EPIDEMIOLOGICAL STUDY OF INFECTIOUS BURSAL DISEASE VIRUS IN SELECTED DISTRICTS OF THE COPPERBELT PROVINCE IN ZAMBIA

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ABSTRACT

Infectious bursal disease (IBD) is a highly contagious disease of young chickens between 3 and 6 weeks of age. It is caused by *infectious bursal disease virus*(IBDV) which occursworldwide affecting livelihoods of resource - compromised poor communities. In Zambia, there is scantily documented information on the epidemiology of IBD. In-depth knowledge on the epidemiology of IBD is needed for effective control measures. This study aimed at molecular detection of circulating IBDV strains, andknowledge assessment of farmers about the disease in Ndola, Kitwe, Kalulushi, Luanshya and Mufulira districts of the Copperbelt province. A cross-sectional purposive study was carried out in the Copperbelt province from February to March, 2015 to determine the occurrence of IBD. The identification of IBDV was done by reverse transcription polymerase chain reaction (RT-PCR) targeting the hypervariable domain (VP2-HVR). A semi-structured questionnaire was administered to 77 respondents who presented poultry related cases to clinics in the selected districts and the information collected was analyzed by statistical package for social scientists (SPSS). A total of 30 bursa of Fabricius samples from young chickens that presented with clinical signs suggestive of IBD were examined. The RT-PCR results revealed two positive samples for IBDV VP2-HVR domain. Questionnaire study revealed that 70.0% (n=10) of the respondents did not know what disinfectant to use; 75.0% (n=57) felt assigning more than one individual to a flock of chickens did not compromise biosecurity whereas 20.3% (n=15) knew the important clinical signs of IBD, compared to 60.1% (n=46) and 70.7% (n=54) that knew clinical signs related to chronicrespiratory disease and ND respectively. Ninety six percent (n= 73) adhered to vaccine cold chain practices. These findings indicate that IBD viruses circulated in the Copperbelt province and chicken farmers had low awareness of IBD and respective

disease control measures. Further studies to characterize the circulating IBD viruses to unravel more information for the rational IBD control strategy in Zambia are required.

DECLARATION

I, Kenneth Chawinga, do hereby declare to the Senate of Sokoine University of Agriculture that this dissertation is my own and original work done within the period of registration and that it has neither been submitted nor currently being submitted in any other institution.

_____ _____

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The declaration is hereby confirmed by:

_____ _____

Dr. Christopher Kasanga Date

(Supervisor)

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DEDICATION

To Almighty God, for his unending wonders and miracles in my life. To my beloved wife Anna Phiri Chawinga, to whom words are insufficient to express my sincere and heartfelt gratitude. To my beloved parents, Chibukepo Chabinga and Clara Zgambo, who have been by my side through difficult and joyous moments.

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LIST OF ABBREVIATIONS AND SYMBOLS

ACP	African-Caribbean-Pacific
AU-IBAR	African Union-Inter-bureaufor Animal Resource
AVE	ribonuclease free water buffer
AVL	viral lysis buffer
AW	wash buffer
BALT	Bronchial Associated Lymphoid Tissue
BF	Bursa of Fabricius
CALT	Conjunctiva Associated Lymphoid Tissue
cDNA	complementary deoxyribonucleic acid
dNTP	deoxynucleoside triphosphate
dsRNA	double stranded ribonucleic acid
FVM	Faculty of Veterinary Medicine
GALT	Gut Associated Lymphoid Tissue
GRZ	Government of the Republic of Zambia
HVR	hypervariable region
IBD	Infectious Bursal Disease
IBDV	Infectious Bursal Disease Virus
IgM	Immunoglobulin M
MAL	Ministry of Agriculture and Livestock
min	minute
ND	Newcastle disease
OIE	Office International des Epizooties (International Organization of Animal Health)
PCR	Polymerase Chain Reaction

rpm	revolutions per minute
RT-PCR	reverse transcription polymerase chain reaction
sec	seconds
SUA	Sokoine University of Agriculture
USA	United States of America
SVP	sub-viral particle
V	Voltage
vIBDV	classical virulent Infectious bursal disease virus
VP1	viral ribonucleic acid polymerase
VP2	viral protein two
VP3	viral protein three
VP4	viral protein four
VP5	viral protein five
vvIBDV	very virulent Infectious Bursal Disease Virus
µl	microlitre

CHAPTER ONE

1.0 INTRODUCTION

1.1Background

Infectious bursal disease virus (IBDV) is the aetiological agent of infectious bursal disease (IBD), also known as infections bursitis or avian nephrosis. It is a highly contagious disease of young chickens, usually between three and six weeks of age, characterized by high morbidity and mortality (Dinev, 2007).

Infectious bursal disease is a disease of worldwide importance due to the huge losses as a result of opportunistic infections encountered by poultry farmers. The disease is especially a problem in developing countries due to challenges, including, but not limited to lack of appropriate vaccines that would be effective against evolving strains of the virus (Mohamed *et al*., 2014). Limited knowledge of farmers about the disease and non adherence to cold chain requirements of the vaccine are also responsible factors for IBD prevalence (Mbuko *et al.,* 2010).

There are two distinct serotypes of IBDV namely, serotype 1 and serotype 2. Infectious Bursal Disease is caused by serotype 1 which has three pathotypes classified as classical virulent, antigenic variant or very virulent IBDVs (Kasanga *et al*., 2013).

In Zambia, IBD outbreaks have been reported to occur since early 1990s in various parts of the country (Kasanga *et al.,* 2008). This is so, especially in poultry kept in backyard farming setups for commercial purposes. Unlike in conventional and large scale poultry farms that can afford veterinary services, in small holder, backyard poultry farming setups, such is not the case. The main contributing factors for IBD occurrence in Zambia include

limited knowledge on poultry diseases by farmers, absence of prompt reporting of outbreaks, lack of appropriate vaccines, lack of proper differential diagnosis with other well known and regular disease outbreaks such as Newcastle disease. This could be further exacerbated by compromised biosecurity, especially intensively kept broiler and traditionally kept chickens, which might be harboring the very virulent IBDV variants (vvIBDV).

However, despite of such problems leading to IBD occurrence, very limited research and studies have been undertaken on molecular detection and characterization of the field strains. Only a few such studies on IBDV genome reasortment have been carried out in Lusaka and Mazabuka districts (Kasanga *et al.,* 2013; Kasanga,2015). The control of IBD needs rapid detection, an in-depth knowledge of molecular characteristics and antigenicity of field isolates as well as poultry keepers/farmers perceptions and common control practices. The present study investigated the molecular detection of circulating IBDV strains and farmers' perception on IBDoccurrence in Zambia.

