table below. The content of each tubes was homogenized by voltexing.

Table showing volumes of samples and dyes and loading wells

S/N	Sample Description	Quantity of protein (ng/µL)	Volume of sample to make 25µg (µL)	Volume of Buffer to make 25µL	Well Position
1.	Loading Buffer	-	0.0	25	Well 1
2.	Protein Marker	—	7.0	0.0	Well 2
3.	Supernatant of cyst fluid	398-	6.3	18.7	Well 3
4.	Sediment of cyst fluid	3860	6.6	18.4	Well 4
5.	50% diluted cyst fluid supernatant	3005	8.3	16.7	Well 5
6.	Supernatant of whole cysts3960	3960	6.3	18.7	Well 6
7.	Sediment of Whole Cysts	3894	6.4	18.6	Well 7
8.	Supernatant of Meat crush	3990	6.3	18.7	Well 8
9.	Loading Buffer	—	00	25	Well 9
10	Loading Buffer	—	0.0	25	Well 10

Operation 13: Loading of Samples: The calculated volumes of the sample and the loading dye are added into Nunc® Cryotube and labelled on the top of the tubes accordingly. The mixture is homogenized by voltexing, ensuring that bubbles do not form. With the aid of a special tip with a very slender points, the samples are aspirated from the Nunc® Cryotube and loaded into the appropriate wells.

Due to interplay voltage, current and running buffer concentration, sometimes the samples loaded in the outer wells form wavy bands. To avert proteins forming wavy bands, loading dye were loaded into the extreme wells (1, 9 and 10). Please see table above **(column 6)** describing samples and their well positions.

In loading samples, care was taken to avoid samples sipping from one well to another and wells are not overloaded as this could cause samples to spill over when running the electrophoretic process.

Operation 14 Running of the Gel: After the protein markers, samples and dyes were loaded into the wells, the cover of the electrophoretic machine was placed over and secured by locking the lid

with the power cords. The terminals of the power cords corresponding with the terminals of the machine (red cord to positive terminal and back cord to negative terminal). The other end of the terminal was connected into the power source; terminals corresponding (red cored to positive terminal and back cord to negative terminal). The machine was powered on and the voltage set to 100V. This voltage was maintained until the samples had descended past the stacking gel; about 1 inch from the top of the glass, which was marked during the casting of the gel. After the samples descend past the stacking gel, the voltage was increased to between 120 to 150V and the machine was allowed to run until the samples had all descended to the last point of the gel (or until all samples have disappeared from the gel). Another way to know the best time to stop the process is to watch for the time when all the bands in the protein marker has been displayed.

Dismounting the gel system: at the close of the electrophoretic process, the gel machine was disconnected from power source and the cables unplugged. The machine was allowed to cool down before the chamber lid was lifted and the gel stand lifted from the pool of the running buffer.

Operation 15: Staining of Gel: The Coomassie dyes (R-250 and G-250) bind to proteins through ionic interactions between dye sulfonic acid groups and positive protein amine groups as well as through Van der Waals attractions. The Coomassie R-250 was utilized in this research because it can detect protein weight as little as 0.1μg of protein. Coomassie blue stain prepared as shown in operation 10.8 was poured over the gel until the entire gel was submerged in the stain. The staining was allowed to stand overnight with gentle agitation provided using a rocking platform at room temperature of 28°C. The staining continues until the gel is unnoticeable inside the stain because both have same colour.

Fixing the Stain: The recipe for fixing mixture has been described in operation 10.9. An alternative to this recipe was also used. The alternative recipe involved preparing 250ml of fixing mixture by mixing 125ml of 50% methanol with 25ml of 10% acetic acid and 100ml of 40% water. The staining chemical was poured out and the fixing mixture was poured over the gel until the gel was fully immersed in the fixing chemical. The fixing process lasted 60 minutes.

De-staining of Gel: Following fixing, the fixing mixture was discarded and the de-staining mixture was poured over the gel until the gel was submerged inside the chemical. The de-staining process continued until the background was clear; this lasted for 14 hours

Operation 16: Imaging of Gel: Two approaches were used to capture the image of the processed gel. The first was to use the GelMax® Imager UVP; the second was to use a smart camera. For the GelMax® Imager UVP, the gel holder was first moistened using PBS then the gel was placed in the translucent base. The machine was powered on and the image was captured using the installed software. The images were saved in folders as recommended by software manufacturers.
Alternatively, smart camera was used to capture the picture of the gel. The gel was placed on a translucent base and the light were switched on to increase visibility of the gel. With the smart camera the image was captured and saved in folders in the computer.

Operation 17: Transblot of Gel unto the Nitrocellulose Membrane

17.1 Reparation of Reagents: At the start of the transblot process the following reagents were prepared and stored at room temperature until when required.

