THE EFFECT OF REPEAT INOCULATION OF FIELD-GRAZING CROSSBRED CALVES WITH TLR 7/8 AGONIST ON THE DURATION OF PROTECTION AGAINST DISEASES

BY

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THIS THESIS IS SUBMITTED TO THE UNIVERSITY OF GHANA, LEGON IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE AWARD OF MASTER OF PHILOSOPHY DEGREE IN ANIMAL SCIENCE

JULY, 2019

DECLARATION

I hereby declare that this thesis which is submitted to the University of Ghana, for the award of Master of Philosophy in Animal Science degree, is the result of my own original work and that no part of it has been presented for another degree in this university or elsewhere. All assistance towards the production of this work and all the references contained herein have been duly credited.

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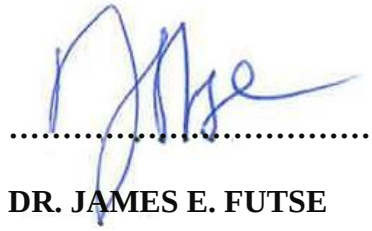

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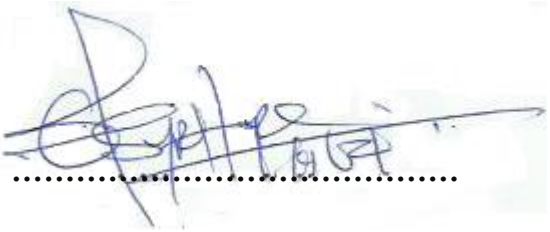

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DEDICATION

This work is dedicated to the almighty God, whose unmerited favour has been a shield about me through my years of study in this university. I also dedicate this to Mr. and Mrs. Ashiabor and my sister, Ashiabor Roselyn.

ACKNOWLEDGEMENT

Great is the Lord and most worthy of praise; His greatness no one can fathom.

To my parents, Mr. and Mrs. Ashiabor, Thank you for your immense ssupport, prayers and faith in me.

I am extremely grateful to my supervisor, Dr. James E. Futse and Dr. Hope Richard Otsyina, for their guidance, patience and advice to the end.

To A.G. Leventis Foundation Scholarship, a great contributor to the success of my project

I am also thankful to the staff of the Department of Animal Science, especially Mr. Jonathan Quaye and Dr Carl S. Beckley whose unwavering support led to a completion of my study here. Words are not enough to expres Hikmat Bilal.Heart felt gratitude to my sister, Ashiagbor Roselyn, whose solid love cannot be comprehended.

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ABSTRACT

Effective control of tick-borne diseases in endemic regions requires control of a complex of pathogens rather than a single pathogen-disease entity. A separate vaccine targeting each pathogen is not feasible for a variety of reasons. Therefore, the most effective means of control is to use one intervention to target multiple pathogens. Presently, we have demonstrated that stimulation of innate immunity using a Toll-Like receptor 7/8 agonist (TLR7/8) protects against multiple pathogen strains including anaplasmosis and ECF in the crossbred calves.

These results, although represented compelling evidence of agonist-induced innate immune protection, the duration of immunity conferred by the agonist is unknown. In most disease endemic areas, challenge will occur within 6 months, and as shown in Ghana, often much sooner. Whether the current formulation and single dose provides innate immune stimulation over this window is a critical question that will require data to resolve. The current study tested the effect of the agonist on disease and disease-associated costs. The cost savings from reduction in treatment costs and avoided death loss can be used to establish a product cost profile. There were three groups of 25 calves. Group 1 was administered with a single dose (1ml) of TLR7/8 emulsion at Day 0, to stimulate the innate immunity prior to exposure to natural disease challenge by vector ticks on the field; Group 2 was identically administered with the TLR7/8 emulsion at Day 0, and repeated at day 28; Group 3 was designated as an untreated control (calves were not injected with TLR 7/8 emulsion). All calves were allowed to comingle and graze on the field. Clinical signs and the need for treatment were assessed daily by the resident veterinary personnel. Specifically, 5 distinct pathogen species were detected in the herd of crossbred calves examined. *Anaplasma marginale* represented the most prevalent pathogen species accounting for >70% of tick-borne infections

among crossbred calves examined. Other infectious diseases detected included Dermatophilosis, Contagious Bovine Pleuropneumonia (CBPP) and Foot and Mouth Disease (FMD). All calves including those stimulated by injection with the agonist were infected with *A. marginale* within 7 days of exposure to field challenge. Calves from all groups were infected with at least one pathogen. There was no statistically significant difference in the mean numbers of pathogen species circulating in calves from various groups. Notably, all 25 (100%) animals from the group that was not injected with the TL7/8 emulsion required therapeutic treatments against at least one disease. In contrast, the need for treatments were 48% and 36% respectively among calves injected with 1ml and 2ml of the agonist. Although, the difference in the need for treatment between the stimulated groups was not significantly different ($p = 0.254$) the survival analysis has revealed that calves stimulated twice had the highest probability (88%) of surviving as compared to those either stimulated once or were unstimulated ($p < 0.001$). Importantly, of the twenty-five unstimulated calves, 15/25 (60%) died of clinical diseases including Anaplasmosis as compared with 14% deaths from the group that received TLR7/8 agonist stimulation. The Odds Ratio for survival is 13.6 (95% confidence interval of 1.2-152.2). While the confidence interval is wide, it does not overlap with 1, the null value, indicating the protection in the group receiving the TLR agonist has a high likelihood of representing a reproducible effect. There were significant differences in IgG2 production with respect to number of stimulations ($p<0.000$). Calves stimulated twice demonstrated a significantly higher percent inhibition of 77.07 as compared to either the cohorts that were stimulated once (68.56) or unstimulated (29.17). Generally, disease-related death of calves within the groups represented the major source of cost to the farmer. This was most significant among the unstimulated group (GHC 18000). The benefit cost ratio revealed that, the group

stimulated twice (6.12) was the most cost effective as compared to groups stimulated once (4.15) and unstimulated (0.45). In summary, this study has identified that the TLR7/8 water-in-oil emulsion provides sufficient innate immune protection to mitigate severe disease upon natural exposure to multiple vector-borne pathogens during the first six to12 months of life. While a single inoculation that provides a long-lasting depot for sustained induction of innate immunity over the six to 12 month vulnerable period would be optimal, a minimally acceptable regimen would be monthly inoculations, feasible in small-holder farms where animals are housed locally and given that the final emulsion cost is estimated between US$0.05-0.20. Together these results provided the rationale for recommending multiple innate immune stimulations of young crossbred calves as the most cost effective approach to protecting field-grazing crossbred cattle against diseases in Ghana.

LIST OF ACRONYMS AND ABBREVIATIONS

AD	-	Anno Domini
BCR	-	Benefit Cost Ratio
CBPP	-	Contagious Bovine Pleuropneumonia
cELISA	-	Competitive Enzyme Link Immunosorbent Assay
CTL	-	Cytotoxic T Lymphocyte
DNA	-	Deoxyribonucleic Acid
ECF	-	East Coast Fever
EDTA	-	Ethylenediaminetetraacetic acid
ELISA	-	Enzyme Link Immunosorbent Assay
FAO	-	Food and Agriculture Organization
FMD	-	Foot and Mouth Disease
IgG	-	Immunoglobulin Gamma
MHC	-	Major Histocompatibility Complex
MoFA	-	Ministry of Food and Agriculture
MSP2	-	Membrane Surface Protein
NK	-	Natural Killer
OD	-	Optical Density
OIE	-	Office international des épizooties
PCR	-	Polymerase Chain Reaction
PPR	-	Peste de Petite Ruminante
PRRs	-	Pattern Recognition Receptors
RBC	-	Red Blood Cell
SAT	-	Southern African Territories

TB	-	Tuberculosis
TBDs	-	Tick- Borne Diseases
TLRs	-	Toll Like Receptors
VACNDA	-	The Vaccine Against Neglected Animal Diseases
VSD	-	Veterinary Services Directorate
WHO	-	World Health Organization

CHAPTER 1

1.0 INTRODUCTION

1.1 BACKGROUND

Cattle sheep and goats are sources of animal protein. Majority of these animals are the indigenous breeds: West African Shorthorn (WASH), Zebu, N'dama, Sanga (MoFA, 2016). These breeds are known for their ability to manage the severe disease and environmental conditions (Terefe *et al.,* 2015) and utilize feed efficiently in case of poor forage conditions (Shabtay, 2015). Tick-borne diseases are a major challenge in the cattle industry leading to economic losses (Gashaw, 2005). Though indigenous breeds of cattle can withstand harsh environmental conditions, they are poor producers of milk and meat (Renaudeau *et al.,* 2012; Dossa and Vanvanhossou, 2016). With increasing population growth, continuous rearing of these animals is expected to result in the shortage of animal protein. In sub-Saharan Africa, perennial shortage of these products led to dependence on the importation of milk, meat and relevant animal products (Thornton, 2010).

However, there is a current approach to solving this protein deficit in developing countries. This approach includes the generation of crossbred cattle through artificial insemination on farms and research institutions. In Ghana for example, Friesian X Sanga F1 cattle are produced at the Amrahia Dairy Farms of Ministry of Food and Agriculture, Kwame Nkrumah University of Science and Technology, Council for Scientific and Industrial Research (CSIR), and University of Cape Coast, (MoFA, 2016).

According to FAO (2018), crossbred cattle are fast maturing, enhanced in milk and meat production as compared to the indigenous cattle. In spite of these advantages there are some problems associated with the production of crossbred. They are very expensive to purchase by low resource farmer, sensitive to tick infestation and inclement weather such as drought, and they require feed supplementation.

Most importantly, crossbred cattle are prone to diseases and parasites (Mureda and Zeleke, 2007). Diseases are economically relevant to the cattle industry. These economic losses manifest as high cost of production in the livestock system which are the cost of control, treatment and prevention (Morris, 1997; Rushton, 2009). Some of these diseases are anaplasmosis, East Coast Fever (ECF), babesiosis, anthrax, trypanosomiasis, contagious bovine pleuropneumonia (CBPP), brucellosis, tuberculosis, and foot and mouth disease (FMD), and other common parasitic diseases. Tick-borne diseases (TBDs) accounts for more than 80 percent of all vector borne related deaths of cattle, among which Anaplasmosis, Babesiosis, Heartwater and Theileriosis are the key TBDs in cattle (Marcelino *et al.,* 2012). The leading control management of ticks is the use of acaricides. In West Africa, tick-borne diseases have repeatedly frustrated attempts to upgrade indigenous cattle breeds through cross-breeding with, exotic highly productive cattle such as Friesians(Tessema and Gashaw, 2010). Control of tick-borne diseases in countries such as Ghana has been achieved through a relatively inefficient combination of strategic acaricides application, drug treatment, and use of mostly poorly productive indigenous cattle breeds.

In recent years, experimental vaccination of small ruminants against Heartwater, caused by *Ehrlichia ruminantium*, has been attempted on a small scale in some parts of West Africa, with limited success (Allsopp, 2009). However, there has been no attempt to immunise cattle (large ruminants) against Heartwater, Babesiosis or Anaplasmosis. Notably, attempts made against diseases using various vaccines have not failed. However, Tropical regions of the world are a home to vectors ticks (WHO, 2014). While they feed on mammals, they also transmit pathogens in the process. In regions that have heavy tick infestations, cattle serve as reservoir for multiple pathogen species (Beckley *et al.,* 2016; Bursakov and Kovalchuk, 2019). There is a need to produce vaccines against individual infections in cattle. Vaccines effectively protect livestock against some diseases such as CBPP and FMD (Lubroth *et al.,* 2007). However, other

re-emerging diseases seem to pose a threat and vaccine production against these different types of infection on a large scale will not be cost effective. This will lead to budgetary constraints on the resource poor farmer.

There is an ongoing approach to control multiple tick-borne disease problems in Africa in the absence of classical vaccines. This process involves the development of a single intervention to target multiple pathogen-disease entities using broad spectrum innate immune stimulation which has solid bases in immunology. Microbes are detected by the immune system using pattern recognition receptors (PRRs). These antigen receptors are specialized in recognizing molecular characteristics common to a wide array of microbes (Sarfo-Owusu, 2016). Notably, the synthetic forms of Toll Like Receptors (TLR) can also serve as an agonist, that when emulsified in an adjuvant will prime the immune system against diseases (Kawai and Akira, 2011). There are some key differences between broad spectrum innate immune stimulation and vaccination. Vaccination targets specific pathogen for destruction by the corresponding immune response. Thereafter, a long-term memory is established to protect the individual against future challenges. Unlike the classical vaccination, stimulation is versatile and targets multiple pathogens. This design has positioned innate immune stimulation by TLR7/8 agonist approach to deliver broad spectrum stimulation against multiple pathogens. That this approach works with cattle was exemplified in crossbred calves. At one site, Accra in Ghana, Friesian X Sanga F1 calves immunized with 1 ml of agonist prep were protected against tick-borne disease challenge on-field (Futse *et al.,* unpublished). At a second site, Morogoro, Tanzania, calves stimulated with TLR 7/8 agonist developed immunity against artificial infection of East Coast Fever (Sarfo-Owusu, 2016). These results, although represent compelling evidence of agonist-induce innate immune protection, the duration of immunity conferred by the agonist is unknown. In most disease endemic areas, challenge will occur within 6 months, and as shown in Ghana, often much sooner. Whether the current TLR7/8 formulation and

single dose provides innate immune stimulation over this window is a critical question that will require data to resolve. The goal of this study is to test the effect of the agonist on disease and disease-associated costs.