1.2 Problem Statement and Justification

The huge losses of poultry in Zambia and other developing countries incurred as a result of IBD is mainly ascribed to firstly to high mortality in chickens of three to six weeks. In addition, severe and prolonged immunosuppression paves way for *E. coli*and other secondary microbial infections (Farooq *et al*., 2003). Furthermore, vaccine failure may be encountered partly due to the emergency of field strains that are antigenically different to the strains used in available vaccine, thereby offering very limited immunity or no immunity at all. The other cause of vaccine failure could be attributed to non-adherence to cold chain requirements, as well as pre-administration reconstitution of the vaccine.The current vaccination protocol against IBD is vaccination of chicks at first day of hatching

and boosted at 21 days post-hatching (Rautenschlein *et al.,* 2005, Besseboua *et al.,* 2015). Outbreaks however, have been reported in vaccinated flocks of chickens, suggesting a possibility of vaccine failure (Islam *et al*., 2008).

The knowledge of poultry farmers on IBD occurrence in Zambia has not been investigated. In this view, it is also imperative to ascertain the awareness of poultry keepers on the knowledge about the disease, its prevention and control measures.

1.5.1 Main Objective

To determine the epidemiology of IBD and its control options in selected districts in the Copperbelt Province in Zambia.

1.5.2 Specific Objectives

(i) To determine the occurrence of IBD in chickens kept in the Copperbelt province of Zambia.

(ii)To determine the awareness of farmers on the occurrence and control options of IBD in Copperbelt province.

(iii) To establish factors for the occurrence/prevalence of IBD in the Copperbelt province of Zambia.

1.5.3 Research Questions

(i) What is the frequency/occurrence of IBDV in the Copperbelt province of Zambia?

(ii) What is the awareness level of farmers on IBDV in the Copperbelt province of Zambia?

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Infectious Bursal Disease Virus

Infectious bursal disease virus (IBDV) is the aetiological agent of infectious bursal disease (IBD), also known as infectious bursitis or avian nephrosis. It is a highly contagious disease of young chickens characterized by high morbidity and mortality of young chickens usually of ages between three to six weeks of age. The disease is also known as Gumboro disease, a name it earned after its first documentation in Delaware, Sussex County, United States of America. The disease is important due to increased susceptibility of infected chickens to other diseases and negative interference with effective vaccination (Saif *et al*., 1991). The bursa of Fabricius (BF) is the primary target organ of IBDV. The virus replicates in mature B – lymphocytes and cause a depletion of these cells in the BF. The immune suppression that results from an IBDV infection has major economic impact on broiler as well as layer chicken industries. Often, the immune suppression goes unnoticed because the disease is sub-clinical in nature. Thus, itis difficult to estimate the true economic impact of IBDV as an underlying cause of opportunistic respiratory and enteric diseases and vaccination failure. In order birds, a transient immune suppression is observed during disease, but because their secondary lymphoid organs have already been seeded with lymphocytes, convalescent birds usually recover most of their humoral and cellular immune functions (Boudaroud and Alloui, 2008).

Infectious Bursal Disease Virus is a segmented, double stranded RNA virus, member of the genus *Avibirnavirus* of the family *Birnaviridae* infecting the IgM-bearing B-lymphocytes in the bursa of fabricius leading to immunosuppression (Caston, 2008). In recent years, very virulent strains of IBDV (vvIBDV) have been reported

furthermorecompounding the problems caused by IBDV.After 1987, pathotypic IBDV variants with enhanced virulence, called very virulent IBDVs (vv-IBDVs) emerged in Europe, and have spread to many places of the world. These strains are also classified into "European vv-IBDVs" and "African vv-IBDVs" (Kasanga *et al.;* 2007, 2013). Australia, New Zealand, Canada and the USA are the only countries not affected by vvIBDVs (Proffitt *et al*., 1999). It is estimated that vvIBDVs are present in 95% of OIE member countries (van de Berg, 2000).

2.2 Viral Structure

The IBDV capsid protein exhibits structural domain that resemble positive sense, single stranded RNA viruses (Vakharia *et al.,* 1994). The structural protein VP2 of IBDV spontaneously forms the dodecahedral T = 1 subviral particle (SVP), and is primarily the immunogen of the virus (Lee *et al*., 2006).

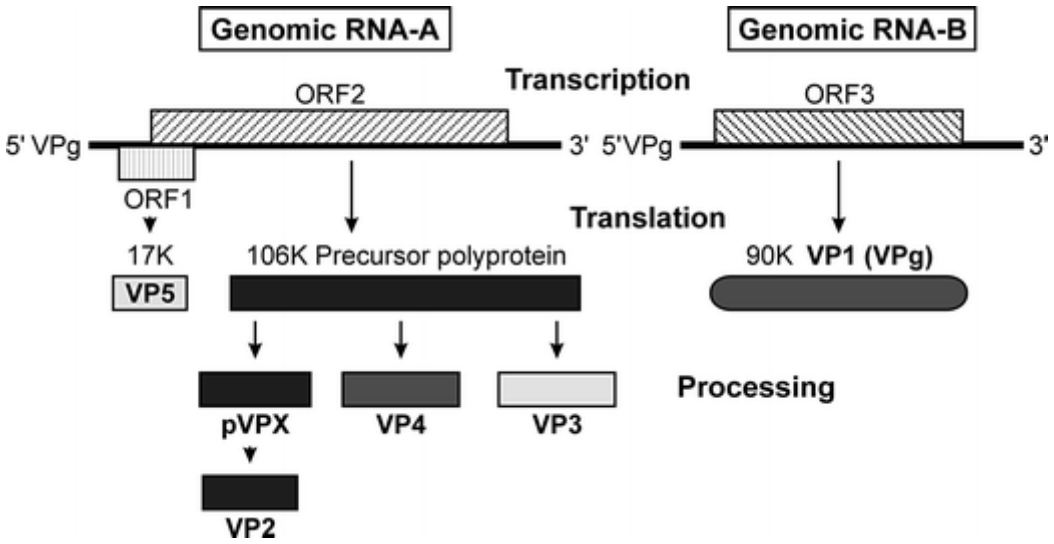

Figure 1: Schematic diagram of IBDV genome

Source: htt://www.linkspringer.com

The IBDV genome segment A contains partially overlapping open reading frames (ORFs), ORF1 andORF2. The small ORF1 encodes a non-structural protein VP5, whereas the large ORF2 encodes aprecursor polyprotein, which is cleaved by autoproteolysis to produce VP2, VP4 and VP3. VP2 and VP3 are the major structural proteins of the virion, and VP4 is the viral protease. Genome segment B, on the other hand, contains one ORF encodes VP1, which is theRNA-dependent RNA polymerase (RdRp) responsiblefor viral genome replication and RNA synthesis (Ismail *et al.,* 1990; Kasanga, 2015).