17.1.1 Preparation of 10X PBST (Phosphate buffered Saline/1%Tween-20).

Stage 1: 1.6 liters of distilled water was poured into 2 liters sterile bottle and agitated while adding 160g of Sodium Chloride ($NaCl_2$) and 4g of Potassium Chloride (KCl_2)

Stage 2: 100 ml of distilled water is heated up to 50°C in 500ml bottle; 28.32g Disodium Hydrogen Phosphate was added and agitated again.

Stage 3: 4g of Potassium Dihydrogen Phosphate is added and dissolved by agitation. The volume is completed to a 2000 ml mark and agitation continued. The pH which is expected to be 6.65-6.90 and the freezing point of -0.53°C ± 0.03°C are recorded

The media is filtered through 0.22µm/ Ap 25 Millipore filter

It is stable for 1 year at room temperature

17.1.2 Preparation of 1X PBST (Phosphate Buffered Saline/0.1% Tween-20).

Exactly 100 ml of 10X PBST and 900 ml of ddH_2O were combined in a 1000ml bottle. The mixture was filtered using filtered paper and the filtrate was store in room temperature until when needed.

17.2 Antigen Application: There are three common methods of transferring antigen to the membrane; these are:

a) **Microfiltration blotting:** The antigens of interest are transferred by a vacuum device such as the Bio-Dot® or Bio-Dot SF onto the membrane. The membrane should be removed from the apparatus for the blocking and all subsequent steps.

b) **Dot blotting:** The membrane sheet is cut to appropriate size. A grid is drawn on the membrane using a pencil. The membrane is wetted by slowly sliding the membrane at an angle of 45° into the PBST. (PVDF membrane must first be wetted in 100% methanol). The wetted membrane is dried using filter paper for 5 minutes. There after antigen sample is applied to each square grid using a syringe or pipet.

c) **Electrophoretic blotting:** The antigens of interest from a gel are transblotted (electrophoretic blotting) to the membrane (i.e. SDS-PAGE, IEF or native gel) using the Trans-Blot® device.

For purposes of this research the Electrophoretic blotting method was used because of convenience, being that it is easy to apply and that it is the available method in the laboratory.

17.3 Membrane Selection: Some membranes take on a 'wavy' appearance in the process of transblot. However, the nitrocellulose membrane was used because it has the ability to remain flat throughout the process.

17.4 Transblot Process:

17.4.1 Transblot Equipment and Kits:

Nitrocellulose Membrane: Selection of the membrane type, pore size and membrane format were taken into consideration to help us choose which membrane to use. Generally, PVDF membrane has a protein binding capacity of 170 to 200 µg/cm2 while nitrocellulose has a protein binding capacity of 80 to 100 µg/cm2. While the binding capacity of PVDF will encourage higher affinity to protein thus ideal for capturing lowly expressed protein, it also produces noisier background. So, for less noisy background the nitrocellulose is preferred. PVDF is less brittle than the nitrocellulose membrane. The pore size of 0.45µm membrane was selected for this research because it is considered suitable for most protein blotting applications. While both nitrocellulose and PVDF membranes are used for Western blotting and amino acid analysis, nitrocellulose is ideal in detecting low molecular weight proteins while PVDF is more suitable for detecting higher molecular weight proteins. Pre-cut membrane was chosen since it is more affordable and flexible. The transblot machine used is the Trans-Blot® Turbo™ Transfer System made by BIO-RAD. It is a 2 chambered machine. Each Chamber can accommodate 2 Trans-Blot® Turbo™ Mini-size Nitrocellulose Membrane (please find Pictures attached).

The **Trans-Blot Transfer Kit** used is the Trans-Blot Turbo Transfer System RTA Transfer Kit made from BIO-RAD

Each **Trans-Blot® Turbo™ Mini-size Transfer Stack** contains: 1 bottom ion reservoir stack; 1 Blotting Membrane; 1 Gel; 1 Top ion reservoir stack. This set is sandwiched inside the Transblot cassette which is made up of 1 positively charged bottom electrode (the anode) and 1 negatively charged top electrode (cathode) (Please see picture attached).

17.4.2. Transblot Reagents

Transfer Buffer: 1 liter of the transfer buffer was prepared by mixing 200ml of 5X transfer buffer with 600ml of nanopure water and 200ml of ethanol (reagent grade of 85% or molecular biology grade of 95-98% purity).