1.2 JUSTIFICATION

More than 300 million people in Africa live below the poverty line. Over 7% of the poorest of the poor depend on livestock production as their source of livelihood (FAO, 2007). Animal production serves as source of food, income, protein, traction and manure for crops (FAO 2011). Mortality and morbidity due to diseases among cattle in Ghana increases the cost of production. This scenario has major implications because it exerts high economic burden on cattle farmers. Some of the common diseases that limit livestock production include Foot and Mouth Disease, Dermatophilosis, Contagious Bovine Pleuro Pneumonia (CBPP), Anthrax and tick-borne diseases (Nene *et al.*, 1995; FAO 2016). In sub-Sahara Africa, infectious diseases are responsible for over 20% of death-related losses of livestock. This problem has resulted in limited productivity of critically needed animal products such as milk and meat with an estimated loss of 20 billion dollars (AU-IBAR, 2013). Currently, there is no applicable control strategy that is globally scalable. Over the years, occasional interventions have been employed to save the livestock industry from major disease outbreaks (MoFA, 2016). Vaccination provided the most dependable approach to control diseases (FAO, 2016). Currently, vaccines readily available have restricted spectrum of targets. As they are antigen-specific by design. Vaccines that were developed for a particular disease do not elicit a wide-spectrum of immune stimulation, rendering them limited in providing protection against a wide pool of pathogen strains, notwithstanding the high cost involved in producing them. Hence, are very difficult to acquire by the poor resource farmer. The current project is responding to the need to develop a novel approach to controlling multiple diseases using single intervention. This goal is achievable by way

of innate immune stimulation of young calves before they are exposed to infectious disease challenge. The need to protect cattle and other livestock against disease is of a major concern to farmers in Africa (Mockshell *et al.,* 2014). Results from the current study will therefore provide a new pathway to achieving this goal and develop a broad spectrum protection for animals. The proposed intervention, unlike classical vaccines that target the pathogen, will stimulate the host cells by targeting the immune host cells instead of the pathogen. It is highly unlikely that these agents (TLR agonist) will develop into antimicrobial resistance when used repeatedly (Jefferson *et al.,* 2005; McElhaney *et al.,* 2006).

Pioneering studies of innate immune stimulation of cattle using single 1ml dose of TLR 7/8 agonist have been carried out in both Ghana and Tanzania (Futse, unpublished; Owusu-Sarfo, 2016). The results have demonstrated that it was possible to protect young calves against prototypical bacterial and protozoan pathogens by innate immune stimulation using TLR7/8 agonist. Although, results from this study strongly supported the implementation of this innate immunity boosting strategy, it has failed to address whether the one-time stimulation is sufficient to ensure a lasting post-stimulation response in cattle. The current study will address this concern and provide actionable vaccination data for nationwide disease surveillance and control. Understanding the duration of protection produced by innate immune stimulation will provide alternative pathways to vaccine development suitable for effective disease control throughout Africa. Not only would data from this work narrow the knowledge gap among scientist and researchers by providing a baseline data to guide vaccines development and deployment against multiple pathogen entities. It will essentially reduce the cost of disease control in the endemic regions of the world to allow farmers achieve food and economic security.

1.3 HYPOTHESIS

The duration of protection of crossbred calves by innate immune stimulation with TLR 7/8 agonist increases with repeat inoculations

1.3.1 OBJECTIVES

1) To determine if a single inoculation of the TLR7/8 agonist is sufficient to provide protection through the first 6 months from conception.

2) To determine if a repeat inoculation of the TLR7/8 agonist at monthly intervals is sufficient to provide protection through the first 6 months from conception.

3) To determine if the TLR7/8 stimulation reduces the cost of producing crossbred cattle in Ghana

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 TYPES OF CROSSBRED CATTLE IN GHANA

Indigenous cattle breeds in Ghana possess qualities that enhance their survival. They are able to withstand adverse climatic conditions, pest and disease tolerance, grow well under little or no feed supplementation. In spite of these, they are poor milk and meat producers which has led to a high demand on protein by a growing population (Simianer, 2000). The resolution of this demand on protein has led to the introduction of breeding programs that involve crossbreeding principles.

There have been different breeding experiments in Ghana of which involves breeds of interest with high physiological characteristics. These breeds include the Sanga, West African Shorthorn, Sokoto Gudali, N'Dama, White Fulani and Muturu (Aboagye, 2002). Also, some temperate breeds were purchased and used in crossing with some of the local breeds (Aboagye, 2002)

In 1964, 30 Black and White cattle were imported from Russia by the State Farm Cooperation to establish a farm at Adidome in the Volta Region. In the Accra plains, Ministry of Food and Agriculture established a farm equipped for 800 cattle on the Accra-Dodowa road at Amrahia (Aboagye, 2002). Due to the demand on milk, Holstein-Friesian was selected and, in 1967, 1974 and 1976 Friesian heifer and bulls calves were introduced from the UK and 400 Friesians, 200 in each of the years, from The Netherlands respectively (Aboagye, 2002). In 1974, Canadian International Development Agency provided assistance to breeding programmes by introducing Holstein-Friesian Heifer calves and bulls (Aboagye, 2002; Alhassan and Owusu, 1980; Buadu, 1977).

Crossbreeding is well defined as the method of generating progeny, particularly through mating two purebred individuals but come from different breeds or varieties (Lasley 1972). It is known to improve upon heterozygosity and increase genetic variation (Aboagye, 2002).

In Ghana, crossbreeding to improve livestock started in 1958 at the University of Ghana Agriculture Research Station (ARS) with the Jersey breed being the first (Aboagye, 2002). Friesians were later introduced through artificial insemination on Ghana Short Horn and Sokoto Gudali cows (Aboagye, 2002).

2.2 DISEASES IN RUMINANT LIVESTOCK PRODUCTION

Animal diseases affects livestock production in two ways; directly that is deaths, morbidity leading to reduction in productivity and indirectly which includes the cost of measures for controlling and prevention (FAO, 2016). These vary from a localized setting to a global point of view (Perry and Sones, 2009). The fiscal impact of these diseases is very challenging to measure, this is dependent on the complex nature of the disease (Bio-Era,2008) but over few years there have been a decline in the burden of livestock diseases that resulted from improved effectiveness of drugs, vaccines and improved diagnostic technologies and services (Perry and Sones 2009). However, emerging and re-emerging of diseases resulting from change in host species (man to livestock), environmental factors, and migration that leads to the introduction of less virulent strains of pathogens into areas where they later become virulent.

In Africa, husbandry practices are managed in an extensive manner, livestock are allowed to graze on pasture lands, these pasture lands provide enough feed during the raining season for animals whereby the farmer will not have to provide supplementary feed, also providing them with poorly constructed structures to house these animals and protect them from thieves (Lawal-Adebowale, 2012). Under this system, animals are exposed to various types of disease that are transmitted by pathogens and parasites that

affect livestock productivity and health (Erasmus and Webb, 2013). Some of these disease that affect cattle production are Contagious Bovine Pleuropneumonia (CBPP), Anthrax, Foot and Mouth Disease (FMD), Brucellosis, Tuberculosis and Tick-Borne disease.

2.2.1 CONTAGIOUS BOVINE PLEUROPNEUMONIA

CBPP is also known as Lung sickness in cattle. *Mycoplasma mycoides* subspecies *mycoides* small colony variant (MmmSc) is the causative agent. Cattle are infected by inhaling droplets and contact with infected animals. It is present in the Western, Eastern, Central and some parts of Southern Africa, which was first defined in 1550 by Gallo (Provost *et al.*, 1987). Some sources through which infection is acquired includes, bronchial secretions, nasal discharges, exhaled air, spread through urinal discharge was not fully confirmed, isolation of microorganisms from the semen has been possible but transmission through semen is unknown (Amanfu, 2009)

CBPP is one of the deadliest disease affecting cattle production in Africa, second to Rinderpest; CBPP is well thought-out to be the utmost severe transboundary disease of cattle (Anon, 2012; Abdela and Yune, (2017). It is considered by the presence of sero-fibrinous, interstitial pneumonia, interlobular oedema and hepatisation giving a marbled appearance of the lung and capsulated lesions termed sequestra in the lungs of affected cattle (OIE, 2009). Incubation period of the pathogen could last between the periods of 3 weeks to 6 weeks, occasionally 6 month of which the animal would start showing signs of high fever, anorexia, fall in milk yield, cessation of rumination, severe depression and sometimes cough (Cfsph, 2005). According to Sacchini *et al.* (2012), CBPP has been eliminated from certain parts of the world with stringent policies such as; test and slaughter, restrictions in importation of live animals, compensations to livestock owners in the 20th Century, it is endemic in Africa (Tambi *et al.*, 2006). It is however difficult to implement these policies in Africa and the disease is very common

in pastoral areas, which has led to the impediment of development of livestock in Africa. In 1970, there was a decline in the prevalence of the disease in Africa, it was later recorded in 1980 and the early 1990s that the disease had re-emerged, and this resulted from economic and financial difficulties faced by the government to fund veterinary services in controlling the re-emergence (Tambi *et al.,* 2006). Abdella and Yune (2017), reported an outbreak of CBPP in about 20 African countries, to mention a few Ghana Burkina Faso, Senegal, and Congo and in 2011, it was recorded that highest outbreak occurred in Burkina Faso and Ghana (Amanfu *et al.,* 1998). The economic importance of CBPP is losses due to the chronic disease which is difficult to assess, others are losses due to mortality, weight loss, reduction in fertility, reduced working rate of animal (Masiga and Domenech, 1996). Tambi *et al.* (2006) projected the price tag of CBPP at 3.7 million euros per 12 Sub-Saharan African countries, proposing that an investment of 14.7 million euros to combat CBPP would avoid a loss of 30 million euros.

The best form of controlling CBPP are stamping out, test and slaughter, animal movement control, quarantine and vaccination with T1 vaccines (Radostis *et al.*, 2000). Also, minimization of contact with infected animals, surveillance programs that would detect the nearest outbreak to enhance quarantine procedures (FAO 2001; FAO 2004; FAO 2007).

2.2.2 FOOT AND MOUTH DISEASE

It is a severe, contagious disease caused by a viral organism which affects pigs, cows, goats, sheep, deer, and other hooved-animals (Veterinary, 2013). However, it does not affect horses, cats or dogs. It is of concern globally which causes economic loss significantly (Veterinary, 2013). It is caused by *Picornaviridae* (Longjam *et al*., 2011).

The virus occurs in seven serological forms and can be differentiated genetically, namely, O, A , C, Asia1, SAT1,2, and 3 , within each subtypes larger numbers have developed (Longjam *et al*., 2011). Foot and mouth disease virus (FMDV) is a single stranded (ss) positive sense RNA virus which possesses a sedimentation coefficient of 146S (Longjam *et al*., 2011; Bachrach,1968). Foot and mouth disease also known as FMD affect extensive areas which makes it very important to the World Health Organization of Animal Health (Rushton *et al*., 2012). It causes production losses and impede livestock trade, a single detection would halt livestock trade (Veterinary, 2013). The first signs of FMD develops within two to fourteen days, these signs would manifest considering favourable conditions for the virus. However, extreme temperature, time and alternating pH can inactivate the virus (Longjam *et al*., 2011; Veterinary, 2013). Clinical signs include; blisters, erosion in the mouth or feet, rise in temperature, foamy and sticky saliva, lameness in feet, difficulty in eating due to lesions in the mouth, cloudy discharges from vesicles, abortion, low milk production in dairy cattle, new-born animals develop heart diseases and die (Jamal and Belsham, 2013; OIE, 2015; Rushton *et al*., 2012; Veterinary, 2013). Moreover, it is transmitted through physical contact of susceptible animal, moving animals in contaminated vehicles, feeding with contaminated meat or feed, direct contact with people in soiled clothes, shoes or handling contaminated equipment, drinking contaminated water, exposure to contaminated biological agents, and in some cases when cows are inseminated with the semen of an infected animal (Veterinary, 2013). According to literature, the first detection of this disease was in Italy by Hieronymus Fracastorius, a monk who discovered the disease in cattle near Verona in 1546, AD. 400 years later, Friedrich Loeffler and Paul Frosch were able to filter the agent and associated it to FMD (Rweyemamu *et al*., 2014; OIE, 2015;)

FMD has been recorded to have low mortality rates but it is still considered a relevant disease of farm animals because it causes greater losses in production and trade. The

virus causes severe lesions in the myocardium of young animals, which leads to death (Domingo *et al.,* 1990)**.** The reason why it is considered the most dangerous viral disease is because it is highly contagious, widely distributed geographically, expansive host range, antigenic variation leading to poor cross-immunity and duration of immunity is very short (Tamilselvan *et al.,* 2009)**.**

In West Africa, serotypes O, A, SAT 1 and 2 have been identified (Sangaré *et al.*, 2004). The current demand for protein, competition for international trade has raised the disease profile in Africa (Sangaré *et al.*, 2004)**.** In South Africa, wildlife plays an important role in the spread of FMDV but this is off no major concern in West Africa, the disease is transmitted by trans-boundary movement due to seasonal changes driving nomads from one region to another in search of pasture (Thompson, 1996; Bastos *et al.*, 2000**).**

Serotypes O, A, SAT 1and 2 were identified in Ghana, using antigen detection ELISA kit for produced by the Istituto Zooprofillatico Sperimentale of Brescia, Italy was positive for serotype SAT 1 in 2018 (Pinto, 2017)**.** The last outbreak was in 2016 Pinto, 2018)

FMD control strategies in Africa is rarely possible due to the difference in serotypes circulating in host species (Rweyemamu *et al*., 2014). The disease can be effectively controlled using, serotype surveillance systems, separation of wild species from livestock, and regular vaccination (Thompson *et al.,* 2003; Bruckner *et al.,* 2002; Jori *et al.,* 2009).