2.2 Transmission / Pathogenesis of IBD

The virus is shed through the fecal route. The incubation period ranges from two to four days and the infected birds start shedding the virus twenty four hours post infection (Lawal *et al*., 2014). The virus can survive in environment with pH ranging from 2 to 12. It can also survive in poultry houses for 122 days after removal of infected birds and for as long as 52 days in contaminated feed and water (van den Berg, 2007).

The clinical disease is associated to birds with the greatest bursal mass, which ideally occurs between 3 and 6 weeks of age. This bursal mass is as a result of large population of maturing IgM- bearing B-lymphocytes (lymphoblasts), the target of infection. Sub-clinical disease may occur before three weeks. At this time, B-lymphoblast population is smaller and its effects are insufficient for generating clinical signs. However, the B-cell destruction and depletion at this stage has more severe consequences as the virus destroys the small population of B-cells, before they are differentiated (Sharma *et al*., 2000).

Upon ingestion, the virus destroys the lymphoid follicles in BF as well as the B-cells circulating in secondary lymphoid organs such as the gut associated lymphoid tissues (GALT), bronchial associated lymphoid tissues (BALT), conjunctiva associated lymphoid

tissue (CALT) and cercal tonsils (Nwaigwe *et al*., 2010). Acute infection and death is due to necrotizing effects of this virus on host tissues. Kidney failure is a common cause of mortality. Morbidity may rise from 10% to 100% with mortality ranging between 20 % to 56%, but going up to 100% with hypervirulent strains (Muskett *et al.,* 1985).

2.3 Risk Factors for IBD

The three main points where risks have been noticed are the breeding farms, the vaccine outlets and at the farm where the risk is twofold, i.e. biosecurity and vaccine handling. The major risk factors however are at the farm and these include, but not limited to;

(i) Few drinkers used for administering vaccine (thereby living out many birds not targeted for the vaccination).

(ii) Presence of disinfectants in water that interferes with vaccine function

(iii) Use of wrong vaccines (i.e. infectious bronchitis vaccines have been used instead of infectious bursal disease by uninformed cadre of farm workers/ managers) and less immunogenic IBD vaccines

(iv) Use of improper diluents and vaccine adjuvants (Mutinda *et al.,* 2014).

2.4 Clinical Signs

Disease may appear suddenly and morbidity may vary from 10% to 100% with mortality typically going up to 60% in classic IBDV infections and up to 100% in vvIBDV infections. Morbidity and mortality is dependent on challenge dose, previous immunity status and presence of concurrent disease (Stuart, 1989). The clinical signs of IBD are not pathognomonic. In acute form, birds are prostrated, debilitated, dehydrated, with water diarrhea and swollen vents stained with faeces. In birds below three weeks, the disease is asymptomatic, but birds have bursal atrophy with fibrotic or cystic follicles and lymphocytopaenia before six weeks and are usually susceptible to other infections that would be contained in immunocopetent birds (Mor *et al.*, 2010).

2.5 Diagnosis

Tentative diagnosis is usually made by obtaining flock history, clinical signs and findings at necropsy. However definitive or confirmatory diagnosis can be done by specific detection and/or isolation and characterization of the IBDV. Immunoflouresence and immunohistochemistry tests based on anti-IBDV labelled antibodies or in-situ hybridization based on labelled complementary cDNA probe are useful in identification of specific IBDV in tissues.Necropsy findings typically shows changes in the bursa of Fabricius such as swelling, edema, hemorrhage and presence of jelly serosa transudate. Eventually the bursa atrophies. Pathological changes, especially hemorrhage might be seen in the skeletal, intestines, kidney and spleen.Because conventional virus isolation and characterization are not practical for routine diagnosis of IBDV, antigen-capture enzyme-linked immunosorbent assay (Ag-ELISA) (Jackwood,2004). Conventional and real-time RT-PCR, amplifying the VP2 hypervariable region, in combination with DNA sequencing of the PCR product, can differentiate classic, variant, and vvIBDV strains, because variant and vvIBDV have characteristic nucleotide and amino acid substitutions (Wu *et al*., 2007).

2.6 Prevention and Control

Peri-focal (ring vaccination) may not be effective in case of an outbreak due to the rapid manner of the spread of wild- IBDV. Passive immunity may protect against challenge with homologous IBDV. Timing of vaccination has proved to be difficult, due to interference with maternally acquired antibodies. Low attenuated vaccines may cause considerable damage to the BF (Eterradosi and Saif, 2008). Various types of vaccines are used to confer immunity to chickens. These include DNA vaccines i.e. construction of plasmid DNA carrying VP2, VP4, and VP3 genes of the standard challenge (STC) strain of IBDV (Chan *et al*., 2001).Inactivated IBD vaccines aremostly used to produce high, long-lasting and uniform levels of antibodies in breeding hens that have previously been primed by live

vaccine or by naturalexposure to field virus during rearing (Malik *et al.*, 2006). The usual programme is to administer the live vaccine at about 8weeks of age. This is followed by the inactivated vaccine at 16–20 weeks of age. Occasionally, inactivated vaccines may be used in programmes combining inactivated and live vaccines, in young valuable birds with high maternal derived antibodies(MDA) levels reared in areas with high risk of exposure to virulent IBDV (Haddad *et al*., 1997).Live attenuated IBD vaccines are produced from fully or partially attenuated strains of virus, known as 'mild', 'intermediate',or'intermediate plus' ('hot'), respectively.

Mild or intermediate vaccines are used in parent chickens to produce a primary response prior to vaccination near to point-of-lay using inactivated vaccine. Application is by means of intramuscular injection, spray or in the drinking water, usually at 8 weeks of age (Muskett *et al*.,1979; Muller *et al*.,2003).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Area

The current study was conducted in the Copperbelt province in Zambia. Apart from Lusaka, the country's capital city, Copperbelt province is the largest poultry raring province, located at 13° 16' 0" South, 28° 25' 0" East. Copperbelt province comprises seven districts and sampling was done in five of the seven districts namely Kalulushi ,12° 50' 0" South 28° 5' 0" East ; Kitwe,12° 49' 0" South, 28° 12' 0" East; Luanshya,13° 8' 0" South, 28° 24' 0" East; Mufulira,12° 33' 0" South, 28° 14' 0" East and Ndola,12° 58' 0" South, 28° 38' 0" East. The sampling areas and flocks were selected from five districts in the Copperbelt (Fig. 2) under the Ministry of Agriculture and Livestock, Department of Veterinary Services.