Transblot process proper

1. One membrane and two transfer stacks were wetted in 30ml of 1X transfer buffer for 5 minutes
2. The first wetted stack was placed on the bottom of the cassette. This serves as the bottom ion reservoir
3. One wet Membrane was placed over this wetted stack
4. The Membrane was carefully overlaid with the gel to avoid trapping any bubbles between the gel and the membrane.
5. A second wetted transfer stack was placed on the gel and the entire stack was gentled rolled using the blot roller in order to expel any trapped bubbles
6. The upper cassette lid was closed, locked firmly and inserted into the machine.
7. The machine was set at Protocol Name: Mixed MW; MW in Kilo Dalton: 5-150; Time: 7 minutes; current: 2.5 A; Voltage: 25 Volts. This is the recommended rates for 2 mini-gel
8. At the end of the transblot process the membrane was dismounted and kept to dry under room temperature of 32•C before un-mounting the sandwich.
9. Carefully the membrane is separated from the gel and the upper and lower ion reservoir stake and transferred to a solution of PBST

Washing the Membrane: The membrane was immersed into Phosphate Buffered Saline Tween (PBST) solution at an angle of 45°. With gentle agitation provided using a rocking platform, the membrane was washed twice in fresh PBST solution for duration of 5 Minutes each time. This

washing helps to remove the transfer buffer and the gel components left over in the membrane. This washing reduces spotted or blotchy background and improves final visibility of the bands.

Operation 18 Blocking of Membrane using PBST-Milk

The PBST solution is poured off and the membrane is fully submerged with the blocking solution. In this research PBST-Milk was used as the blocking solution.

18.1 Preparation of 5% PBST Milk: 2.5gm of Difco™ Skim Milk was added to a 50 ml bottle containing 50ml of 1X PBST. The mixture was homogenized by voltexing. Mixture was autoclaved at 121°C for 15 minutes and stored at room temperature of 28°C until when required.

18.2 Selection of Non-Fat Milk instead of BSA: The Non-Fat Milk was used instead of the BSA because the former is more readily available, cheaper and easier to handle.

18.3 Blocking Proper: About 5ml of the PBST-Milk was poured into the container and the membrane was immersed at an angle of 45°. The solution was gently agitated using a rocking platform and incubated for 1 hour 30 minutes.

18.4 Washing of Membrane: The blocking solution was decanted and the membrane was immersed into PBST solution. With gentle agitation provided using a rocking platform membrane was washed twice in the fresh PBST solution for duration of 5 Minutes each time.

19. Operation 20 Incubating Membrane with Primary Antibody: Primary Antibody used was infected bovine serum.

19.1 Preparation of Primary Antibody:

The process of preparing the serum started from the abattoir. At antemortem inspection, 15 mL of blood was collected from the jugular vein of 10 cattle. Each blood bottle was tagged to tally with the live animals and later traced back to their carcass. The blood from animals that harbored cysts, as detected at postmortem were considered positive. Blood from positive animals were kept overnight in a slanted tube in order to separate the serum from the plasma. After 24 hours the clear serum was carefully separated from the plasma. This positive serum was refrigerated until when needed.

19.2 Dilution Factor of Primary Antibody: BIO-RAD® (n.d.) literature stipulates a dilution factor of 1 part of primary antibody in 100-1000 parts of 1-5% PBST with bovine serum albumin (BSA). However, for the purposes of this research, 1X PBST in 5% Non-Fat Milk (NFM) was used. Please refer to section 19.1 for preparation of PBST-Milk. Preliminary studies optimized a

dilution factor of 1 part of primary antibody in 500 parts of PBST-Milk. To achieve this dilution factor 20µL of primary antibody was mixed with 10mL minus 20µL of PBST-Milk. The mixture was homogenized by voltexing.

19.4 Incubation Proper: Although manufacturer's (BIO-RAD) literature recommends 1 to 2-hour membrane incubation with agitation, researcher's experience shows that an overnight (10-12 hours) incubation with agitation leads to better blocking. So, membrane was incubated for about 10 hours.

Operation 20 Washing off Primary antibody

The primary antibody solution was decanted and the membrane was immersed into PBST solution. With gentle agitation membrane was washed twice in the fresh PBST solution for duration of 5 Minutes each time.

Operation 21: Incubating with secondary Antibody

21.1 Selection of Secondary Antibody: Anti-Bovine secondary antibodies are affinity-purified antibodies with well-characterized specificity for bovine (cow, ox) immunoglobulins and are useful in the detection, sorting or purification of its specified target. Secondary antibodies offer increased versatility enabling users to use many detection systems (e.g. HRP, AP, fluorescence). They can also provide greater sensitivity through signal amplification as multiple secondary antibodies can bind to a single primary antibody. Antisera to bovine IgG were raised by repeated immunization of rabbits with highly purified antigen. Purified IgG was prepared from whole serum by affinity chromatography to generate highly specific reagents. The Rabbit anti-Bovine IgG (H+L) Secondary Antibody HRP was utilized for this research. Its approximate Protein Concentrations (IgG) is concentration 1.0 mg/ml. This product has shown ability to recognize both the heavy and light chains of bovine immunoglobulin G although it may cross react with IgG from other species.