2.2.3 ANTHRAX

Anthrax is an acute, per acute or subacute zoonotic illness that affects herbivores mainly cattle, sheep, goat, deer and occasionally affect birds (Turnbull, 2002). It is caused by a gram positive, rod-shaped microbe that dwells in the soil. It is known as *Bacillus anthracis* (Turnbull, 2002; NCEZID, 2016)**.** Its popularity originated from it being used

as a biological warfare agent (Ravenel, 1940). Infection is established when host releases the vegetative bacilli onto the ground and begins sporulation as a result of exposure to air, spores could remain in the soil for decades and later be carried by another host for multiplication (Ravenel, 1940; Turnbull, 2002). Larger outbreaks in endemic areas are influence by flies. Humans are infected through poor handling of carcasses, bones and hides or dead infected animals (Ravenel, 1940; Turnbull, 2002). Some major factors that influence spore formation of *Bacillus anthracis* are pH, temperature, water activity, and other seasonal factors include grazing, health of the host and population of insects and humans (Ravenel, 1940; Turnbull, 2002; NCEZID, 2016). Animals acquire the disease through ingestion of spores during grazing, inhalation of spores in dust, direct animal-to-animal contact (Ravenel, 1940; Kusiluka and Kambarage, 1996; Turnbull, 2002; NCEZID, 2016;). However, clinical signs usually appear 3 to 7 days after the spores have been ingested or inhaled. Animals die within two days after signs are established (Ravenel, 1940; NCEZID, 2016). Some clinical signs observed are; difficulty in breathing, tremble, seizures and death followed after a few minutes after the animal collapse (Ravenel, 1940; Turnbull, 2002). Finally, dark blood oozes from natural orifices of dead animals. clinical signs are not serious in cats, dogs and pigs (Ravenel, 1940; Turnbull, 2002; NCEZID, 2016).

In Ghana, outbreaks are recorded annually since 1988 including cases related to humans infected through contact with contaminated animals and these are documented in the World Data anthrax site showing the economic impacts of the disease to the livestock industry (Kracalik *et al.*, 2017). Vaccines are produced locally by the Central Veterinary Laboratory in Pong-Tamale, Ghana and are subsidized by government. Regardless of this, occurrences are recorded, especially in cattle (Kracalik *et al.*, 2017).

According to Nsoh *et al.* (2016), 43 outbreaks were confirmed including death amongst cattle, sheep goats, pigs and humans. The peak of outbreaks varies seasonally of which

are highest at the end of the dry season and the beginning of the rainy season (Chikerema *et al.*, 2013)

Control measures are targeted at halting the progression of the disease among livestock, these measures are to be dynamically implemented (Turnbull, 2002). Anthrax can be controlled by; destroying pathogen hotspot areas, moving animals from infected, incinerating or burying infected dead animals, (Turnbull, 2002).

2.2.4 BRUCELLOSIS

It is a disease caused by a facultative intracellular gram-negative coccobacillus bacterium of genus *Brucella*. It affects different animal species and humans as well. *Brucella abortus* causes of late abortion in pregnancies of bovines, leading to possibilities of infertility or sterility (Hesterberg *et al.*, 2008). Brucellosis was first identified in 1886 through the isolation of the pathogen from infected Soldiers in Malta by Sir David Bruce (Mangen *et al.*, 2002). It is known to be a prevalent zoonotic disease, which has globally burdened poor people and livestock efficiency (McDermott *et al.*, 2013a, 2013b; WHO, 2005).

In Africa, *Brucella spp* have been associated with the formation of hygromas in cattle which is not consistent with the infection features (Mangen *et al.*, 2002).

The bacteria are expelled with the foetus, placenta and uterine fluid during the time of calving or abortion or parturition. It can also be excreted in the milk of cows that are infected (Charters, 1980; DFRA, 2002). It can be transmitted to cows through infected bulls during mating (DFRA, 2002). In exception of infection through contact, contaminated water and feed are other relevant sources (Morgan and MacKinnon, 1979). Despite inadequate amount of data capturing brucellosis and its impact on human and animal health, the little data available is evident that the disease is very important and a widespread problem in Africa (Ducrotoy *et al.*, 2017). There is serological evidence of the wide distribution of brucellosis over Sub-Saharan Africa.

However, they are scattered over space and time (Ducrotoy *et al.*, 2017). A decade ago, data were collected and interpretations of the figures were be done cautiously because of doubts in the test application and authentication. Despite these diagnostic uncertainties, a number of studies recorded that the seroprevalence of the disease was associated with the number of abortions that occurred (Akakpo, 1987; McDermott and Arimi, 2002; Megersa *et al.*, 2011a; Muma *et al.*, 2012). On small ruminant in Sub-Saharan Africa, it is has been established that brucellosis in small ruminants is caused by *Brucella Melitensis* (Ducrotoy *et al*., 2017)**.**

South Africa is the only country that has implemented the programs that would enable a gradual eradication of this disease. In some sub-Saharan African countries stamping-out and vaccination as a mode of controlling the disease is done on ad-hoc basis as carried out in Botswana, Namibia, Lesotho and South Africa (Ducrotoy *et al.*, 2017)**.** Other countries like Zimbabwe carried out vaccination on targeted cattle production systems not to eradicate the disease, but to control or reduce its prevalence (Emslie and Nel, 2002). Where vaccinations were implemented and carried out B. abortus S19, B. abortus RB51 and RB51 which was later introduced in Mozambique and Zambia (McDermott and Arimi, 2002; Muma *et al.*, 2012; Davey, 2014; Ekron, 2008).

2.2.5 TUBERCULOSIS

Mycobacterium Tuberculosis complex are a collection of microbes that causes Tuberculosis (TB) (Addo *et al.*, 2016). They are; *Mycobacterium bovis, Mycobacterium tuberculosis, Mycobacterium avium* (Kaneene and Theon, 2004). The rate of occurrences over the years is increasing due to the detection of multi-drug resistance among strains of the causative agents (Cosivi *et al.*, 1998). *Mycobacterium tuberculosis* causes tuberculosis in man, *Mycobacterium avium* causes tuberculosis in birds and *Mycobacterium bovis* is associated with tuberculosis in cattle**.** However other animals such as; dogs cats, horses, pigs, squirrels, lions to mention a few serve as reservoir for

bovine tuberculosis (Gutpa *et al.*, 2009). Tuberculosis is an airborne infection, carried by airborne particles and their route of infection is not through physical contact with infected person but through the inhalation of droplet released from an infected individual (Center for Disease Control and Prevention, 2013). These airborne particles are called droplet nuclei. Droplet nuclei are released through sneezing coughing, shouting or singing, depending on the environmental conditions, these particles can remain in the air for several hours. Not all people infected with TB exhibit signs and symptoms but those who do show signs of coughing that lasts for 3 weeks and longer and chest pains (Center for Disease Control and Prevention, 2013).

In Ghana, there are three common strains of *Mycobacterium* found in patients with TB, *Mycobacterium tuberculosis, Mycobacterium africanum and Mycobacterium bovis* (Addo *et al.*, 2007). However, Mycobacterium bovis which is virulent in cattle, affects both man and animals with a nature similar to Mycobacterium tuberculosis (Kaneene and Pfeiffer, 2006; Biet *et al.*, 2005). In recent times, bovine tuberculosis is becoming endemic in developing countries, reason being the difficulty in implementing policies properly (Cosivi *et al.*, 1998). According to Addo *et al.* (2007), Mycobacterium bovis forms 3 percent of the pulmonary tuberculin cases recorded in Accra. A current estimation suggests that one-third of the people living in the world have TB with about 5 to 10 percent of these individuals who may be negative to HIV but likely to be infected with tuberculosis (WHO, 2005). Bovine tuberculosis is obtained by drinking unpasteurized milk of infected animals, eating improperly cooked infected animals, inhalation from animals (Ayele *et al.*, 2004). Also, bovine tuberculosis is very common among herdsmen who spend their whole lives with their animals, this offers enough time for zoonotic transmission of the infection (Amemor *et al.*, 2017).

In tuberculosis diagnosis, in vivo intradermal comparative tuberculin skin test is the standard used in cattle but it lacks specificity and sensitivity (Lilenbaum *et al.*, 1999). Recently, gamma interferon (γ-IFN) test has been utilized for recognition of bovine TB

which identifies the cytokine γ-interferon which is essentially discharged by T-cells after an in vitro incitement with Bovine Purified Protein Derivative (BvPPD) and avian Purified Protein Derivative (AvPPD).(Ryan *et al.*, 2000; Gormley *et al.,* 2006). In Ghana, tuberculin skin testing of cattle is done sporadically. From 2010 to 2016, 516 TB cases were recorded from abattoirs and slaughter houses (Veterinary monthly summary reports, 2010). According to Amemor *et al*. (2017)**,** 19 percent prevalence of bovine tuberculosis was found in cattle which is higher than the ones investigated by others whose result was based on routine data from slaughter houses using a tuberculin test. Addo *et al*. (2016) stated otherwise in prevalence but further established that the reduction in percentage prevalence does not disprove other works, rather the kind of test used is more specific than that of the intradermal tuberculin test. In addition, reduction of prevalence percentage can be associated with several reasons; age of the animal, breed type, size of the herd and farming practices (Bonsu *et al.,* 2000).

2.2.6 DERMATOPHILOSIS

Dermatophilosis is a highly contagious skin infection that is caused by an aerobic bacterium (Dalis *et al.,* 2010). *Dermatophilosis congolensis* is a gram-positive aerobic bacterium that produces zoospores (Hirsh *et al.,* 2004). It was observed in Belgian Congo in the year 1915 in cattle (Shoorijeh *et al.*, 2008). According to Dejene *et al.* (2012), animals that are commonly infected are sheep, horse, cattle and sometimes dogs.

In cattle, it is called cutaneous streptotrichosis, in Sheep it is known as mycotic dermatitis, and rain scald in horses. Although, it is known as Senkobo in central Africa and Kirchi in Nigeria, many rural people know it to be Dermatophilosis (Radostits et al., 2007). It usually appears as proliferative or hyperkeratotic dermatitis, which is followed by a crust. It invades the skin and causes sever skin diseases (Yeruham et al., 2003). It is a very important disease in the tropics and subtropical regions of the world,

being that, it affects many ruminant species (Andrew et al., 2003). Factors that influence the disease occurrence include; rainy seasons, high relative humidity, injury to the skin, compromised host immune systems as a result of stress, and above all tick infestation (Yeruham et al., 2003). The organism can live on the epidermis of the skin without causing damage unless it is exposed to favourable conditions (Hirsh et al., 2004). Mode of transmission is through, shearing, contact with infected animal and dipping (Quinn et al., 2002). However, a moisturized environment enhances the release of zoospores (Radostits et al., 2007). The disease is properly diagnosed when there is an appearance of lesions on the animal affected and showing the presence of the causative agent beneath the affected areas (Kahn, 2005). The need for treating the infection is required if the integrity of the skin is severely compromised. The only intervention adopted by physicians is the use of antibiotics in treating infected animals (Awad *et al*., 2008). Losses are made due to the depreciation of the skin, making it difficult to obtain hide and in some cases, weaker animals die (Stewart, 1997)

2.2.7 TICK-BORNE DISEASES

Ticks are very small insect-like creatures found in natural vegetative areas, they feed by attaching themselves to humans or animals skin and sucking blood for several days (Parola and Raoult, 2001)**.** Generally, ticks do not cause disease but when infected they can pass a wide range of bacteria or virus to their host, they serve as vectors of diseases (Parola and Raoult, 2001)**.**

Tick-borne diseases are a major threat to domestic animal health and are of major economic importance to developing countries especially to the rural people, not only affecting their food supply but their daily earnings and other agricultural activities (Jongejan and Uilenberg, 2004; Ghosh and Nagar, 2014). Losses associated with tick-borne diseases were pegged between US$ 13.9 billion and US$ 18.7 billion in 1997 (de

Castro *et al.,* 1997). Eighty percent of the world's cattle population are affected by tick-borne diseases especially in the tropics and the subtropics (de Castro *et al.,* 1997)

Theileriosis, Babesiosis, Anaplasmosis and Heartwater *also* known as cowdriosis are the four important group of tick-borne diseases in the world causing management and health problems in affected areas. East Coast fever (ECF), tropical Theileriosis and Babesiosis are caused by protozoan parasites such as *Theileria parva*, *Theileria annulata* and *Babesia bovis* or *Babesia bigemina*, correspondingly, whereas Heartwater and Anaplasmosis are caused by the Rickettsiales; *Ehrlichia ruminantium* and Anaplasma marginale, respectively in cattle (Marcelino *et al.,* 2012).

2.2.7.1 THEILERIOSIS

Theileriosis is a disease caused by a parasite known as *Theileria;* a protozoan parasite affects both red and white blood cells of cattle. They are transmitted during feeding of arthropods, particularly ticks (Mans *et al.,* 2015). The two most important species in cattle and water buffalo are *T. parva*, which causes East Coast fever, and *T. annulata*, which causes tropical theileriosis (Fever, 2009)**.** In Ghana, tick-borne haemoparasite isolated from blood of cattle sheep and goats sampled from slaughterhouses had *Theileria* present (Macfie 1915). According to Assoku (1979), two species of *Theileria* were found after a comprehensive study of *Borrelia* and *Eperythrozoon* **.**It affects all age range of domestic ruminants especially cattle. However pregnant, lactating and stressed cows are prone to infection as a result of reduction in immunity (Jeanmonod *et al.,* 2018). According to Africa and Africa, (2012) cattle are infected when they are bitten by ticks that have already fed on infected buffaloes (serve as reservoir for pathogen). This implies that the disease is only found in cattle grazing around areas where buffaloes are or have once been present (Africa and Africa, 2012)**.** Some clinical signs are; depression, listlessness, isolation, Enlargement of local lymph glands, decreased milk production, anorexia, lacrimation, Gums and mucous membranes of

eyes may be pale, weakness leading to general difficulty in walking, Blocked cough, difficulty in breathing, and foamy fluid from the nose, nervous signs such as walking in circles and paralysis. About 80 percent of animals showing signs die within 3 to 4 days (Africa and Africa, 2012; Fever, 2009).