3.2 Study Design

A purposive cross-sectional study design was used with semi structured questionnaire was administered to 76 respondents in the selected districts to examine the poultry keepers' perception about IBD. The respondents were poultry keepers that had brought their sick or dead chickens to the government clinic in Ndola District.

3.3 Samples and Sampling

The bursa of Fabricius samples were collected from chickens with ages ranging from 3 to 6 weeks in Copperbelt province in Zambia. A total of 30 samples (5 bursae from each affected flock) were collected. The bursa of Fabricius samples were taken from broiler chickens presented to veterinary clinics with clinical signs suggestive of IBD.

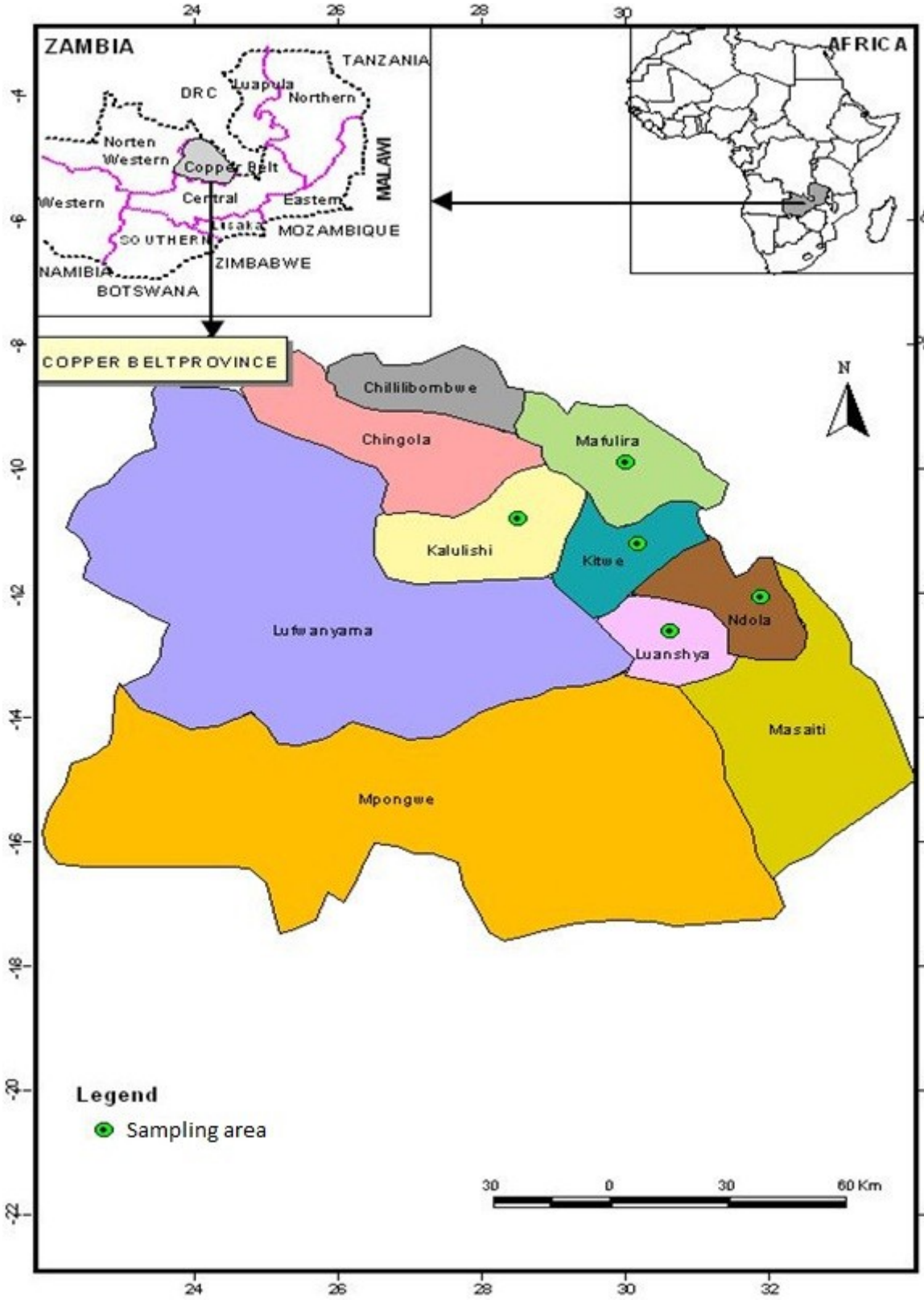

Figure 2: Map of Zambia, showing Copperbelt province and specific districts from where sampling was done

Source:https://en.wikipedia.org/wiki/Districts_of_Zambia

3.2 Sampling Procedure

Sampling in the current study was done in the months of February and March, 2015. All sick and dead chickens between the ages of three and six weeks brought to the above mentioned clinics had postmortem conducted on them. In case of live and moribund cases, consent had to be granted by the owners for humane slaughter and postmortem to be conducted. Thereafter, humane slaughter was by way of dislocation of the atlantoaxial joint of the vertebra column. After this, chickens were placed on a dissection board and positioned in dorsal recumbancy for postmortem examination. A thorough external examination of the chickens was done and the pathological lesions noted. A deep incision was then made on the region between the abdominal cavity and pectoral muscles, exposing the viscera. The bursa of Fabricius, which lies at the dorsal cloacal end of intestines were removed.

3.2.1 Sample Packaging and Transportation to the Laboratory

After the process described above, the bursae were collected aseptically and packaged in a properly labeled plastic container and stored under ice. Sample packages were labeled with the following information; location, management system and biosecurity measures. From the initial site of necropsy, samples were transported to the University of Zambia (UNZA), Samora Machel School of Veterinary Medicine, Department of Disease Control laboratory, where they were stored at -20°C until further shipment to Sokoine University of Agriculture (SUA), Faculty of Veterinary Medicine (FVM) Morogoro, Tanzania where sample analyses were done. Shipment from UNZA was done with samples under dry ice. Once at SUA, samples were stored at -20°C at the Department of Microbiology and Parasitology in the virology laboratory till analysis.