21.2 Preparation of Secondary Antibody Mixture: The recommended dilution factor is 1:100-10,000. However, dilution factor of 1part of secondary antibody to 5,000 parts of PBST-Milk was ideal, and this was adopted. The manufacturer's recommend to cover with 0.1mL of secondary antibody mixture per cm^2 of membrane. A membrane size of 67.5 cm^2 required 6.7 mL of the mixture. However, an approximated volume of 5mL was prepared for each membrane. To achieve

this volume for two slides, 2µL of secondary antibody was added in 10ml minus 2µL PBST-Milk. The mixture was homogenized by voltexing.

21.3 Incubation Proper: The secondary Antibody mixture was poured into the container containing the membrane, and the mixture was gently agitated using a rocking platform while incubated for 2 hours.

21.4 Washing off Secondary Antibody: Secondary antibody mixture was decanted and the membrane was immersed into PBST solution. With gentle agitation, membrane was washed twice in the fresh PBST solution for duration of 5 Minutes each time.

Operation 22: Amplification of conjugate formed by serological test

Objective: to amplify the conjugates formed by the serological reactions of primary and secondary antibodies

Components Amplified Opti-4CN substrate kit:

1. Bio-Rad Amplification Reagent, 53ml
2. Streptavidin-HRP, 0.5ml
3. Blocker 20g
4. 2x Amplification diluent 106ml
5. Opti-4CN substrate, 12.5ml

Dilution Factor and Preparation of BIORAD Amplification Mixture

An average of 85µL 1x BAR solution is needed to soak $1cm^2$ of membrane. Dilution factor stipulates 2 part of 2x Amplification Diluent mixed with 1 part of 4x BAR and 1 part of double distilled water (ddH_2O). Therefore, to produce 10mL BAR solution requires mixing 5mL of 2x Amplification Diluent with 2.5mL of 4x BAR and 2.5mL of ddH_2O. The mixture was homogenized by voltexing

Incubation with Amplification Mixture: Membrane was incubated while immersed under the amplification mixture for 10 minutes with gentle agitation at room temperature.

Operation 23: Washing with 20% DMSO-PBST: Dimethyl sulfoxide is an organosulfur compound with the formula $(CH_3)_2SO$. This colorless liquid is an important polar aprotic solvent that dissolves both polar and nonpolar compounds and is miscible in a wide range of organic solvents as well as water. It has a relatively high melting point of 198°C and a melting point of

19°C. These characteristics make DMSO apt for electrophoretic process because the process involves both polar and non-polar compounds at high temperatures.

Dilution Factor and Preparation of 20% DMSO-PBST Wash: Exactly 100mL of DMSO and 400mL of 1XPBST was combined to give 500 mL of the mixture. The mixture was homogenized.

Wash Proper: The membrane was immersed in a solution of 20% DMSO-PBST for duration of 5 minutes twice with gentle agitation at room temperature

Washing off DMSO-PBST with PBST for duration of 5 minutes twice: The DMSO-PBST Soap solution was decanted and the membrane was immersed into PBST solution. With gentle agitation the membrane was washed twice in the fresh PBST solution for duration of 5 Minutes each time.

Operation 24: Incubation with Streptavidin- HRP: Streptavidin-Horseradish Peroxidase conjugate is typically used in the immune-detection of biotinylated proteins. This conjugate is suitable for use in immunoblotting, ELISA, immune-microscopy. It is recommended to use 10 mL of working solution per reaction.

Dilution Factor and Preparation of Streptavidin-HRP: About 85µL of the Streptavidin-Horseradish Peroxidase conjugate mixture was needed per 1 cm^2 of the membrane. Based on the dilution factor of 1 part of Streptavidin-HRP with 1000 parts of 1% of PBST-Milk, to make a 10mL solution, 10µL of the conjugate was mixed with 10mL minus 10µL of PBST-Milk.

Addition of the Substrate: The membrane was immersed in the Streptavidin-Horseradish Peroxidase conjugate mixture for duration of 30 Minutes with gentle agitation at room temperature.

Washing off Streptavidin-HRP with PBST for duration of 5 minutes twice: Streptavidin-HRP mixture was decanted and the membrane was immersed into PBST solution. With gentle agitation membrane was washed twice in the fresh PBST solution for duration of 5 Minutes each time.

Operation 25 Colorimetric Detection:

Dilution Factor and Preparation of Colorimetric Detection Mixture: The dilution factor is 1 part of Opti-4CN diluent concentration added to 9 part of $ddtH_2O$, then add 0.2µL of Opti-4CN substrate to form a 10mL of the Colorimetric solution. So, for the making of 20mL of the colorimetric solution, 18mL of $ddtH_2O$ was added to 2mL of Opti-4CN diluent concentration and then 0.4µL of Opti-4CN substrate was added to the mixture. The mixture was homogenized.

Colorimetry Proper: Membrane was covered with the Colorimetric Detection mixture. The colorimeter mixture was agitated until colour change was obvious

Imaging of the Membrane: Two approaches were used to capture the image of the processed membrane. The first was the use of the GelMax® Imager UVP; the second was the use a smart camera. For the GelMax® Imager UVP, the holder was first moistened using PBST then the Membrane was placed in the translucent base. The machine was powered on and the image was captured using the installed software. The images were saved in folders as recommended by software manufacturers.