Globally there is a variation in the disease caused by the parasite but in Africa, East Coast Fever is severe (Fry *et al.,* 2016). Every year, over one million cattle die out *Theileria parva* infection in the sub-Saharan Africa, this has led to severe economic losses of which pastoral farmers are disadvantaged (Fry *et al.,* 2016). Mortality is very high in exotic breeds of cattle are more expensive, indigenous breeds however have been estimated to about 10 percent but can rise to a 90 percent in susceptible host (Lawrence *et al.,* 1988). In cases where mortality may not be present, there may be some less quantifiable losses such as; low milk and meat yield, paralysis, low fertility, exposure to other diseases caused by other parasites (Pegram *et al.,* 1989). The major control of this vector is the use of acaricides, which over the years have proven to be less efficient (Cox, 1991). On the other hand, immunization of cattle with live sporozoites followed by treating disease symptoms with long-acting oxytetracycline enhances the immune system of animals that survive (Brown *et al.,* 1977). This approach that induces insusceptible response, is mediated by major histocompatibility complex (MHC) CD8 T cells (CTL) particular for *T. parva* (McKeever *et al.,* 1994)

2.2.7.2 BABESIOSIS

It is a tick-borne disease of cattle that is caused by a protozoan parasite scientifically classified under genus *Babesia,* order *Piroplasmida,* phylum *Apicomplexa* (Cycle, 2013). In cattle, there are three primary species that causes clinical signs of babesiosis; *Babesia bovis* and *Babesia bigemina* are prevalent in tropical and subtropical regions, *Babesia divergens* is equally present in Europe and north Africa (Cycle, 2013). Mortality and morbidity are significant in cattle. However, economic losses are

considerable, when animals are migrated to endemic regions with no immunity (Rovid, 2018). The disease can be managed and treated but controlling the causative agent is a major problem. United States was able to eradicate the disease by targeting and eliminating the vector (Ticks) limiting their existence in a quarantine buffer zone between U.S and Mexico which took 40 years (Rovid, 2018). *Babesia* is transferred by ticks. The main vectors for *B. bigemina* and *B. bovis* are *Rhipicephalus microplus*, formerly known as *Boophilus microplus*, and in some areas, *R. annulatus*, formerly known as *Boophilus annulatus* (Rovid, 2018). There are no indications that bovine babesiosis possess zoonotic properties. However, babesiosis divergens causes severe illness in immunocompromised people (Rovid, 2018).

In cattle, clinical signs are visible two to three weeks after tick bite but inoculation with infected blood takes ten to twelve days for incubation of *B. bovis* and four to five days for *B. bigemina* (Rovid, 2018). The development of anaemia is sometimes rapid. Other signs include jaundice, weakness, abortion or temporal reduction in fertility among bulls, Haemoglobinuria and Hemoglobinemia are clinical signs in less acute stages of animals infected with *B. Bigemina*. Nervous signs such as incoordination, teeth grinding and manic behaviours are observed. It may cause other respiratory distress. It is important to note that B. *bigemina* and *B. divergens* do not cause similar changes in RBCs, and neurological signs in cattle are not common either. These may occur if the brain of the animal is completely deprived from oxygen (Brain Anoxia). Due to the constriction of the anal sphincter, pipe stem diarrhoea has been reported to be the earliest stages of babesiosis caused by *B. divergen.* Late stages of the disease results in dehydration, constipation and animals that survive are emaciated and weak (Rovid, 2018). There are dissimilarities in the rigorousness of the disease among individual animals, cattle younger than 9 months do not show clinical signs when infected with *B. divergens.* It has also been reported that mild illness coupled with a little fever, anorexia leading to recovery have been very common among some animals infected with *B.*

divergens (Rovid, 2018)**.** The most precise test in identification of the pathogen is PCR, this enables the identifying and distinguishing between the species of the parasite by amplifying and sequencing some closely related segments of *Babesia* (Rovid, 2018).

In Ghana, several studies have identified the presence of Babesia in cattle (Nagano *et al.,* 2013; Owusu, 2015; Beckley *et al.,* 2016).

The disease is effectively controlled by early detection, vaccination of calve using live attenuated *B. bovis* (Rovid, 2018).

2.2.7.3 ANAPLASMOSIS

This is a disease caused by the bite of ticks. The family, *Anaplasmataceae and* order, *Rickettsiales* is where the pathogen that causes the disease originates from (Mensah-Bonsu, 2016). Literature records a minimum of about twenty species of ticks that are vectors of the pathogen but the major transmitting tick is the *Boophilus microplus* (Aubry and Geale, 2011**).** Another mode of transmission is mechanical which is via fly or fomite bites or the use of unsterilized objects contaminated with the blood of an infected animal (Kumar *et al.,* 2015). Bovine anaplasmosis is caused by *A. bovis, A. phagocytophilum, A. marginale and A. centrale* (Matsumoto *et al.,* 2006). It is of major economic importance in countries such as India and a global menace (Rodríguez *et al.,* 2009; Brown 2012)**.** Anaplasmosis is known to affect all age range of cattle but some level of resistance is exhibited by adult cattle (Futse *et al.,* 2003). Mostly, *rickettsia Anaplasma marginale* infects cattle with respect to all species of tick-borne *Anaplasma* (Kocan *et al.,* 2003). Pathogenesis is established by *A. marginale* infecting matured erythrocytes of bovine replicating alongside; endothelial cells are also a possible site for infection. However, there is no literature regarding endothelial invasion in vivo by marginale (Wamsley *et al.,* 2011). Levels of bacteremia of red blood cells increases after two to six weeks, these levels peak to about 10^9 per ml of blood. However, signs of anaemia vary with respect to infected animals (Abbott *et al.,* 2005). Phagocytic cells

from the spleen that aid in the removal of *A.marginale* infected cells result in anaemia (Han *et al.,* 2010). Animals that have intact spleens are able to resolve infection but are not able remove infection completely making these animals spend their lifetime with bacteremia around 10^2 to 10^7 bacteria per ml of blood (Han *et al.,* 2010). In the sera of infected cattle with *A. marginale*, MSP2 is the predominant protein found (IJdo *et al.,* 1997). According to Palmer *et al.* (2009), antigenic variation of MSP2 and MSP3 of *A. marginale* enables the host to be permanently infected with the parasite. Study has also shown that MSP2 antigens have evolved enabling them to evade anti-MSP2 antibodies in response to A. marginale infections (French *et al.,* 1999).

Clinical signs begin to manifest 4 to 9 days throughout the developmental stage. During this period, the infected animal's body destroys the parasites and in doing this, some RBCs are destroyed leading to anaemia. Temperature increases (fever) and a decline in milk production in lactating cows. Most cattle farmers are able to detect these signs when the animal becomes weak, and lags behind. Other signs include anorexia, refusal to drink water, the skin around the teats, eyes, lips, muzzle become pale. Rapid weight loss, weakness, constipate and die within the first to fourth day after showing clinical signs (Whittier *et al.,* 2009)

Over the years, the complete eradication of anaplasmosis has been unattainable; this is due the non-existence of cattle reservoirs and many different vectors (Radostits *et al.,* 2006). The recommended approach in controlling anaplasmosis are; controlling of the arthropods, chemoprophylaxis, vaccine usage and culling out infected cattle from the herd **(**Radostits *et al.,* 2006). Recent approach has seen the use of live and inactivated vaccines that rely solely on cattle blood. Nevertheless, these vaccines cannot be standardized and stand a risk of transmitting pathogens to cattle. Morever, some researchers have stated that because *A. marginale* strains are isolated by geographical parameters, it does not always confer cross-protection and therefore, protection provided by these vaccines is based on isolate specificity (Kocan *et al.,* 2000). These

vaccines inhibit the manifestation of clinical signs but they do not prevent the disease **(**Eshetu, 2015**)**. Other countries engage therapeutic agents such as antimalarial, which are of no significance to the disease control (Potgieter and Stoltsz, 2004). In 2010, Akhter recorded that Imidocarb dipropionate is effective in treating and preventing bovine *anaplasma* infection.

2.2.7.4 HEARTWATER

It is a disease found in wild and domestic animals such as goat, cattle, sheep and deer. *Ehrlichia ruminantium* is the causative agent. They are gram negative, pleomorphic cocci and obligate intracellular parasites that are passed on to animals through the bites of ticks that serve as vectors. Heartwater is common in Africa and can be found in a few Caribbean islands but cannot be found in America. Ticks from the *Amblyomma* family are the target source of infection. There are two species from this family, *Amblyomma hebraum* ticks in southern Africa and *Amblyomma variegatum* that is common in Sub Saharan Africa and the islands in the Indian Ocean and Caribbean (Walker and Olwage, 1987; Stachurski *et al.,* 2013). This disease has been the major hindrance of introducing high-producing animals to improve and replace the local breeds in Africa (Provost and Bezuidenhout, 1987). Approximately 44.7 million is spent on controlling this disease (Minjauw *et al.*, 2000). According to Roth *et al.* (2012)**,** heartwater is listed among 12 vital animal transboundary diseases. Due to the antigenic variability of the pathogen, it is very difficult to produce effective vaccines to control the diseases, other methods such as recombinant, attenuation and inactivation has not been successful either (Faburay *et al.,* 2007; Adakal *et al.,* 2010)

After natural infections, incubation periods are within 2 to 3 weeks but varies from 10 days to a month (OIE World Organization for Animal Health, 2009)**.** However, intravenous inoculation has an incubation period between 10 days in sheep and goats and 10 to 16 days in cattle (OIE World Organization for Animal Health, 2009)**.**

However, the incubation period is dependent on the dose of active pathogen inoculated into a susceptible host. With high doses, the outcome can be death whilst low doses may not result in death rather providing protection to the animal (OIE World Organization for Animal Health, 2009). Upon infection, heartwater occurs in four different clinical forms; per acute, acute, subacute and mild forms known as heartwater fever (Heartwater, 2008; OIE World Organization for Animal Health, 2009). Per acute cases are usually seen among infected animals in Africa, there is brief periods of fever, convulsion, respiratory distress, lacrimation, severe diarrhoea in some cattle breeds, followed by sudden death. More often, pregnant cows are susceptible (OIE World Organization for Animal Health, 2009). For acute cases, animals die within a week, this form is common in both exotic and indigenous breeds of cattle, sheep and goats (OIE World Organization for Animal Health, 2009). Pyrexia, listlessness, diarrhoea, dyspnoea, nervous signs such as restlessness, animal walks in circles, cattle may be aggressive and show anxiety. Subacute cases are rare. However, animals show signs of prolonged fever, coughing and mild incoordination (Heartwater, 2008; OIE World Organization for Animal Health, 2009). Mild forms or subclinical infections can be observed in young calves and some breeds which are naturally resistant. Mostly, a brief fever and the animal recovers (Heartwater, 2008; OIE World Organization for Animal Health, 2009). There are no commercial vaccines for heartwater. Methods of immunization are infection and treatment by the use of infected blood, animals that show signs of the disease are treated with tetracycline. Albeit, a first generation of vaccine made up of inactivated bodies of *E. ruminantium* have been purified and emulsified in an adjuvant (Montanide ISA 50) have showed to confer protection in the field. Other three different isolates Senegal, Gardel and Welgevonden were attenuated and have exhibited to provide good protection whilst significant protection were obtained from DNA vaccinations but field experimentations have revealed that antigenic diversity should be considered in effectively formulating vaccines (OIE

World Organization for Animal Health, 2009). Other traditional methods in controlling heartwater are; the use of acaricides in the control of the ticks, and in other regions were ticks are endemic especially Africa, ticks are left on animals to boost their immune systems (OIE World Organization for Animal Health, 2009)

2.3 DIFFICULTIES IN THE CONTROL OF TICK-BORNE DISEASES

Disease causing parasites are a major problem globally and are known to be an obstacle to the health and production performance of animals. These parasites are either endo-parasitic or ecto-parasitic, that is: living inside the body of their host or living on the body of their host, and causing severe damage. Among these ecto-parasites, ticks are classified to be harmful and of economic importance, they suck the blood of their host (mammals, birds and reptiles) and transfer pathogens in their saliva through their feeding process (Furman and Loomis, 1984). The complexity of the problem associated with ticks and their related disease of cattle has led to the demand in securing interventions to control and reduce the losses they produce in cattle (George *et al.*, 2004). The transmission of tick borne diseases and infestation of ticks are a major problem to the cattle industry in the tropical and subtropical regions worldwide (Rajput *et al.*, 2006). It has become the topmost priority for countries found in those regions (Lodos *et al.*, 2000). Over the years, some methods employed in the eradication of tick and tick borne diseases included the use of chemicals such as acaricides to control ticks and the use of vaccines (Rajput *et al.*, 2006). Notwithstanding this, ticks have become generally resistant to acaricides, which is becoming an economic threat globally. For instance, stockholders solely depend on acaricides but do not possess the knowhow on how to detect tick resistant to acaricides (George, 2000). Resistance of these ticks has reached a level where when acaricides are introduced to an area, after five to ten years, some ticks develop resistance. This has led to the need for producing new products to combat ticks. Researches have been carried out to determine the kind of chemical

agents tick are resistant to and many more (Rajput *et al.*, 2006). In 1983, Wharton concluded after reviewing alternative methods, established that tick resistant breeds should be used in breeding programs but many questions were posed in respect to resistance being an acquired characteristic. Many breeding techniques have been successful but productivity among breeds were affected (Rajput *et al.*, 2006). Moreover, a considerable progress has been made in crossing beef and dairy cattle that has limited the effects of ticks and maintained high productivity (Turner, 1975; Hayman, 1974; Mason, 1974). The immunization of bovine species against tick-borne diseases has seen different approaches. Willadsen and Kemp (1988), attempted the use of complex tick extracts. Tick-borne disease vaccines over the years are difficult to produce. Although tick-borne diseases are important in all domestic animals, most vaccines that are developed target the cattle industry, such as Babesiosis (*B. bovis*, *B. bigemina*), Theileriosis (*T. parva*, *T. annulata*), Anaplasmosis (*A. marginale*) and Cowdriosis (*C. ruminantium*) (Rajput *et al.*, 2006). In Eastern, Central and Southern Africa, FAO have implemented a coordinated multi-donor programme for integrated tick and tick-borne diseases. A three strain *Theileria parva* stabilized vaccine was developed and used in conjunction with antibiotic treatment, known as the infection and treatment method. Boleni strains were isolated in Zimbabwe and used as vaccines (FAO, 1998). Although attempts have been made in vaccine developments, many side effects have been encountered and they are too expensive to produce are some related problems (CRC-VT, 2001)

2.4 ECONOMIC IMPACT OF DISEASES ON THE CATTLE INDUSTRY

Diseases hamper the economic growth of many developing countries (Fonkwo, 2008). Some of these disease outbreaks affect the economy of the animal market significantly through the cost incurred by treating them (McLeod *et al.,* 2016).