3.2.2 Questionnaire Administration

A semi structured questionnaire, consisting of both closed and open ended questions was administered by the district livestock officer in the selected district with the aim of finding out the poultry keepers' perception about the IBD namely, knowledge about the disease, duration that individual farmers have kept chickens, biosecurity measures employed, maintenance of vaccine cold chain from the source of purchase to administration.

3.2.3 Laboratory Analyses

RNA Extraction

Five hundred and sixty microlitre of prepared Buffer AVL containing carrier RNA was pipetted into a 1.5ml microcentrifuge tube.140 µl of tissue supernatant was added to Buffer AVL- carrier RNA in the microcentrifuge tube. This was then mixed by pulse – vortexing for 15 seconds.

Carefully, the QIAamp Mini spin column was opened and 500 µl of Buffer AW2 added. The cap was then closed and centrifuged at full speed (20 000 x g; 14 000rpm) for three minutes.

To eliminate any chance of possible Buffer AW2 carryover, the QIAamp Mini spin column was placed in a new 2 ml collection tube. This was then centrifuged at full speed for one minute.

The QIAamp Mini spin column was placed in a clean 1.5 ml microcentrifuge tube. The old collection tube containing the filtrate was discarded. The QIAamp spin column was then carefully opened and 60µl of Buffer AVE equilibrated to room temperature was added. The cap was closed and incubated at room temperature for 1 minute. The contents were then centrifuged at 6000 x g (8000 rpm) for 1 minute.

A double elution using 2 x 40µl of Buffer AVE was performed so as to increase the yield of viral RNA.Viral RNA labeled according to sample from where it was extracted was then stored at -20°C awaiting Reverse Transcription.

3.2.4 cDNA Synthesis

The extracted viral RNA was converted to cDNA by using the Thermal Cyclerfollowing the protocol outlined below (Applied Biosystems). TenµL of 2 X RT master mix was pipetted into 30 tubes (corresponding with the number of RNA samples).Ten µL of the IBDV RNA samples was pipetted into all the 30 tubes, pipetting up and down two times to thoroughly mix the contents, containing the master mix. All the tubes were then sealed and centrifuged briefly to spin down the contents and eliminate any air bubbles. While conducting the above steps, the tubes were placed on ice until they were ready to be loaded into the Thermal Cycler. For different stages in cDNA Reverse Transcription, refer to Table 2 below.

Table 1: Master mix for conversion of viral RNA to cDNA

No	Component	Volume (µL)
1	10 x RT Buffer	2.0
2	25x dNTPs Mix (100mM)	0.8
3	10xRTRandom Primers	2.0
4	Reverse Transcriptase	1.0
5	Nuclease Free Water	4.2
Total Volume per reaction		**10**

Table 2: Thermal Cycler conditions for cDNA Reverse Transcription

Step 1	Step 2	Step 3	Step 4	
Temperature (^{o}c)	25	37	85	4
Time Min)	10	120	5	∞

RNA is first incubated with a primer at 70 degree to denature RNA secondary structure and then quickly chill on ice to let the primer anneal to the RNA. (2) Other components of RT are added to the reaction including dNTPs, RNase inhibitor, reverse transcriptase and RT buffer. (3) RT reaction is extended at 42 degree for 1 hr. (4) Heat the reaction at 70 degree to inactivate the enzyme.

3.2.5 Detection of IBDV by PCR

Conventional PCR was run using TakaRa PCR Thermal Cycler Dice™, Version III, Model TP600/ Gradient from TAKARA BIO INC. PCR reactions were carried out on a final volume of 20 µlper reaction using master mix as shown in Table 2 below. Each reaction consisted of an initial denaturing step, 35 cycles of denaturing, annealing and extension, followed by a final extension step as illustrated in Table 3.

Table 3: Conventional PCR master mix components for detecting IBDV

No	Component	Volume (µL)
1.	1 x Ready Mix	12.5
2.	Forward Primer	0.5
3.	Reverse Primer	0.5
4.	cDNA	4.0
5.	Water	9.5
Total Volume per reaction		20

Table 4: Pair of Primers used in the present study

Primer Pair	Primer sequences(5' -3'direction)	Targetgene	Target species	Product size (bp)
V1	CCA GAG TCT ACA CCA TAA	VP2-HVR 472bp	IBDV	
V2	CCT GTT GCC ACT CTT TCGTA (Lin *et al*)			

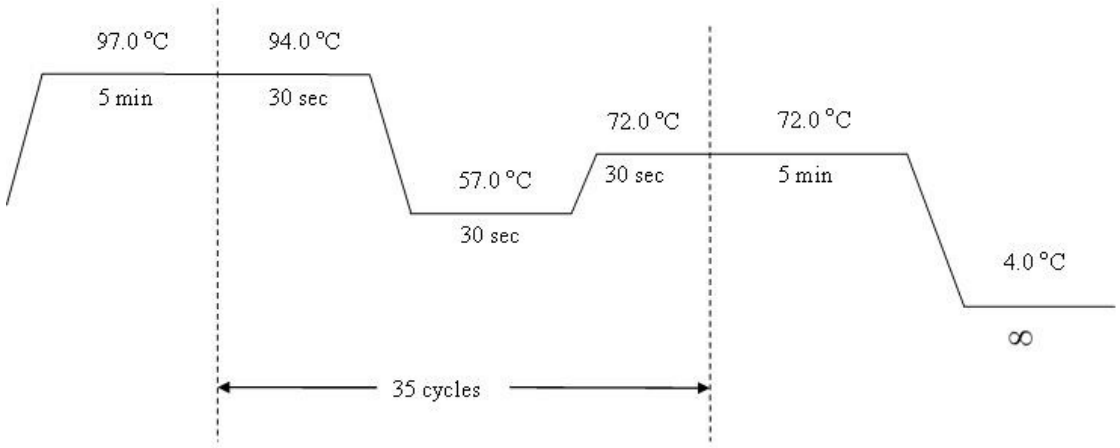

Figure 3: Conventional PCR cycling conditions used in the present study. The reactions consisted of 35 cycles, each composed of denaturing at 94 ^{o}C for 30 sec., annealing at 57.0 ^{o}C for 30 sec. and extension at 72.0 ^{o}C for 30sec., all these stages preceded by an initial denaturing step at 97.0 ^{o}C for 5 min. and followed by a final extension step of 5 min. at 72.0 ^{o}C.