Alternatively, smart camera was used to capture the picture of the membrane. The membrane was placed on a translucent base and the light were switched on to increase visibility of the gel. With the smart camera the image was captured and saved in folders in the computer.

APPENDIX III

ANCILLARY EXPERIMENTS AND HALL OF SHAME

EXPERIMENT 1 : DETERMINATION OF EFFECT OF FREEZING ON QUANTITY OF PROTEIN IN CYSTS

Introduction: Literature is replete with the recommendation that freezing beef infested with *Taenia saginata* cysticercosis can recover the meat and make it fit for human consumption (Ostertag, 1897 in Ransom, 1914; Georgsson *et, al.*, 2006; Livestock and Meat Industries Act 2007). The Botswana government through the Livestock and Meat Industries Act (2007) recommends that in an event that ten or more bladder worm cysts are found in the head, the tongue, liver or stomach and intestines, the part so infected together with the viscera shall be condemned as unfit for human consumption. However, meat can be subjected to cold storage to the satisfaction and under the control of the Director of Veterinary Services or local authority, and in which cold storage the carcass and viscera are subjected, for a period of 14 continuous days, to a continuously maintained temperature of or below minus 10^0C.

Another recommendation is for the carcass to be frozen, to the satisfaction and under the control of the Director of Veterinary Services or local authority, in a blast freezing tunnel in such a manner that the temperature at the thermal center of the carcass or viscera is reduced to not more than minus 15^0C within 24 hours of the commencement of the blast freezing and that immediately thereafter the carcass and the viscera are retained in cold storage at a temperature of not more than minus 10^0C for a continuous period of not less than 72 hours

Another recommended method is boiling and sterilization (Livestock and Meat Industries Act 2007).

Perroncito (1877) found that the cysticerci in an artificially infested calf were all dead when frozen for fourteen days after the slaughter of the animal (Ransom, 1914). However, Zschocke (1896) showed that cysticerci from beef remained infective fourteen to sixteen days after slaughter (Ransom, 1914). Ostertag (1897) in a related study concluded that a lapse of twenty-one days after slaughter is amply sufficient to ensure the death of the beef cysticercus, and on the other hand that fourteen days is not sufficient, although he agreed with Perroncito that the parasites may have lost their vitality within 14 days or less (Ransom, 1914). Reissmann (1897) reported that beef cysticerci inserted into the depths of large pieces of meat which were then kept at temperatures of from -8 to -10^0C do not survive when thus exposed for three days. Ransom (1914) claimed that if measly beef

carcasses are exposed for six days to a temperature not exceeding -9.44^{0}C the vitality of the cysticerci will be destroyed.

It is not clear whether the freezing process, in addition to affecting the vitality of the cyst, also affects its infestivity, and whether this effect would manifest as changes in protein content of the cyst. However, since it is known that certain proteins are responsible for the infestivity of the cyst, this work is based on the hypothesis that it is probable that freezing will lead to alteration in the quantity or characteristics of the infestive protein in the cysts. The objective of this mini study was to determine if freezing causes significant change in the quantity of protein contained in the cysts and to estimate duration of freezing. It was hoped that this change may explain the loss of vitality of the cysts and thus validate the practice of freezing infested carcass as a means of recovering beef before passing for human consumption.

Objective: to determine if the quantity of protein in cysts decreases with freezing.

Null Hypothesis: Freezing cyst across time does not reduce the protein content of the cyst

Alternate Hypothesis: Freezing cysts across time reduces protein content of the cyst

Methods:

1.1 Collection of Samples: The Cysts were harvested from infested carcass and conveyed in cold packs to maintain the cold chain.

1.2 Decontamination of Samples Using normal Saline

a. The samples were washed in saline
b. Cysts were sorted with spatula and dissected out of tissue
c. Saline was drained from cysts

1.3 Stabilizing the Cyst in PBS

The decontaminated cysts were washed in cold phosphate buffered saline (PBS) by rocking the petri dish gently. This washing was repeated 2 times with fresh PBS each washing.

Cysts was transferred from the petri dish unto clean tissue papers using non-toothed forceps to avoid bursting the friable shell. In this paper the shells were allowed to drain off the PBS.

1.4 Storage of Sample: The cysts were transferred into Nunc® cryotube and labelled according to their intended holding time; from Day 0 to Day 28. They were stored at a temperature of -15°C until when required.

