INFECTIOUS BURSAL DISEASE IN INDIGENOUS VILLAGE CHICKENS, DUCKS AND TURKEYS IN EMBU COUNTY, KENYA: STATUS; KNOWLEDGE, ATTITUDES AND PRACTICES OF VALUE CHAIN ACTORS

A THESIS SUBMITTED TO THE UNIVERSITY OF NAIROBI IN PARTIAL FULFILLMENT OF REQUIREMENTS OF THE MASTER OF SCIENCE DEGREE IN APPLIED MICROBIOLOGY (VIROLOGY OPTION)

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DECLARATION

This thesis is my original work and has not been presented for a degree in any other University.

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DEDICATION

I dedicate this thesis to my loving husband Simon Nderitu and our beloved daughter Elizabeth Njeri.

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LIST OF ABBREVIATIONS AND ACRONYMS

AGPT	agar gel precipitation test
BF	bursa of Fabricius
CEB	chicken embryo bursa of fabricious
CEF	chicken embryo fibroblast
CEK	chicken embryo kidney
ELISA	enzyme- linked immuno absorbent assay
IBD	infectious bursal disease
IBDV	infectious bursal disease virus
IHA	hemagglutination inhibition test
Km	kilometres
KNBS	Kenya National Bureau of Statistics
KPHC	Kenya Population and Housing Census
Mls	millilitres
OIE	World Organisation for Animal Health
OR	Odds Ratio
RPM	revolution per minute
RT-PCR	reverse transcription polymerase chain reaction
VvIBDV	very virulent infectious bursal disease virus
FAO	Food and Agriculture Organization
SPF	specific pathogen free

ABSTRACT

Indigenous village chickens are mainly raised in rural settings under free range systems alone or as mixed flocks with ducks and turkeys. They are routinely exposed to overwhelming number of microorganisms, some of which are highly pathogenic such as infectious bursal disease (IBD) virus. The IBD infected ducks and turkeys do not show overt (IBD) clinical signs and they may act as a source of infection to naive chickens kept in such mixed flocks. It is not clear what poultry value chain actors know on IBD maintenance and spread and the status of the disease in Embu County, in chickens, ducks and turkeys, is also not known. The objectives of this study were to determine knowledge, attitudes and practices of value chain actors on IBD in indigenous village chicken, ducks and turkeys and status of the disease in these birds in Embu County.

Structured questionnaires, direct observation, sero-survey and reactive Bursa of *Fabricius* homogenates were used to collect the respective data. The questionnaire study involved a total of 93 poultry value chain actors including: 47 farmers, 39 traders and slaughter personnel and 7 animal health providers. As the disease status sera were collected from 97 indigenous village chickens, 32 ducks and 13 turkeys; Indirect enzyme linked immunosorbent assay (ELISA; IDEXX IBD-XR from IDEXX laboratories USA) was used to screen for IBD virus (IBDV) antibodies in the birds' sera; Agar gel precipitation test (AGPT) was used to detect presence of IBDV in reactive homogenised bursae, collected from chicken during post-mortem. Fisher's and Chi-square tested for association between the risk factors and transmission of IBD. Odds ratio greater than one ($OR > 1$) and p-value less than 0.05 ($p < 0.05$) were taken to be significant.

The risk factors for IBD endemicity identified among the poultry value chain actors were: sourcing birds from other farmers and traders with no known disease or vaccination history (68.1% farmers and 100% traders); moving with gathered chickens from one farm to another during procurement (46.15% traders); lack of disinfection between farms (89.74% traders;

57.1% animal health providers); low use of vaccines against diseases (8.5% farmers); feeding dogs on sick and dead birds (farmer 25.3%; traders 35.89%; slaughter personnel 12.82%); disposal of manure (without making compost) in the farm (95.7%); direct mixing of new and unsold birds from the market with home flocks (55.3% farmers; 21.51% traders) and mixed rearing of different species of birds by farmers (38.3%). There was a significant association ($p<0.05$) between traders sourcing of chickens from local farmers ($p= 0.0107$), feeding sick and dead birds to dogs (O.R=2.75), low IBD vaccine use by farmers (O.R=3.07) and maintenance and spread of IBD in indigenous chickens. The seroprevalence rates were 65% in chicken, 6% in ducks and 92% in turkeys and there was significant difference ($P>0.05$) between age groups of individual species of bird (indigenous village chickens, ducks and turkeys). Agar gel precipitation test results were all negative for IBDV antigen.

In conclusion: presence of IBDV antibodies in the named birds suggested an on-going viral circulation in chickens with ducks and turkeys possibly acting as virus reservoirs for the primary source of new IBD outbreaks in chickens. Some practices undertaken by poultry value chain actors because of their attitudes and due to lack of knowledge have promoted maintenance and spread of IBD in indigenous village poultry. There is therefore a need for awareness-creating campaigns and training on biosecurity to build prevention capacity at community level and routine surveillance coupled with vaccination against IBD in indigenous village chickens, ducks and turkeys in order to control the disease.