3.2.6 Gel electrophoresis and visualization of PCR products

Ten μl of each PCR product were run on a 1.2% agarose gel electrophosis in TAE buffer at 100V for 45 min using Mupid-One electrophoresis System (advance, Tokyo, Japan), along with a 100 bp DNA ladder (Promega, Madison, USA), after being mixed with Ethidium bromide for 25min. Figure 4 under results section.

CHAPTER FOUR

4.0 RESULTS

4.1 Awareness of Respondents on IBD

4.1.2 Common disease conditions encountered

On common disease occurrence, 60.1 % (n=46), 29.3 %(n=22) and 70.7 %(n=54) gave clinical signs and symptoms which were consistent with ND, IBD and CRD respectively.

4.1.3 Hygiene and knowledge in the use of disinfectants and biosecurity

While all the respondents knew that good hygiene practices reduce occurrence and incidence of poultry diseases, majority, 70 % (n=54), did not know what disinfectant to use. Seventy five percent felt assigning more than one individual to a batch or poultry house did not compromise biosecurity.

Table 5: Chemicals used for disinfecting chicken houses and equipment by respondents in the questionnaire study

Means of Disinfection	% of Respondents
Lime	34.7
Chlorine	8.3
Dettol®	0.8
Jik®	6.7
Hot water	0.8
Ordinary soap	1.7
Zecro®	0.8
Virrokill®	16.7
Doom ®	0.8
Microl®	13.3
Virid®	6.7
Biodan®	4.2
Formalin®	5.0

4.1.4 Knowledge of respondents about IBD

While respondents were knowledgeable about IBD, relatively fewer respondents i.e.30 % (n=23) were educated about IBD as compared to 91 %(n=70) that were educated on other poultry diseases that need vaccination such as ND.

4.2 Risk Factors for the Occurrence of IBD in the Copperbelt province

4.2.1 Chicken rearing experience among the respondents

Of the 77 respondents that voluntarily took part in the questionnaire study in Ndola district of the Copperbelt province of Zambia, Table 6 below shows the trend on how long they have been raring chickens (ranging from 1993 to 2015).

Table 6: Respondents experience (measured in years) in raring poultry

Y	**1993**	**1994**	**2001**	**2002**	**2005**	**2006**	**2009**	**2010**	**2011**	**2012**	**2013**	**2014**	**2015**
E	22	21	14	13	10	9	6	5	4	3	2	1	0
N	13	15	2	1	1	1	1	2	3	5	7	9	17
%	16.9	19.4	2.6	1.3	1.3	1.3	1.3	2.6	3.9	6.5	9.1	11.7	22.1

Y = Year **E** = Experience of raring chickens in years **N**= Number of poultry keepers

%=% of respondents with corresponding experience

4.2.2 Type of Chickens kept

Of all the respondents interviewed, 92.2% (n=71) said they kept broilers, 2.6 %(n=2) layers and 5.2% (n=4) local breeds.

4.2.3 Adherence to vaccine cold chain and reconstitution

Almost all the responds, 96% (n=74), acknowledged the importance of adherence to cold chain requirements. On vaccine reconstitution and administration the majority, i.e.

87%(n=67) did not mind to check or find out if there were any chemicals added to water that could affect the vaccine and 60%(n=46) felt the duration under which the vaccine should be administered did not matter. Seventy three percent did not withdraw water prior to administration of the vaccine so as to ensure maximum coverage.

4.3Detection of IBDV Genome from Field Samples

Out of 30 samples that were collected, (n=2) tested positive for IBDV by RT-PCR targeting the VP2-HVR. These were from Kitwe district (n=1) and Ndola district (n=1) in the Copperbelt province.

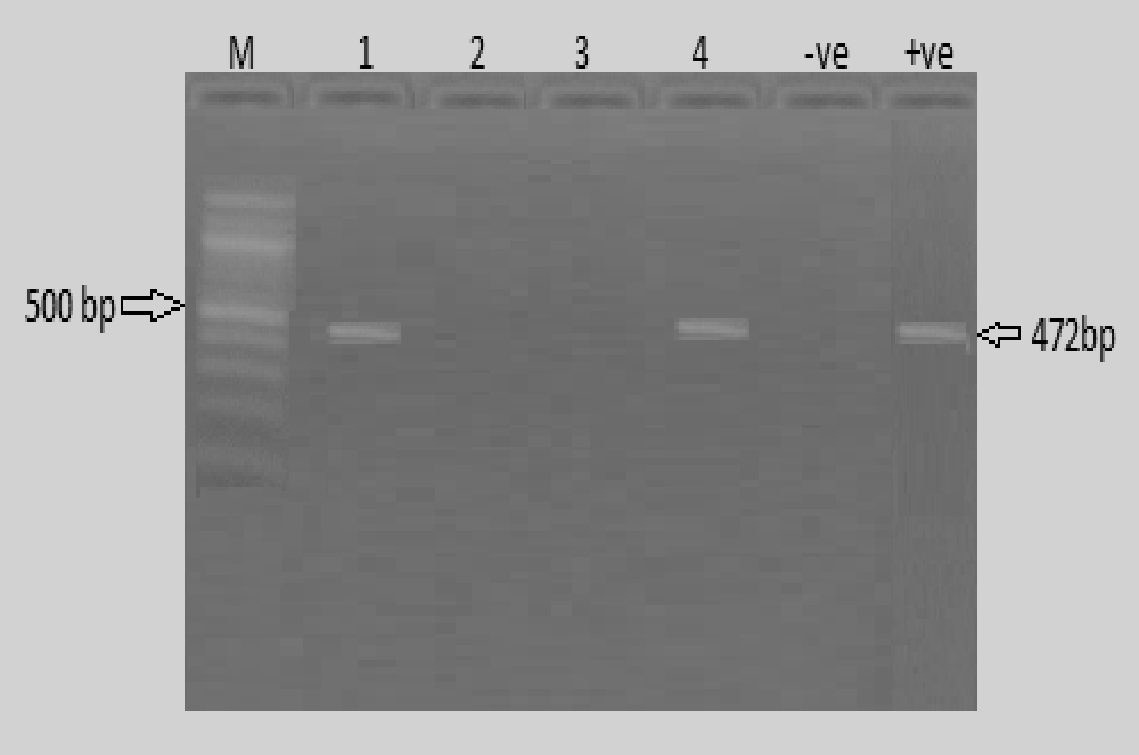

Figure 4: A representation of RT-PCR amplicons obtained from amplification of the IBDV-VP2-HVR gene. An agarose gel of 1.2% was used for analysis of PCR product. M is 100 bp markers, Lane 1 and 4 are positive samples. Lane 5 (-ve) is a negative control and Lane 6 (+ve) is a positive control

CHAPTER FIVE

5.0 DISCUSSION

The present study aimed to ascertain the presence of the IBDV in chickens in selected districts of the Copperbelt province in Zambia as well as to assess the factors that perpetuate the presence of the virus among vaccinated broiler chickens. A total of 0.10% samples were positive to IBDV in the Copperbelt Province. This compares with results obtained from similar studies in Zambia where IBDV was detected in vaccinated broiler chickens in Lusaka and Mazabuka (Kasanga *et al*., 2013) and in Lusaka (Ndashe *et al*.,2015).