1.5 Thawing of Sample: Sample was removed from the freezers where they had been stored and thawed at room temperature.

1.6 Preparing the Samples: whole Cyst samples were prepared by crushing the cysts under pressure using mortar and pestle. The crushed cyst was mixed using PBS. Mixture was centrifuged at speed of 10,000rpm at 4°C for 3 minutes. The supernatant was separated from the sediments into separate Nunc® Cryotube. The cysts were prepared during Day 0; Day 7; Day 14; Day 21 and Day 28 of freezing the cysts

PROTEIN QUANTIFICATION

1.7 Adding Samples: Exactly 1µL of each sample supernatant as well as the sediments was added to the assay tubes containing the correct volume of Qubit® working solution, then homogenized. The final volume in each tube was 200µL. All together 10 samples were read.

1.8 Inserting tubes containing Samples: In the home screen we selected the "Run samples" Tubes containing samples were inserted one after another.

1.9 Selecting sample volume and units: On the assay screen, sample volume and units were selected using the + or – buttons on the wheel. From the dropdown menu, units for the output sample concentration was set ng/µL

1.10 Reading samples: By choosing the "Read button" each sample was read. The reading takes about 3 seconds. The instrument displayed the results on the assay screen. This process was repeated until all samples were read.

Results Determination of Effect of freezing on quantity of protein in cysts

Table showing quantity of protein in cysts across days of freezing

S/N	Sample Number	Sample Description	Quantity of Protein ng/µL
1.	Sample 1	Supernatant Day 0	2250
2.	Sample 2	Supernatant Day 7	2184
3.	Sample 3	Supernatant Day 14	2180
4.	Sample 5	Supernatant Day 21	2140
5.	Sample 6	Supernatant Day 28	2000
6.	Sample 7	Sediments Day 0	2320
7.	Sample 8	Sediments Day 7	2200
8.	Sample 9	Sediments Day 14	2312
9.	Sample 10	Sediments Day 21	2250
10.	Sample 11	Sediments Day 28	2219

DISCUSSION

The results of this experiment show that quantity of protein in the cyst does not change significantly or uniformly following freezing over a period of 28 days. It therefore follows that since the quantity of protein in the cysts does not depreciate significantly, then the loss of vitality of the cysts as described by Ranson (1914) may not have resulted from outright loss of the protein but rather from attenuation of the infestivity of such protein. Which means that although the protein of interest may be present in the cyst, the protein may have lost its infestivity. It could also be that freezing destroys the proteins and/or denatures the protein by re/dis-organizes its genome thus, interfering with the infestivity of the cysts. Georgsson *et. al.*, (2006) claim that freezing of naturally contaminated carcasses followed by storage at -20^{0}C for 31, 73, 122 and 220 days showed statistically significant ($P \leq 0.05$) reductions in Campylobacter counts initially as compared with counts found on fresh product Inferring from this work, it is also probable that freezing the meat beyond experiment time of 28 days may not cause decrease in protein quantity but may cause denaturing of protein.

EXPERIMENT 2 : OPTIMIZING PROTEIN QUANTITY IN SAMPLE AND LOADING BUFFER VOLUME FOR EACH ELECTROPHORETIC WELL

Literature recommends loading in each gel well, 4-10µg of protein contained in 25µL sample (McCauley, 2019). Highly concentrated or highly diluted samples may not achieve this combination. Some samples produced poor gel picture even when loaded at the stipulated quantity and volume. This inconsistency may have been caused by differences in nature of sample, method of preparing sample, denaturing potency of loading buffer or other electrophoretic processes. It was therefore imperative to develop a workable combination of protein quantity, sample volume and loading buffer volume that can give good gel pictures. The optimal protein quantity and loading buffer volumes for each well was determined by comparing gel pictures (showing movement of the protein bands across lanes) with the volume of sample loaded, along with the quantity of loading buffer.

Objective: to estimate the optimum protein quantity for different samples that can be loaded into each gel well needed to achieve best gel picture

Null Hypothesis: The quantity of protein loaded into each gel well does not affect the picture of the well

Alternate Hypothesis: The quantity of protein loaded into each gel well affects the picture of the well

3.0 Methods

3.1 Following methods used in operation 5, supernatants and sediments of whole cyst and cysts fluid were prepared. These gave 4 samples. Supernatants were separated from the sediments by aspirating it using pipette.

3.2 Furthermore, each of the 4 samples were diluted with 2 different volumes of distilled water to give 4 different concentrations of each sample. This gave a total of 8 samples.

3.3 Following the methods described in operation 8 the protein quantities in each sample was determined and recorded.

3.4 Calculation of volumes of sample to add into each well: Arbitral quantities of 50µg, 30µg, 10µg and 5µg were set to be loaded into each well. By simple arithmetic of dividing stipulate protein quantities with protein quantities (µg\µl) of sample their volumes (in µl) were worked out. For example, to load a 25µg of protein from a sample with protein quantity of 3.980 µg\µl, we divided 25 by 3.9 giving 6.3µl of the sample.

Table1 showing protein quantities and volumes of loading buffer for each gel well.