Animal diseases affect the economic, resource distribution in the private and public divisions (McLeod *et al.,* 2016). Farmers are affected by the cost incurred during treating disease outbreaks, death of productive animals and other management practices that aid in curbing the spread of diseases. For instance, Otte *et al.* (2007) noted that HPAI resulted in the depopulation (through natural or control-oriented mortality) of 25–30% of the poultry population in Thailand and Vietnam while, in Egypt, some 80% of the layer stock and 10% of the national poultry population was culled or died. Outbreaks of diseases immediately affect the production sector by reducing the capacity of animal products obtained from the animal industry. These goes further to reduce the demand on animal product (Perry and Grace, 2009). When diseases affect livestock, clinical and subclinical signs of the disease may result in the severe productivity losses. Animals that die, attract losses in areas of productivity (Hurtado and Giraldo-Rios, 2018). However, animals that do not die from diseases become less productive, that is reduction in milk production, wool, traction, eggs and meat (Thornton, 2010). A highly pathogenic form of avian influenza known as the "fowl plague" first appeared in Italy around 1878. Since then pathogenic avian influenza was first recognized in the United States in 1924-25. In 1983, an outbreak of highly pathogenic avian influenza occurred in Pennsylvania that took two years to control and required the destruction of some 17 million chickens (Avian, 2006). The direct costs of the outbreak were $62 million, and the indirect costs were estimated at more than $250 million (Polyak, 2004). Similarly, an outbreak of mad-cow disease in England in the 1990s cost between $9 billion and $14 billion in compensation costs to farmers and laid-off workers, and another $2.4 billion from loss of export markets (Parker, 2002).

2.5 VACCINATION AGAINST DISEASES

Vaccines are biologically prepared substances that are able to improve immunity. They usually contain an agent that mimics a disease causing organism, usually made from a

killed or weakened form of that pathogen, its toxins or surface proteins. In most cases, less virulent strains of the said pathogen are used (Baxter, 2007). These agents are detected as foreign materials the host system, targeted and destroyed. However, a memory is created to ensure immediate removal of the said pathogen if there is a future infection (National Institutes of Health, 2007). The world as experienced a range of successes obtained from vaccines. According to WHO, more than 10 million deaths have been prevented by use of vaccines (Andre *et al.,* 2008). There is a difference in the criteria of producing vaccines for animals and humans (Knight-Jones and Rushton, 2013). Most diseases of wildlife that vaccines are produced for are based of the zoonotic factors. On the other hand, vaccines produced for livestock are aimed at productivity, and eliminating the risk of zoonosis (Narrod *et al.,* 2012).

Veterinary vaccines make up 23 percent of the world market for animal health products. A consistent growth over the years, facilitated by; advanced technology in vaccine development, drug resistant pathogens and emergence of new diseases (Meeusen *et al.,* 2007). On the other hand, Veterinary vaccines are very efficient in disease elimination, the only problem associated with vaccine development is presence of multiple strains of pathogens.

2.6 BROAD SPECTRUM INNATE IMMUNE STIMULATION AS A SOURCE OF PROTECTION AGAINST DISEASES

The immune system is made up of two mechanisms. The innate immune response, which is relatively rapid but nonspecific, and not always effective, and the adaptive immune response; which is slower in its development during an initial infection with a pathogen, but is highly specific and effective at attacking a wide variety of pathogens (Clark and Kupper, 2005). The innate immune system serves as the first line of defence against different kinds of microbes, by first eliminating any microorganism that makes its way through the physical barrier (Spiering, 2015). The innate immune response

comprises phagocytic cells, and the cytotoxic Natural Killer cells. They recognize patterns of pathogen-specific molecules that are foreign to the body. These are bacterial cell wall components or bacterial flagellar proteins, which are detected by receptors found on these cells (Medzhitov, 2007). A pattern recognition receptor (PRR) is a membrane-bound receptor that recognizes characteristic features of a pathogen and molecules released by stressed or damaged cells, commonly known as PAMPs and DAMPs (Akira *et al.,* 2001).

Pathogen associated molecules are recognized as danger signals by pattern recognition receptors (PRRs) on innate immune cells that initiate host defense reactions. Among PRRs, Toll like receptors (TLRs) plays essential roles in protective responses against infectious diseases (William *et al.,* 2004; O'Neill *et al.,* 2013). Bacterial infections initiate a broad range of TLR activation, including TLR2, TLR4, TLR5, and TLR9 (O'Neill *et al.,* 2013; Kawai and Akira, 2011). Alternatively, virus particles activate the innate immune system via nucleotide receptors, including endosomal TLR3, TLR7, TLR8, or TLR9 and various cytoplasmic recognition molecules (Gürtler and Bowie, 2013). When TLRs are activated, multiple signalling pathways are deployed to protect host barrier tissues from external microbial attack are deployed. This approach has been explored as cures against transferrable illnesses and the disease severity they can establish (Steinman and Banchereau, 2007). The goal of these remedies is to provide protection, thereby imposing restrictions to severe damages caused by the pathogen. The functions of Toll-like receptors (TLR) are critical to the host immune defences. Synthetic forms have been developed, serving as immunotherapeutic agents (van der Poll and Opal 2008). This new approach helps in fighting against infectious ailment, by priming the innate immune system of individuals that are ligands, which attach to the receptors of cells and perform agonist or antagonistic functions (Mifsud *et al.,* 2014). They directly target the host cells instead of microbes, reducing the risk of developing into antimicrobial resistance (Mifsud *et al.,* 2014). The fast and wide nature of the

inborn resistant framework demonstrates that use of these agents will give a more extensive range of protection and could be utilized in blend with other antimicrobial agents including vaccines. The prophylactic administration of these agents could likewise be valuable for those most vulnerable to diseases, such as, the old, who are inadequately receptive to immunization (Jefferson *et al.,* 2005; McElhaney *et al.,* 2006).

CHAPTER 3

3.0 MATERIALS AND METHODS

3.1 LOCATION OF THE STUDY

The experiment was conducted at the Amrahia Dairy Farm of MoFA and the Department of Animal science microbiology laboratory, University of Ghana, Legon. Amrahia is about 27km from Accra on the Accra-Dodowa road (APD, 2014). It lies on latitude 5° 46' 55" North and longitude 0° 08' 25" West at altitude 80m above sea level. Temperature generally fluctuates between 25.1°C minimum in August and 33°C maximum in February and March being the hottest months.

3.2 STUDY ANIMALS

A cohort of 75 Friesian x Sanga F1 calves (3-6 months old) obtained from a study herd owned by the Animal Disease Biotechnology Laboratory of the Department of Animal Science, University of Ghana, were used. The animals were age-cross matched. There were 3 groups of 25 age-matched calves.

3.3 INNATE IMMUNE STIMULATION OF CROSSBRED CALVES

Innate immune response of crossbred calves was stimulated using TLR 7/8 agonist emulsified in saponin. Calves in Group 1 were administered with a single 1 ml dose of TLR 7/8 agonist at day 0 to stimulate the innate immune response. Calves in Group 2 were administered with 1 ml emulsion at Day 0 and repeated at Day 28. The third group of calves (Group 3) were used as control (not administered with the agonist). Animals were allowed to graze from the third day and maintained on the field for six months. Animals were examined daily for clinical signs (fever, parasite detection). The need for treatment was assessed by clinical presentations and post-mortem findings. Treatment cost related to tick-borne infection and other related diseases was recorded.

3.4 BLOOD SAMPLING

Peripheral blood samples were obtained from 75 crossbred calves. PCR and serology were performed in order to verify the infection status of calves prior to immune stimulation. Blood samples were collected via jugular venipuncture from the seventy-five crossbred-calves every two weeks over six months. The samples collected were transferred to EDTA and gel tubes for DNA and serum extraction. Samples collected were transported on wet ice to the Animal Science microbiology laboratory.

3.5 SERUM EXTRACTION

All seventy-five blood samples collected in plain tubes were left on the bench, undisturbed, at room temperature overnight allowing the blood to clot. After 24hrs, the serum was poured gently into Eppendorf tubes and stored at -20^{o}C

3. 6 GENOMIC DNA EXTRACTION

EDTA-tubes containing blood were transferred to the biotechnology laboratory of the University of Ghana for genomic DNA extraction according to the manufacturer's specifications QIAGEN blood kit (QIAGEN Inc, Valencia, U.S.A). Five millilitres of the whole blood was added to fifteen millilitres of Red Blood (RBC) Lysis solution and allowed to incubate for 10 minutes at room temperature. During incubation, the samples were inverted several times to guarantee thorough lyses of erythrocytes. The samples were centrifuged at 4,400 rpm for 30 minutes. The supernatant was thrown away after centrifuging, leaving behind the cell pellet in a residual liquid between 100-200 µl. The pellets were vigorously vortexed in the residual supernatant. Four millilitres of Cell Lysis solution was then added to the re-suspended cells and pipetted up and down to lyse them. The samples were cooled to room temperature before adding 3ml of protein precipitation solution to the cell lysate. The tubes were vortexed vigorously at a high speed for 20 seconds to mix the protein precipitation solution uniformly with the lysate

and then centrifuged at 4,400 rpm for 30 minutes. This was done to precipitate the protein, which was visibly seen as a tight dark brown pellet. The supernatant containing the DNA was decanted and transferred into a 15 ml tube containing 10 ml of isopropanol. The tubes were gently inverted 50 times enabling the DNA to be visible as a white pellet. The visible DNA was carefully removed using a pipette and transferred into 1.5ml tube. It was air-dried for 10 minutes before being reconstituted in 500 µl of DNA hydration solution. The DNA pellets from different samples were incubated at 65°C for 1 hour in a water bath to allow complete dissolution in the hydration buffer before stored at -20 °C.

3.7 PRIMER SELECTION AND SPECIFICITY

PCR primers specific for *Anaplasma marginale, Babesia bigemina* and *Theileria spp.* were used simultaneously to amplify DNA fragments respectively from genomic DNA templates obtained from individual calves. To determine whether each primer is specific for the gene of interest, equivalent amounts of the primer pairs were applied to 100ng of genomic DNA obtained from the organism. PCR amplifications were performed in reaction volumes of 25 µl using 0.5 µM of each primer. Amplification reactions were performed using an initial denaturation step of 95 ºC for 5 min, followed by 30 cycles of 94 ºC for 5 sec, 55 ºC for 30 sec, and the initial extension at 72 ºC for 1 min. The products were finally extended at 72 ºC for 7 min before holding at 4 ºC. The amplicons were size-separated by agarose gel electrophoresis, excised from the gel and purified (QIAGEN), and the ends were extended at 72 ºC for 20 min. The same primer pairs were applied, prospectively, to genomic DNA samples obtained from unrelated DNA samples from *Theileria* species and *Babesia bigemina* to confirm that there will not be amplification.

Table 1: PCR primers

Pathogen species	Gene(length)	Forward primer(5'-3')	Reverse primer (3'-5')	Reference
Anaplasma marginale	msp1b (265bp)	gctctagcaggttatgcgtc	ctgcttgggagaatgcacct	Bilgic *et al.*,2012
Theileria spp.	Cytochrome b (462bp)	actttggccgtaatgttaaa	ctctggaccaactgtttggc	Bilgic *et al.*,2012
Babesia bigemina	Cytochrome b (1125bp)	tggcggcgtttattagttcg	ccacgcttgaagcacagga	Bilgic *et al.*,2012

3.8 PCR ASSAY SENSITIVITY

The sensitivity of PCR assay was determined using 10-fold dilutions of genomic DNA from A. *marginale*, *B. bigemina* and *Theileria* spp. Genomic DNA samples containing initial amounts of 100 ng were serially diluted to 10 ng, 1 ng, 0.1 ng, 0.01 ng stocks. First, the lowest detection limit of each primer was determined by PCR using the serially diluted samples, starting from samples containing the highest concentration of pathogen DNA (100 ng) to those that contained the least amount of the target DNA. Secondly, the sensitivity of detection of individual pathogens was determined in the background of one another to mirror the detection during mixed infection. Reciprocal PCR amplifications were conducted using respective primers for each pathogen gene with reciprocal amounts (100 ng of pathogen A, with 0.01 ng of pathogen B; 100 ng of pathogen B with 0.01 ng of pathogen A) of genomic DNA template. Briefly, the highest amount of genomic DNA (100 ng) from one organism was paired with the lowest amount from the other organisms in a duplex PCR reaction. The PCR reaction mixture was performed in a total volume and the amplicons were size-separated by ethidium bromide-stained agarose gel electrophoresis.