However, unlike in the two studies referred to above, in which characterization of the circulating IBDV was done, the scope of the current study did not include molecular characterization or sequencing of the IBDV genome. Further, in the current study, the study design used was purposive, cross sectional study. Therefore sampling only involved vaccinated broilers that either presented with clinical signs or postmortem lesions consistent with IBD. To establish the actual prevalence therefore a further broader study involving a larger sample size would be required. Additionally, the low prevalence observed in the current study could be partly ascribed to wrong tentative diagnosis that was one of the criterions for inclusion. Postmortem lesions of IBD closely resemble ND (Dolka *et al.,* 2012). The time during sampling coincided with the usual time of ND outbreaks which is endemic in Zambia.

Further, to have an indepth knowledge that would add to the rational control strategy of IBD, a study on the molecular characterization and sequencing of the circulating viral genome would be needed. This is important as the detection of IBDV occurred in chickens

duly vaccinated against IBDV, implying that the circulating field pathotypes are not homologous to strains used in vaccine manufacture. Also this could be ascribed to the fact that protection of chickens against IBDV relies primarily on vaccination. Live vaccines are used in chicks for active immunization and inactivated vaccines are used in breeder hens so that MDA can pass to their progeny and protect them during the first few weeks of life. Live vaccines are of different levels of attenuation, and are popularly known as mild, intermediate and intermediate plus vaccine in order of increasing residual pathogenicity. The latter vaccines can cause some degree of bursal lesions and subsequent immunosuppression (Islam, 2014).Therefore even if compliance to vaccination may be high as seen in the results under study, immunosuppression may in fact be due to the effect of the vaccine used. Thus, to alleviate this problem apart from conventional live attenuated and inactivated vaccines, efforts have also been made to develop vaccines using recombinant DNA technologies. Such vaccine candidates include DNA vaccine, subunit vaccine, genetically modified live virus vaccine and vectored vaccine (Martinez-Torrecuadrada *et al*., 2003).

In the current study, it was revealed that the awareness levels of farmers regarding the occurrence and control options of IBD is relatively low, i.e. at 30% of the respondents compared to 70% awareness about ND.

A similar study in Madina town in Pakistan showed similar trends in awareness levels of poultry keepers towards IBD in particular and poultry diseases in general. The study noted that there are a lot of problems faced by poultry farmers regarding rearing and IBD management knowledge. Hence there is need to find out those areas of poultry faming in which poultry farmers needs to be educated (Razzaq*et al*., 2011).

In both cases overwhelming majority agreed on regular training of disease management. Respondents in both studies also pointed out that government should launch readily accessible disease control extension service.

In the current study, the following factors for occurrence of IBD were identified through a semi structured questionnaire namely; weak biosecurity measures, use of inadequate drinkers, presence of disinfectants in drinking water and in some cases, lack of adherence to vaccine cold chain as well as vaccine reconstitution protocols.

Thesefindings were similar to other studies done elsewhere that revealed that the underlying cause of high morbidity and mortality rates were found to be; improper vaccination, poor biosecurity measures, existence of vvIBDVs, poor vaccine storage, use of fewer drinkers for vaccine administration, traces of disinfectants in drinkers and water used to administer vaccine and use of improper diluents (Mor *et al*., 2010; Mutinda *et al*., 2014).

The challenge to minimizing the risk factors associated with IBD in the Copperbelt Province remains the inadequate extension staff to serve the poultry keepers whose numbers are on the increase.The control of IBD worldwide is mainly achieved through adherence to biosecurity measures as well as vaccination.

CHAPTER SIX

6.0 CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusion

From the present study, it was found that IBD viruses were circulating in the Copperbelt province, whose pathotypes are not yet known. Furthermore, the awareness level of farmers regarding the occurrence and control options of IBD was relatively low, corresponding to 30% of the respondents.

Risk factors for the occurrence of IBD in the Copperbelt province were mainly weak biosecurity measures, use of inadequate drinkers,presence of disinfectants in drinking water and in some cases, lack of adherence to vaccine cold chain and non-compliance to vaccine reconstitution protocols.

6.2 Recommendations

(i) Carry out studies to ascertain molecular characteristics, including sequencing of the circulating IBD viruses in the Copperbelt Province in order to gather more information regarding knowledge and understanding of the pathotypes involved.

(ii) Molecular epidemiological studies to understand the occurrence and pattern of spread of IBDV field strains in the Copperbelt Province and Zambia in general are required so as to recommend for the rational IBD control method.

(iii)Farmer education by way of robust IBD extension service and short courses of lead poultry farmers is required. The lead poultry farmers would in turn be trainers of trainers on IBD and associated viruses.

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APPENDICES

Appendix 1: Semi structured questionnaire for collecting social data about IBD in Ndola district of the Copperbelt province-Zambia

SOKOINE UNIVERSITY OF AGRICULTURE

FACULTY OF VETERINARY MEDICINE

DEPARTMENT OF MICROBIOLOGY AND PARASITOLOGY

SEMI STRUCTURED QUESTIONNAIRE (TO BE ADMINISTERED BY CAMP EXTENSION OFFICERS)

This questionnaire seeks to assist in collection of information from poultry keepers in order to come up with risk factors associated with Gumboro disease (Infectious bursa disease). The other objective is to assess the knowledge of poultry farmers on Gumboro disease. The information collected and collated will be used to advise the relevant department, National epidemiological information center-NALEIC in order that they come up with alternative control measures.

1. When did you start rearing chickens.....

2. What kind of chickens do you rear [A] Broilers [B] Layers [C] Local chickens [D] Others

Specify.....

3. What are the common diseases that you encounter?

(a) Name(s) of diseases (including names in local languages) Most important clinical signs

(i).....

(ii).....

(iii).....

(iv).....

(v).....