S/n	Sample type	Quantity of protein (ng\µl)	Volume of samples in µL at different Concentrations			Volume of Loading Buffer in µL to make up for 25µL		
			50µg	*30µg*	*10µg*	*50µg*	*30µg*	*10µg*
1.	Inner fluid Supernatant	4380	11.6 (1)	7.7	2.5	13	17.3	23.7
2.	Inner Fluid Sediments	3960	12.6 (2)	7.7	2.5 (4B)	13	17.3	23.7
3.	Whole Cyst Supernatant	3980	12.6 (3)	7.7	2.5	13	17.3	23.7
4.	Whole Cyst Sediments	3960	12.6 (4)	7.7 (10)	2.5	13	17.3	23.7
5.	Inner Fluid Supernatant + 50% distilled Water	3280	15.6 (5)	9.4 (9)	3.1		15.6	22.0
6.	Inner fluid Sediment + 50% distilled Water	2200	22.7 (6)*	13.6	4.5 (2B)	5	12.4	12.7
7.	Whole Cyst Supernatant + 50% distilled Water	3005	16.7 (7)	10.0	3.3 (3B)	9.3	15.0	21.7
8.	Whole Cyst Sediments + 50% distilled Water	2960	17.2 (8)	10.3	3.4	8.8	14.7	21.4

Key: Volumes of samples and well number in red

3.5 Calculation of Volume of Sample Loading Buffer: Since 30µL is the optimum sample volume for each well, the remainder of 30µl from the sample loaded became the volume of the

loading buffer. For example, if 25µl of sample is loaded, then 5µl of loading dye would be added. Please find table

3.6 Loading Samples: Fifteen (15) Nunc® Cryotube were labelled on top from 1 to 15, which were the numbers of the well in the gels to load the sample. After calculation, the volumes of samples and the corresponding sample loading buffer were added to the tubes as listed in the table1. The content of each tubes was homogenized and loaded in the appropriate wells

RESULTS OF OPTIMIZATION OF PROTEIN QUANTITIES AND VOLUME OF LOADING BUFFER LOADED FOR EACH WELL

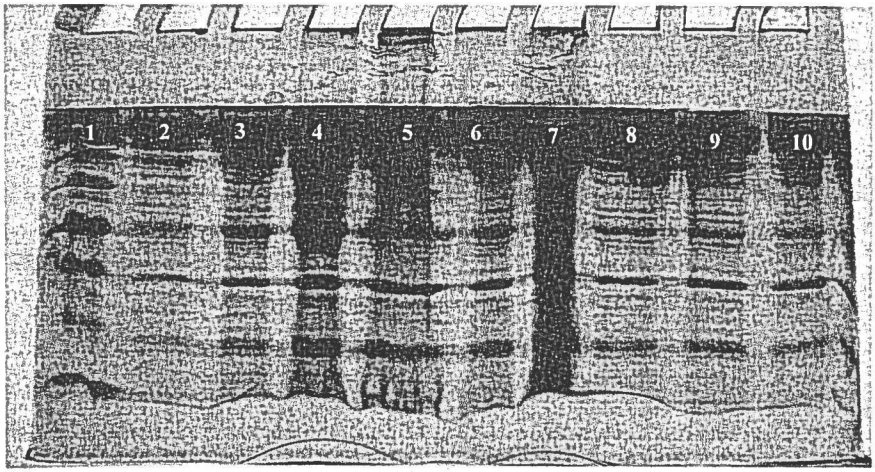

Figure1 picture of gel wells that were loaded with different protein quantities. Wells 1 to 8 had protein quantities of 50*µg*. They were over crowed at the top and showed protein bands not separating distinctively. However, wells 9 and 10 were loaded with protein quantities of 30*µg* showed clear and distinct protein bands

KEY

W1: 11.6µL containing 50µg of cyst fluid supernatant
W2: 12µL containing 50µg of cyst fluid Sediments
W3: 12.6µL containing 50µg of whole cyst supernatant
W4: 12.6µL containing 50µg of whole cyst sediments
W5: 15.6µL containing 50µg of Inner fluid supernatant + 50% distilled water
W6: 22.7µL containing 50µg of fluid sediment + 50% ddH_2O
W7: 16.7µL containing 50µg whole cyst supernatant + dd50%H_2O
W8: 17.3µg containing 50µL of whole cyst sediments + dd50%H_2O
W9: 9.4µg containing 30µL of inner fluid supernatant + 50% ddH_2O
W10: 7.7 µg containing 30µL of whole cyst sediments