3.9 COMPETITIVE ELISA (cELISA)

A competitive Enzyme-linked-immunosorbent assay (cELISA) was used to measure antibodies related to *Anaplasma* using the *Anaplasma* Antibody test Kit cELISA v2 manufactured by Veterinary Medical Research & Development (VMRD). The inhibition percentages were calculated using the manufacturer's formulae.

3.9.1 cELISA TEST PROCEDURE

The reagents, serum samples and plates were warmed at room temperature (23 ± 2˚C) before starting the test. Control samples (Positive and Negative) were loaded onto the plate in duplicates and triplicates respectively per the manufacturer's directive. 1X Antibody-peroxidase conjugate was prepared by adding 1 part of 100X Antibody–Peroxidase conjugate to 99 parts of Conjugate diluting buffer. 1X wash solution was prepared by diluting 1 part of wash solution concentrate with 9 parts deionized or distilled water.

50µl of controls and serum samples were carefully loaded into each well coated with *Anaplasma* antigens and marked for easy identification. The sides of the wells were tapped gently to guarantee the loaded samples had coated the bottom of the plates. It was ensured that samples did not spill and incubated for one hour. After one hour, plates were washed manually by removing sera and sharply striking the inverted plate 4 times on a clean paper towel, 1X Wash solution was immediately pipetted into each well using a multichannel pipette. The wells were emptied and the plate was struck sharply on a clean paper towel 4 times to ensure there was no sera residue. The wells were filled using the same method with the 1X Wash solution one additional time for a total of two washes. 50 µl of the diluted (1X) Antibody- Peroxidase conjugate was added to each well and the side of the plate was tapped gently to ensure that the bottom was completely coated and incubated at room temperature (23 ± 2◦C) for 20 minutes. After 20 minutes, plates were washed four times. 50µl of Substrate solution was pipetted into

each well on the plate and the plate was tapped gently to ensure the bottom of the wells were coated and incubated for 20 minutes at room temperature (23 ± 2◦C). After 20 minutes, stop solution was added to the wells containing the substrate solution and Optical densities were read on a microplate absorbance spectrometer of wavelength 630nm.

3.9.2 % INHIBITION (% I)

The percentage inhibition was calculated using a formula provided by the manufacturer

% I = 100 * [1- (SAMPLE OD ÷ NEGATIVE CONTROL OD)]

3.10 DATA COLLECTION AND ANALYSIS

The need for treatment was analysed as 'A' binary (Yes/No) data; First day of treatment, number of days of treatment, and cost of treatment. Cost of treatment was taken into consideration to run a cost benefit analysis**.**

3.10.1 COST BENEFIT ANALYSIS

The cost for treatment of calves, which were judged by the veterinarian as diseased after inoculation with the adjuvant once or twice, was compared to the cost of treatment of calves in the unstimulated group.

Benefit Cost Ratio (BCR): It is the ratio of project benefit to project cost and it involves summing the total discounted benefits for the project over its entire life span and dividing it by the discounted cost of the project. BCR is specified below as:

$$BCR = \frac{\left[\sum B_i / (1+d)^i\right]}{\left[\sum C_i / (1+d)^i\right]}$$ Summed over i=0 to n months

Where:

B_i is the project's benefit in year i, where i = 0 to n months; C_i denotes the project's cost; n is the total number of years for the project/life span; d represents the discount

rate. A BCR greater than 1 implies the project is profitable and worth undertaking, but BCR less than 1 means the project is unprofitable.

3.11 ETHICAL CONSIDERATIONS

The study followed ethical principles and regulations of University of Ghana to ensure quality and integrity of the work.

CHAPTER 4

4.0 RESULTS

4.1 PATHOGEN PREVALENCE AMONG GROUPS

Initial samples from all crossbred calves showed the absence of tick-borne pathogens (Fig 1 and 2). Figure 1 and 2 show a photomicrograph of PCR product electrophoresed on 1% agarose gel. Figure 1 is an example of 265bp fragment for *Anaplasma* and Figure 2 shows 462bp fragment for *Theileria.* Using this, the infection status of every animal was established. Other pathogens present such as FMD, Dermatophilosis, and CBPP were examined by clinical presentations and post mortem findings.

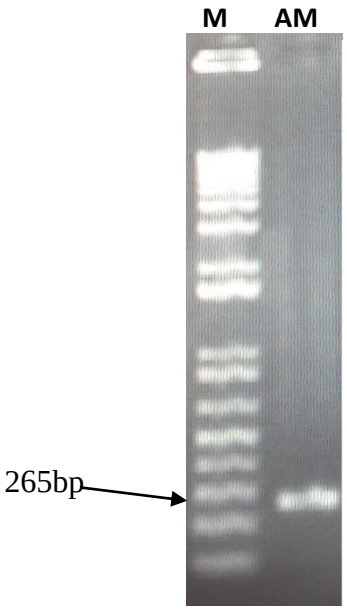

Figure 1: Gel photo showing PCR fragment of *Anaplasma Marginale*

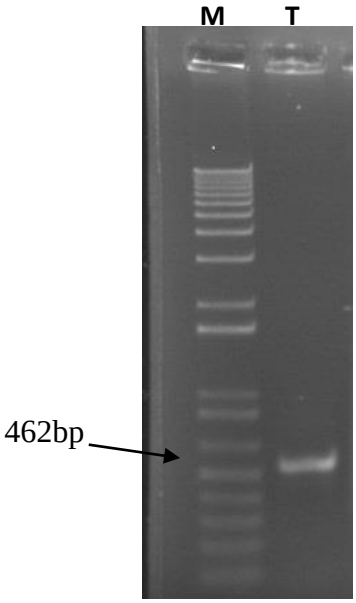

Figure 2: Gel photo showing PCR fragment of *Theileria Spp*

Legend

M **1 Kb plus ladder**

AM ***Anaplasma Marginale***

T ***Theileria Spp***

After a period of six months, Anaplasma (78.31%) was the most prevalent pathogen within the groups, followed by Dermatophilosis (14.46%), Theileria (2.54%), CBPP (2.41%) and Foot and Mouth disease (2.28%) (Figure 3)

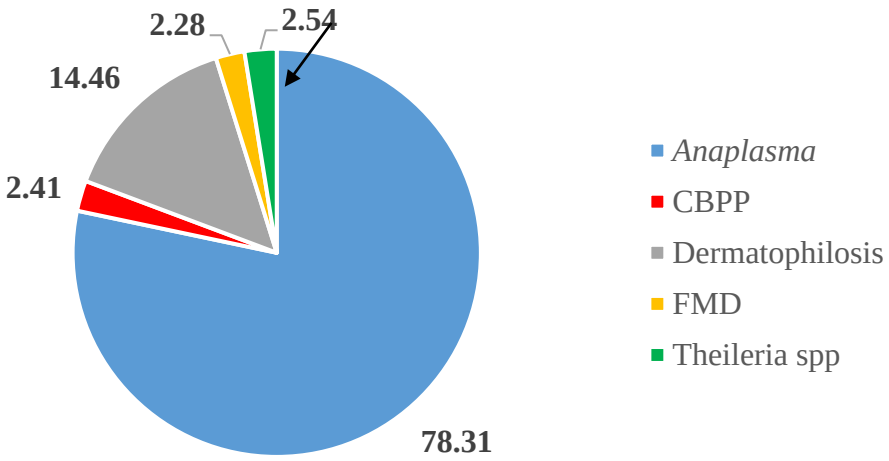

Figure 3: Pie chart indicating in percentages the highest occurring pathogen

4.3 EFFECT OF REPEAT INNATE IMMUNE STIMULATION OF AVERAGE PATHOGEN LOAD

The average number of individual pathogens was monitored at monthly intervals, before and after stimulation of crossbred calves with the TLR 7/8 agonist. This was to determine whether the effect of stimulation affected the average number of pathogens found within the group. The correlation between increasing months and the number of times stimulated with TLR 7/8 was tested by calculating the average number of pathogen occurrence within the individual groups over the number examined. However, at the beginning of the experiment, crossbred calves were clean (Table 2.0). At the end of the first month, each group of crossbred calves tested positive for tick-borne pathogens in their blood. The predominant tick-borne pathogen was *Anaplasma*. Crossbred calves that were stimulated once were further divided into two groups and one group was stimulated a second time with TLR 7/8 agonist. After the second month, the average pathogen load had increased in the unstimulated group from 1.08 to 1.95;

Anaplasma spp being the predominant followed by Theileria, CBPP, and Dermatophilosis. The group that was stimulated once had an average of 0.6 to 1.2 (Table 2. Figure 4). This significant increase was due to an increase in tick-borne infections followed by CBPP and FMD. Pathogens increased significantly in the unstimulated group compared to the stimulated groups (Figure 4)

Table 2: Effect of innate immune stimulation on pathogen Load

	GROUPS											
	Unstimulated				Stimulated Once				Stimulated twice			
Months	0	1	2	3	0	1	2	3	0	1	2	3
No Examined	25	24	20	10	25	25	25	21	25	25	25	22
Anaplasma	0	9	12	10	0	15	22	21	0	17	17	19
Theileria Spp	0	0	1	1	0	0	0	2	0	0	1	1
CBPP	0	5	10	2	0	0	4	0	0	0	3	0
FMD	0	5	11	4	0	0	3	6	0	0	1	2
Dermatophilosis	0	7	5	3	0	0	1	2	0	0	0	2
A.P.L	0	1.08	1.95	2	0	0.6	1.2	1.48	0	0.68	0.88	1.09

Key: No- Number, A.P.L- Average Pathogen Load, CBPP- Contagious Bovine Pleuropneumonia, FMD- Foot and Mouth Disease

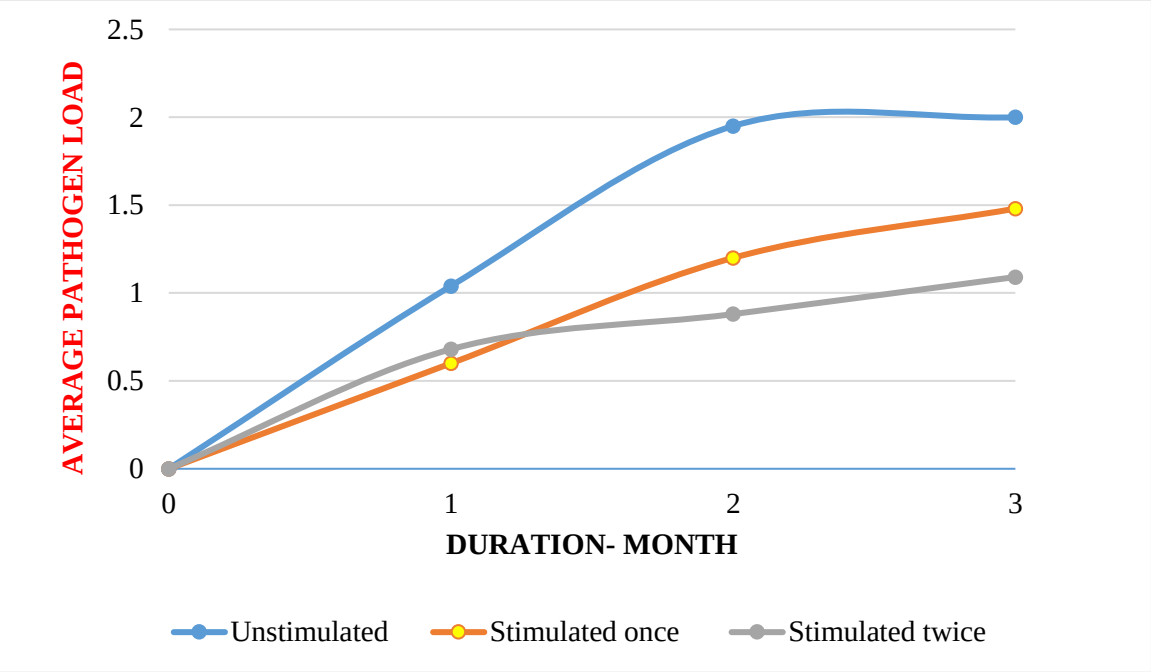

Figure 4: The effect of stimulation on Average pathogen Load

The y-axis represents the average pathogen load and the x-axis represents the duration in months. The blue line represents the unstimulated group, the orange line represents the group stimulated once and the grey line represents the group stimulated twice

4.4 EFFECT OF STIMULATION ON THE NEED FOR TREATMENT

After six months of observation, crossbred calves were monitored for the presence of disease among the various groups and a veterinarian passed out the need for treatment. All crossbred calves that were not stimulated (100%) with TLR 7/8 agonist, at one point, needed to be treated for a disease. On the other hand, some crossbred calves which were stimulated once (48%) and twice (36%) were presented for treatment. Though the rate of treatment varied numerically between the stimulated groups, chi-square analyses revealed that these differences were not significant; $\chi 2 = 1.299$, df =1 and p=0.254 (Table 3.0).