4. Do you vaccinate your birds?

5. What are the names of the vaccines you use?

6. Which vaccines of the mentioned do you think is important?

7. Do you disinfect the poultry house and equipment, e.g drinkers and feeders in between batches?

8. What are the disinfectants that you use (give both trade and generic names)?

10. How many people are allowed to look after the chickens?

11. Is the vaccine cold chain from the distributor to the farm maintained?

12. Are the instructions for the administration of the vaccine followed?

Appendix 2: Consent agreement

SOKOINE UNIVERSITY OF AGRICULTURE

FACULTY OF VETERINARY MEDICINE

DEPARTMENT OF MICROBIOLOGY AND PARASITOLOGY

Informed consent agreement for study participants

Title of study: Epidemiology of Infectious Bursal Disease in the Copperbelt Province.

Study rationale and aim: We are carrying out research on Infectious Bursal Disease. The aim of this study is to understand the spread, public awareness, risk factors and control measures. These data are important as they will increase awareness about the implications and productivity losses related to this disease, so to be able to implement control strategies that will reduce these impacts. In order to better understand the above, we will ask you about general knowledge of the disease including your experience regarding poultry raring.

Potential risks: No risks are anticipated in this study except minimal interruption in your time to participate in this survey.

Potential benefits: You and your village will be informed of the broad results obtained and what they mean. We will also discuss with you if there are any actions you might want to take to reduce the impact of IBD. In the end, this study will lead to better control of IBD in the Copperbelt province and Zambia at large. However, you will not see this benefit during the study.

Compensation: Your participation in this study will be voluntary. However, recommendations will be provided to you regarding ways of preventing and controlling IBD. Your participation in this study is not compulsory, and you have the right to decline to participate in this study or to not answer any of the questions that we will ask you. There will not be any repercussions on the quality of veterinary care you are receiving from withdrawal from this study.

Data confidentiality: Your answers are completely confidential and your name will not be included in any reports of these results. Your individual answer will not be shared with anyone. In presenting or publishing this study, your household will be represented by a code number, so that any facts about you or your household are kept private.

Contact person in case of emergency: For any emergency cases, please contact……..on +255…….. (phone n.) or through …….(email address).

Participant's information

Village/town name: _____

Village leader (where appropriate): Name _____ **Phone** n. _____

Study participant: Name _____ **Phone** no. _____

I hereby give full approval to the researchers of the Sokoine University of Agriculture to conduct this study in my household.

I understand the background and objectives of this research project and I am fully aware of the nature of the research and my role in it.

Signature or thumbprint of participant: _____Date:

_____

Signature of witness:_____

Date:_____

Research team statement

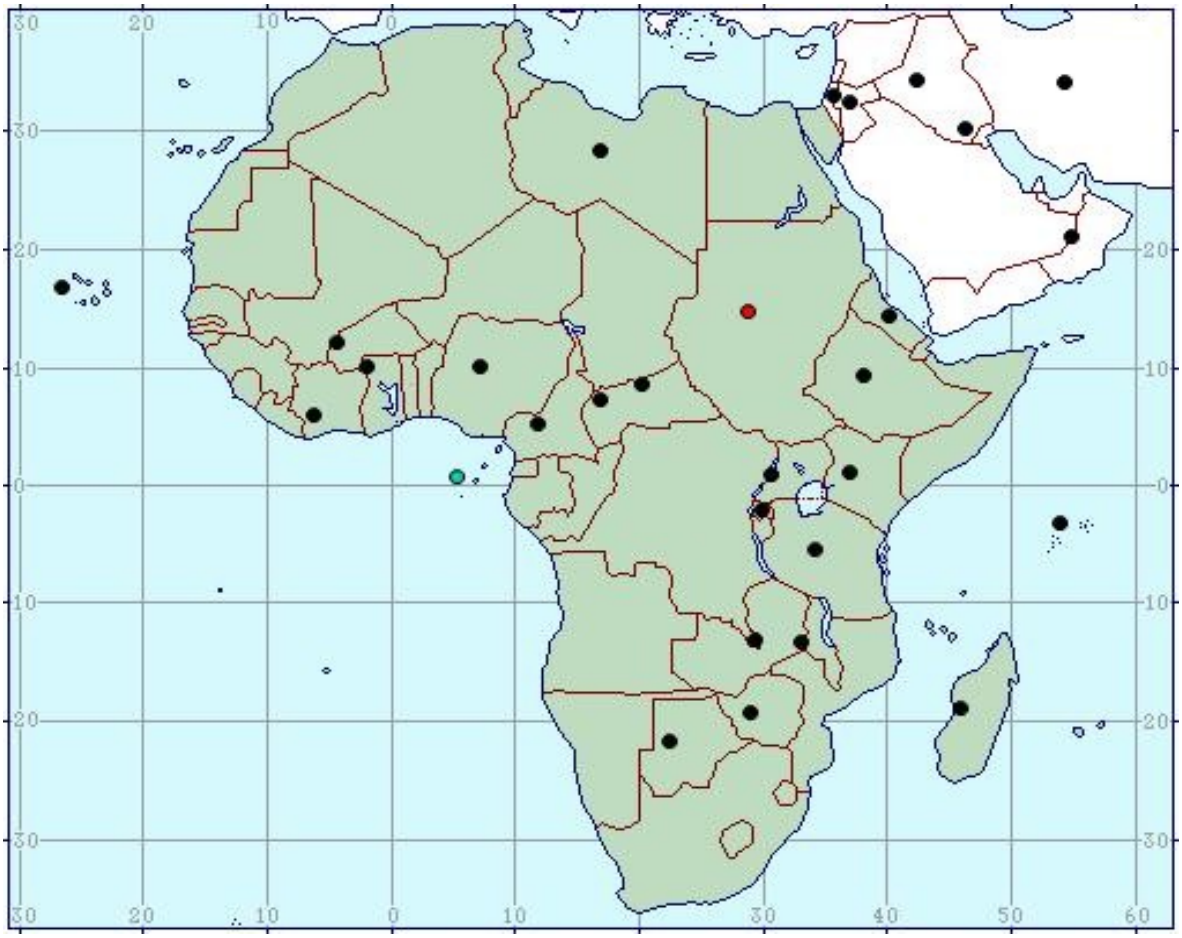
Name of the research officer:_____ Signature:

_____

Contact details: _____

I hereby confirm that I have explained the objectives, potential risks, benefits and any compensation of this study to the participants in a language they understand

Appendix 3: Distribution of vvIBDV in Africa



Legend ●= Present, no further details

●= Confined and subject to quarantine

Source: AU-IBAR