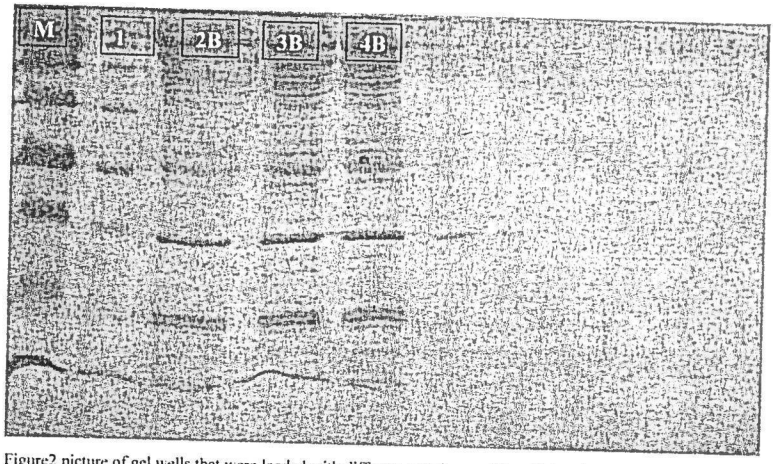

Figure2 picture of gel wells that were loaded with different protein quantities. Gel wells having protein quantities of 30μg to 10μg were not over crowded at the top of the well and showed distinct protein bands. However, wells that were loaded with protein quantities of 10μg or less were clear and showed distinct protein bands

KEY

W1B: spilled sample
W2B: 4.5µL containing 10µg of inner fluid sediment + 50% distilled water
W3B: 3.3µL containing 10µg of whole cyst supernatant + 50% distilled Water
W4B: 2.5µL containing 10µg of inner fluid sediments

DISCUSSION AND CONCLUSION

Well 1 was loaded with 11.6µl of sample 1, whereas wells 2 to 4 were loaded with 12.6µl of samples 2, 3 and 4 each. Wells 5, 6, 7 and 8 were loaded with 15.6µl, 22.7µl, 16.7µl and 17.2µl of samples respectively. Wells 9, 10, 2B, 4B and 4B were loaded with 7.7µl, 9.4µl, 4.5 µl, 3.3µl and 2.5µl or samples respectively. Although, there is apparent spillover of samples due to shaking hands during loading, the overall picture of the gel reveals that samples loaded at quantities between 10µg and 30µg gave better pictures; with 10µg tending towards under loading while 30µg tending towards overloading. Obviously concentrations of 50µg was too concentrated and so produced busy bands. Literature already prescribed loading samples at concentration level of 4µg to 10µg. Going by this results, the optimized quantity for loading the researchers samples are in the range of 10µg to 15µg. This result is imperative and the methodology recommended for young entrants in Electrophoretic process. As earlier observed, literature recommended proteins quantities and loading volumes may not produce good gel picture. This can be caused by differences in nature of sample particles, method of preparing sample, denaturing potency of loading buffer or other electrophoretic processes. The quantities observed to give good gel picture was adopted in most experiments in this study.

EXPERIMENT 3: ALTERNATIVE METHOD OF QUANTIFICATION OF CALIBRATION OF QUBIT FLUORIMETER:

The continuous use of standard kits to calibrate the Qubit fluorimeter is costly in addition to being cumbersome. There have been times when one of the standards may be unavailable. At such a time or for the reasons of saving cost the researcher has found the method of using 3 similar samples prepared under the same condition to calibrate the machine useful. If the machine is calibrated, the protein quantities in the three samples should be the same. This experiment was carried out and the results are as shown below.

Results of protein concentration from three similar sample

S/N	Description of Sample	Quantity of Protein (ng/μL)
1	Sample tube 1	741
2	Sample tube 2	742
3	Sample tube 3	742

Following the above results, the machine can be said to be well calibrated as it could measure the three samples from the same stock, three different successive times and give the same results under the same condition. The slight drop of 1 ng/μL observed in sample 1 could be because sample 1 was the first sample to be prepared and due to keeping the protein content may drop.

EXPERIMENT 4: INVESTIGATION OF EFFECTS OF CHLING ON IMMUNOGENICITY OF PROTEINS OF *C bovis*

Samples were prepared and proteins were quantified as described in operations 1-8 above.

Electrophoresis was run as described in operations 10 to 16 above

Table2 showing protein quantities and volumes of loading buffer for each gel well.

S/N	Sample Description	Quantity of protein (ng\μl)	Volume of Sample to achieve 10μg (μl)	Volume of Dyes to make up 20μl	Well Position
	Protein Marker	–	7.0	–	Well 1
Sample 1	Cyst fluid at Day 47	2250	4.3	17.0	Well 2
Sample 2	Cyst fluid at Day 40	1124	8.9	11.1	Well 3
Sample 3	Cyst Fluid at Day 30	2280	4.4	15.6	Well 4
Sample 4	Cyst Fluid at Day 21	2200	4.5	15.5	Well 5
Sample 5	Cyst fluid at 14	2000	5.0	15.0	Well 6
Sample 6	Beef + PBS	2320	4.3	15.7	Well 7
Sample 7	Loading Buffer	–	0.0	20.0	Well 8
	Loading Buffer	–	0.0	20.0	Well 9
	Loading Buffer	–	0.0	20.0	Well 10