Table 3: The need for treatment among groups over a period of six months

GROUP (n=25)	Untreated	Treated	Treatment Rate (%)
Unstimulated	0	25	100
Stimulated once	12	13	48
Stimulated twice	16	9	36

n= number of animals

4.5 EFFECT OF INNATE IMMUNE STIMULATION ON SURVIVAL RATE

Unstimulated crossbred calves had a 40 per cent chance of survival after treatment of diseases compared to the groups stimulated once and stimulated twice with survival rates of 84 and 88 per cent respectively (Figure 5.0).

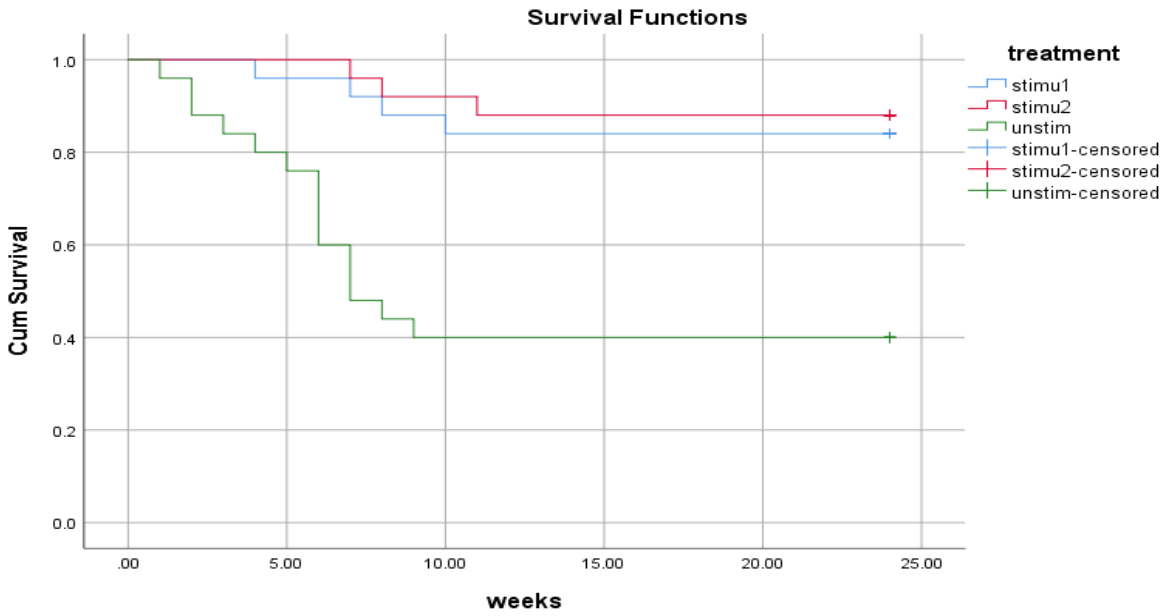

Figure 5: Survivor curve showing the probability of survival among crossbred calves.

The y-axis indicates the percentage survival and the x-axis is the duration in weeks. Unstimu= Unstimulated, Stimu1= stimulated once and stimu 2= Stimulated twice. There were significant differences between the groups (P< 0.00)

4.5 EFFECT OF STIMULATION ON THE DEVELOPMENT OF ADAPTIVE IMMUNITY

Despite the detection of several diseases among the groups, the prevailing infection was *Anaplasma* (Figure 3.0), which required treatment. Two groups were tested initially, the unstimulated (n=25) and stimulated group (n=50), per cent inhibition for antibodies was calculated at weekly intervals for two weeks. Antibodies for crossbred calves before inoculation varied between groups with a margin of 1.26%. In spite of this,

differences were not significant (p=0.668). After the first inoculation, there was a rapid rise in the per cent inhibition of the group that had been stimulated, compared to the unstimulated group (Figure 6).

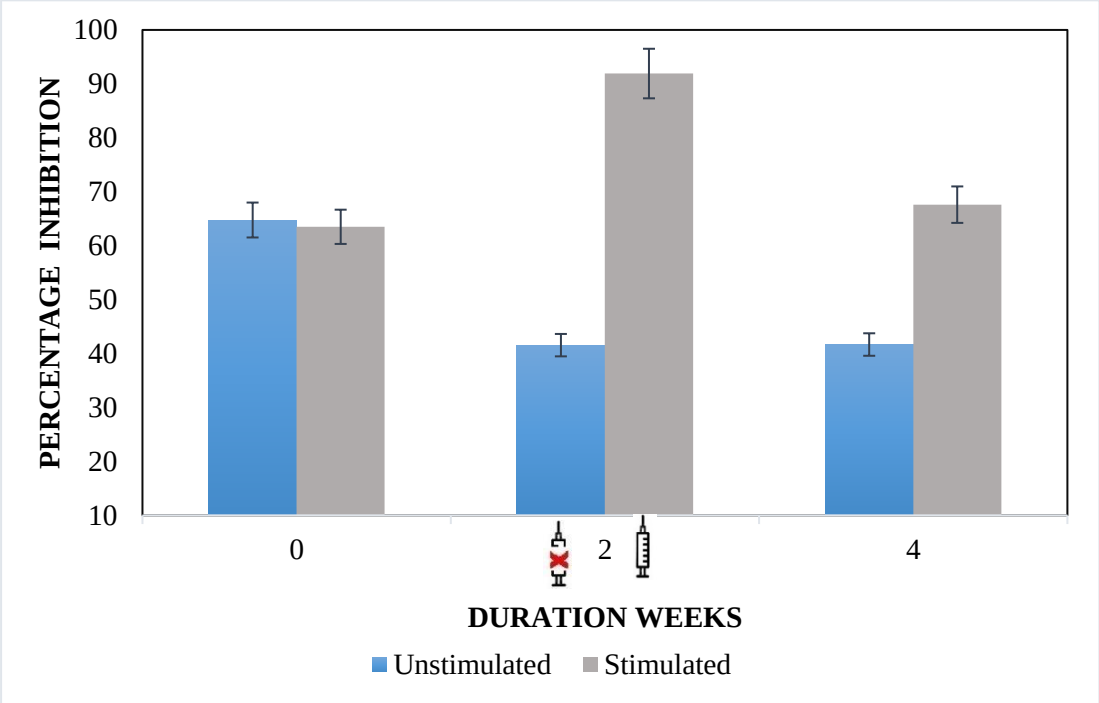

Figure 6: Antibody percentages of unstimulated and stimulated field-grazing crossbred calves

The y-axis indicates in percentages the antibodies from calves and the x- axis is the duration in weeks. The blue bars represent the mean inhibition percentage of unstimulated crossbred calves and the grey bars represent the mean inhibition percentages of stimulated crossbred calves.

4.5.1 EFFECT OF MULTIPLE STIMULATIONS ON THE DEVELOPMENT OF ADAPTIVE IMMUNITY

Antibody levels declined in the stimulated group after 4 weeks by 30 % (Figure 6). To determine whether there was a difference in antibody percentage inhibition with respect to repeated stimulation, a third group (Stimulated twice, n= 25) which received a second inoculation was observed bi-weekly alongside the other groups, unstimulated (n= 25), and stimulated once (n= 25). At week six, percentage inhibition for the group stimulated twice, rose to about 95% and declined to 91% at week 8. Crossbred calves were monitored closely for a period of 8 weeks and it was observed that antibodies were significantly different among unstimulated (n= 25), stimulated once (n=25) and stimulated twice (n=25) with a p value of 0.00. Antibodies for the various groups declined gradually over time. (Figure 7)

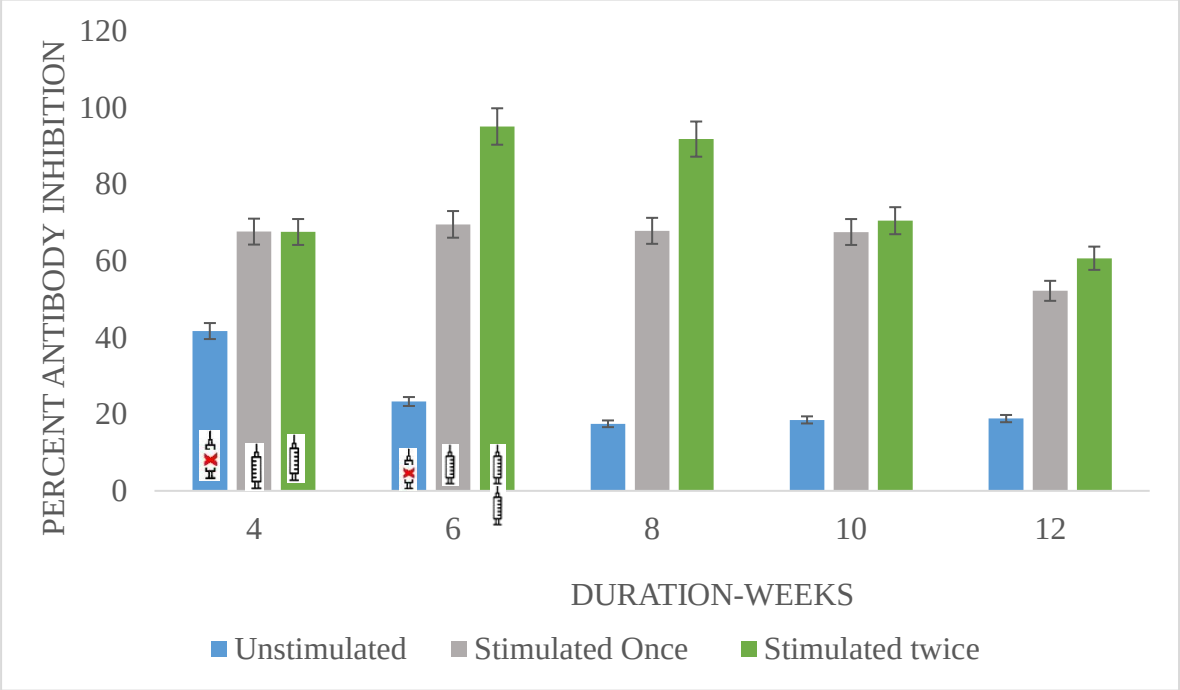

Figure 7: Average percent inhibition of antibodies measured from field-grazing crossbred calves following different levels of stimulation.

The y-axis indicates antibody titres expressed in percentages and the x-axis indicates duration expressed in weeks. The blue bars represent the mean inhibition percentage of unstimulated crossbred calves, the grey bars represent the mean inhibition percentages of crossbred calves stimulated once and the green bars represent the mean percentage inhibition of groups stimulated twice. = Unstimulated, = stimulated once and = stimulated twice

4.5.2 SUMMARY OF ANTIBODY PERCENTAGES ACROSS GROUPS

The average antibody percentage for a period of 6 months was calculated. Unstimulated crossbred calves had a percentage inhibition of 29.17% compared with calves from groups that were either stimulated once (68.56%) or stimulated twice group (77.07%). In summary, there were significant differences in the antibody titres between all groups of calves ($p < 0.001$). (Figure 8)

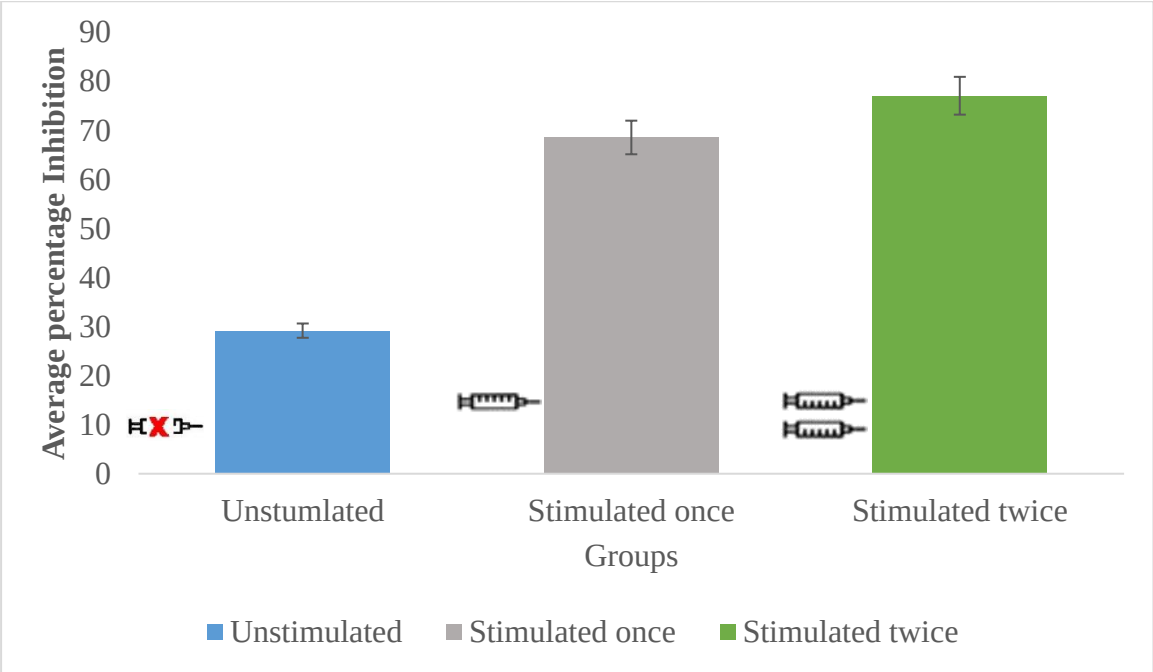

Figure 8: Summary of percent inhibition for the period of six months

The y-axis indicates the average percent inhibition for the entire duration of 12 weeks and the x- axis indicates the groups under study (n= 75). There were significant

differences in the antibody percentages between the group stimulated once and twice ($p < 0.001$). The blue bars represent the mean inhibition percentage of unstimulated crossbred calve, the grey bar represent the mean inhibition percentages of crossbred calves stimulated once and the green bar represents the mean percentage inhibition of groups stimulated twice

4.6 COST OF TREATMENT

The unit cost per treatment of the disease and the number of days required for treatment were considered. The costs of treating various diseases were as follows: CBPP, GHC 20.00 per day for three days, *Dermatophilosis*, GHC 24.00 per day for three to five days, FMD and Tick-borne diseases were, GHC 15.00 and GHC 6.00 respectively for one day (Table 4). The death of a crossbred calf was accounted for as its market value.

The total cost of treating twenty-five crossbred calves that were diagnosed with CBPP was GHC 1, 100.00. In spite of treatment, they died. Twenty cases of *Dermatophilosis* were treated at a cost of GHC 360.00. Nineteen cases of Foot and Mouth disease and Tick-borne diseases were treated, summing up to GHC 284.00 and GHC 114.00 respectively. Death was inevitable, a number of calves were recorded dead in the unstimulated group, costing GHC 18,000.00, stimulated once GHC 4,800.00 and stimulated twice GHC 3,600.00. Animals that were isolated for treatment were provided with feed and water, at a cost of GHC 600.00.

Table 4: Cost of disease treatment among groups over six months

GROUPS	Number of animals Examined	Diseases	Number of animals treated	unit cost day GHC	No of Days Treated 1	2	3	Total Cost of Treatment GHC
NOT Stimulated	25	CBPP	17	20	340	340	0	680
		Dermatophilosis	16	24	384	384	384	384
		FMD	11	15	165	0	0	165
		Tick-borne Diseases	11	6	66	0	0	66
		Death after treatment	15	1,200	0	0	18,000	18,000
		Culled	20	200	200	0	0	200
		Total Cost						**19,495**
Stimulated Once	25	CBPP	4	20	80	80	80	240
		Dermatophilosis	2	24	48	48	48	144
		FMD	6	15	90	0	0	90
		Tick-borne Diseases	4	6	24	0	0	24
		Death	4	1,200			4,800	4,800
		Culled	12	100	200	0	0	200
		Total Cost						**5,498**
Stimulated Twice	25	CBPP	3	20	60	60	60	180
		Dermatophilosis	2	24	48	48	48	144
		FMD	2	15	30	0	0	30
		Tick-borne Diseases	4	6	24	0	0	24
		Death	3	1,200			3,600	3,600
		Culled	7	200	200	0	0	200
		Total Cost						**4,178**

4.7 COST AND BENEFIT OF THE PROJECT

The cost and benefits derived from either the experimental group that received immune stimulation or the control group that were not stimulated were computed. Crossbred calves that died or were treated for diseases, were valued as cost incurred. However, benefits were derived from the number of calves who neither died nor received treatment for diseases. Treatments and death cases recorded for groups that received TLR 7/8 agonist were minimal (44%) compared to unstimulated crossbred calves (Table 5).

Table 5: Cost and Benefits

Groups	Number of animals Examined	Agonist and Diseases	Cost GHC	Benefits GHC
UNSTIMULATED	25	TLR 7/8 Agonist/ml	0	1375
		CBPP	680	480
		Dermatophilosis	384	648
		FMD	165	210
		Tick-borne Diseases	66	84
		Culled	200	0
		Death after treatment	18,000	6,000
		Total Cost	**19,495**	**8,797**
STIMULATED ONCE	25	TLR 7/8 Agonist	1,375	0
		CBPP	240	1,260
		Dermatophilosis	144	1,656
		FMD	90	285
		Tick-borne Diseases	24	126
		Culled	200	0
		Death	4,800	25,200
		Total Cost	**6,873**	**28,527**
STIMULATED TWICE	25	TLR 7/8 Agonist	2,750	0
		CBPP	180	1,320
		Dermatophilosis	144	1656
		FMD	30	345
		Tick-borne Diseases	24	126
		Culled	200	0
		Death	3,600	39,600
		Total Cost	**6,928**	**43,047**

4.8 BENEFIT-COST RATIO FOR VARIOUS GROUPS

According to Zangeneh *et al.* (2010) a project is defined as beneficial when the cost-benefit ratio >1. From the current study, the cost benefit ratio of the unstimulated group was 0.45 as compared with 4.15 and 6.12, respectively, from those that were stimulated once and twice (Table 6). From the study, it was observed that it would be beneficial to stimulate crossbreds twice.

Table 6: Benefit-Cost Ratio

	Unstimulated	Stimulated Once	Stimulated Twice
Total Benefit GHC	8,797.00	28,527.00	43,047.00
Total Cost GHC	19,495.00	6,873.00	6,928.00
Cost-Benefit Ratio	0.45	4.15	6.12

CHAPTER 5

5.0 DISCUSSION

5.1 EFFECT OF STIMULATION WITH TLR 7/8 AGONIST ON PATHOGEN LOAD

An observation made from the study was that crossbred calves allowed to go grazing tested positive for tick-borne pathogens when screened and Anaplasma was the most prevalent pathogen. This observation was consistent with findings made by Beckley *et al.* (2016) where Anaplasma was the prevalent tick-borne pathogen constituting 36.3 percent of the population of cattle sampled in Ghana. Other studies also found similar trends of anaplasma being the most prevalent tick-borne pathogen in cattle (Tana-Hernández *et al.,* 2017; Zabel and Agusto, 2018). In contrast, some studies carried out in Ghana found the most prevalent tick-borne pathogens to be *Theileria* followed by *Babesia*, while Anaplasma was confirmed to be the least prevalent (Assoku, 1979; Bell-Sakyi *et al.,* 2004). This could be because Giemsa staining techniques were used in their case compared to molecular techniques, which were used in this study.

Over time, the number of pathogens circulating in individual crossbred calves increased. This accumulation in pathogen numbers was designated as the pathogen load and it was essentially the same between cattle from both experimental and control groups. Pathogen load is essential because, the already primed immune system of crossbred calves would be able to encounter these pathogens and provide protection at the adaptive level. Innate immune stimulation with synthetic TLR7/8 agonist played an important role in disease control among crossbred cattle. This was in agreement with works by Jefferson *et al.* (2005) and McElhaney *et al.* (2006). Because agonists bind to

host cells, they interact with PRRs on cells, which are responsible for detecting distinct structures on pathogens (Goubau *et al.,* 2014).

From the study, the average number of pathogens found in the groups stimulated once (1.2) and twice (1.08) were minimal as compared to the unstimulated group (2). FMD and CBPP were common among the unstimulated group. According to literature, these diseases encountered during the experiment are said to be acquired through different routes of infection such as aerosol, direct contact, and ingestion. For pathogens to invade a host successfully there should be a complete destruction of the first line of defense. These minimal infections could be attributed to effective nature of how the immune system works, blocking pathogens from penetrating physical barriers, through which they cause infection (Janeway *et al.,* 2001).These agonists have been observed to activate multiple signaling pathways leading to the production of antimicrobial peptides, that work against pathogens. (Wu *et al.,* 2014; Aujla *et al.,* 2008; Evans *et al.,* 2010).

5.2 EFFECT OF REPEAT STIMULATION ON THE NEED FOR TREATMENT AND SURVIVABILITY

From the study, 100 % of the unstimulated group, 48 % of the stimulated once and 36% stimulated twice required treatment throughout the six months of observation. The intervention also exhibited an increase in the survival rate among the stimulated once (84%) and stimulated twice (88%) groups and a decrease in the unstimulated group (40%). The perceived reduction in the need for treatment could be attributed to the stimulation of the innate immune system with TLR 7/8 agonist. This corresponds with other studies, indicating that TLR 7/8 agonist improves health status of calf and mouse model (Sarfo, 2016; Zhang and Matlashewski, 2018). Wu *et al.* (2014) also found that

the administration of TLR 7/8 agonist activated a wide range of immune cells and cytokines that are responsible for protection against bacteria and viruses.

5.3 EFFECT OF REPEAT STIMULATION ON THE DEVELOPMENT OF HUMORAL IMMUNITY

Before inoculation, the IgG2 levels in the serum of experimental calves were within the range of 60 to 62 per cent. These high levels of IgG2 identified were assumed to have originated from ingested milk rich in colostrum (Hurley and Theil, 2011). Two weeks after the first stimulation, IgG levels in serum of the unstimulated crossbred calves were observed to dwindle. This observation is consistent with literature, according to which IgG antibodies obtained from colostrum have a half-life of twenty days (Murphy *et al.,* 2014).

However, contrary to the unstimulated group, IgG2 levels in the stimulated group peaked to 91.2% 2 weeks after inoculation. This could be because Toll like receptors serve as mediators between the innate and the adaptive immune system. They induce interferons and other cytokines, producing inflammatory responses, and activate the adaptive immune system (Akira *et al.,* 2001). The high levels of IgG2 observed in stimulated crossbred calves were translated into protection against diseases, which was evident by the reduction in the need for treatment and the enhanced probability of survival in the face of multiple infections. A second stimulation of crossbred calves with TLR 7/8 agonist saw a rapid rise in IgG2 levels (95%) in the sixth week, though it declined to 92% in the eighth week, but was not significant (Figure 7). This observation suggests that, for higher antibodies in crossbred calves, there is a need for a second inoculation. Some studies have exhibited the efficacy of TLR 7/8, in producing high levels of IgG in mouse models and in humans. In this case, specific antibodies were produced against specific antigens encountered. Progress in research has broadened the understanding of innate immunity and its ability to shape adaptive immune response (Medzihtov, 2007; Wang *et al.,* 2013).

5.4 EFFECT OF REPEAT STIMULATION ON THE COST OF TREATMENT

The study revealed that, the unit cost for treating diseases and the number of days required for treatment were high among the unstimulated group with a total of GHC 19,495.00, compared to the groups stimulated once (GHC 6,873.00) and stimulated twice (GHC 6,928.00). The huge cost incurred from the unstimulated group resulted from disease related death. It is difficult to quantify the economic impact of diseases because of the complex nature they may possibly have (Bio-Era, 2008). The current study conducted a cost-benefit analysis, establishing the cost effectiveness of innate immune stimulation. Controlling diseases among cattle is a major problem encountered by many rural farmers. In some communities, veterinary services are expensive. Over the years, vaccines and drugs have been produced against some diseases, but the only limitation is the ability to protect cattle against a broad spectrum of diseases, which is very expensive to purchase by the poor-resource farmer (Paton and Taylor, 2011). Regular vaccinations have catered for losses of cattle to certain diseases flagged by the World Health Organization (CBPP and FMD) (Donadeu *et al.,* 2019). However, other diseases, caused by ticks are treated with broad-spectrum antibiotics. From the experiment, crossbred calves that were unstimulated incurred a lot of cost (GHC 1495.00) in treating diseases compared to groups stimulated once (GHC 698.00) and twice (GHC 578.00), through the use of a wide range of antibiotics. The cost effectiveness of a vaccine is determined by several factors, such as the durability of the disease severity in a particular area, price, mode of delivery of vaccines (Dubé *et al.,* 2013).

From the study, the highest cost incurred was due to mortality from diseases, and this was significant among the unstimulated group. This emphasizes the need to quantify losses from the stimulated herd since it provided some level of protection against diseases among crossbred calves that received the TLR 7/8 agonist. From the study, the

benefit cost ratio revealed that the intervention was beneficial (Table 6). However, the benefit of this intervention is approximately 14 and 9 times higher when stimulated twice and once, respectively. Therefore, a rural farmer can make a choice between either stimulating once or twice based on his or her income. This will enable them avoid the abuse of cheaper forms of medications (Ekunseitan *et al.,* 2016). In general, TLRs that are used as vaccine adjuvants have been declared beneficial in terms of reducing the amount of antigen or injections required to provide protective immunity. Hence, reducing the cost of producing vaccines.

CHAPTER 6

6.0 CONCLUSION AND RECOMMENDATION

6.1 CONCLUSION

In summary, this study has identified that the TLR7/8 water-in-oil emulsion provides sufficient innate immune protection to mitigate severe disease upon natural exposure to multiple vector-borne pathogens during the first six to twelve months of life. While a single inoculation that provides a long-lasting depot for sustained induction of innate immunity over the six to 12 month vulnerable period would be optimal, a minimally acceptable regimen would be monthly inoculations, feasible in small-holder farms where animals are housed locally and given that the final emulsion cost is estimated between US$0.05-0.20. Together these results provided the rationale for recommending multiple innate immune stimulations of young crossbred calves as the most cost effective approach to protecting field-grazing crossbred cattle against diseases in Ghana.

6.2 RECOMMENDATION.

The strategy is that the innate immune stimulation afforded by the TLR7/8 agonist provides protection against severe disease during the most vulnerable period of a calf's life. This "short-term" protection during the first six months of life when all-cause mortality is greatest and natural challenge with multiple vector-borne pathogens occurs is then "replaced" with immunity due to natural pathogen exposure, immunity that is antigen-specific and life-long. With these findings, there is a need to;

- ✓ Stimulate crossbred calves before field-grazing in disease endemic areas
- ✓ Sensitize cattle farmers on the need to stimulate the innate immunity of young calves prior to exposure to natural challenge on the field
- ✓ Implement innate immune stimulation as a means of eliminating multiple pathogens

- ✓ A technological alternative would be to modify the formulation to enhance depot effect and length of innate stimulation in collaboration with our partners at the Infectious Diseases Research Institute in Seattle

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