CHAPTER ONE: INTRODUCTION

In developing countries nearly all families at the village level own free range indigenous poultry since they are easy to manage, require little space and relatively low initial capital (Nduthu, 2015). In Kenya, there are about 32 million poultry, of which 70% are of indigenous type; comprising chickens (which are the majority), ducks, turkeys and geese (Nyaga, 2007; Kenya census report, 2010). Indigenous poultry adapt well to different environments and can survive on limited feed resources obtainable through their daily free ranging habits (Kingori *et al.*, 2010). This free range system exposes them to precarious conditions such as diseases, inadequate feeding, poor housing, predation and extreme weather changes (Kingori *et al.*, 2010). These conditions lower the birds' production; subsequently lowering economy of the needy mostly women and youth, who mainly own these birds (Guèye, 2005).

Diseases such as IBD and Newcastle have been identified as the main stumbling block for indigenous village poultry production (Olwande, 2014). Viral diseases (including infectious bursal disease and Newcastle disease) causing huge economic losses to the poultry industry in Kenya (Nyaga, 2007; Njagi *et al.*, 2010). Olwande (2014) reported IBD as the second most important disease of indigenous village chickens responsible for marked economic losses in Nyanza, Kenya, after Newcastle disease. Infectious bursal disease outbreaks have been linked to relatively higher mortalities in indigenous chickens compared to layers and broiler chickens by Mutinda *et al*. (2013) and Mutinda (2016) in Kenya.

Infectious bursal disease is a highly contagious, acute viral disease of young chickens that can result to 100% morbidity and mortality reaching up to 100% in susceptible flock (Mutinda, 2016). Chickens are the only birds known to develop clinical disease and distinct lesions when exposed to IBD virus (IBDV) (AU-IBAR, 2013); even in cases of natural and experimental infections, clinical disease is usually not observed in ducks and turkeys (Oladele *et al.*, 2008).

However, presence of antibodies to IBDV in ducks and turkeys has been reported by Oluwayelu *et al*. (2007) in Nigeria and Barnes *et al.* (1982) in Iowa respectively. This indicates that these birds may be involved in the maintenance and spread of the virus to chickens in the mixed flock production systems in rural settings. Therefore screening for anti-IBDV antibodies and presence of IBDV in indigenous ducks and turkeys, in addition to chickens, can give an insight of their role on chicken morbidity and mortality due to IBD. This screening has not been undertaken in Embu County or elsewhere in Kenya in indigenous chickens, ducks and turkeys.

Indigenous poultry diseases are spread by movement of birds and their products, input equipments and fomites. These movements are driven and controlled by people involved in the poultry value chain (Murekefu, 2013). Understanding people's behaviour to IBD in this chain, with emphasis on people's knowledge, attitude and practices can facilitate in identifying the risks factors involved in disease maintenance and spread and help in formulating control measures for IBD. As there are normally limited successful and sustainable vaccination programmes for these indigenous birds (Nyaga, 2007). This information has not been collected and documented for Embu poultry value chain actors.

This study, therefore, was conducted in order to investigate status of IBD in indigenous village chickens, ducks and turkeys in Embu County, Kenya. It also documents the knowledge, attitudes and practices of value chain actors in regard to IBD spread in indigenous village poultry in Embu County. This information will contribute in designing and implementation of a suitable control IBD program.

1.1 HYPOTHESIS

Indigenous village chickens, ducks and turkeys have no antibodies against IBD and the value chain actors have no knowledge on IBD issues in Embu County, Kenya.

1.2 OBJECTIVES

1.2.1 General objective

To determine status; knowledge, attitudes and practices of value chain actors, in Embu County on IBD in indigenous village chicken, ducks and turkeys

1.2.2 Specific objectives are to:

1. Determine knowledge, attitudes and practices of value chain actors on IBD in indigenous village chickens, ducks and turkeys in Embu County
2. Determine IBD status in unvaccinated indigenous village chicken, ducks and turkeys in Embu County

1.3 JUSTIFICATION

Indigenous village chickens, comprise the highest number of poultry in Kenya due to their low capital investment and space requirement. Some farmers also keep indigenous ducks and turkeys. These poultry impact significantly on the small holder farmer's welfare, rural trade, and food security. Infectious bursal disease is the second most important viral disease of indigenous village chickens associated with marked economic losses. It is not clear what indigenous poultry farmers and other value chain actors know about it. Further, the status of the disease among indigenous village chickens, ducks and turkeys in Embu County has not been determined. This study, therefore, conducted to establish the disease status and knowledge, attitudes and practices of value chain actors in Embu County, Kenya. This will facilitate identification of critical control points which are essential in designing a policy document on how the disease should be managed and controlled both at local and national level. Any efforts towards increasing productivity of these birds will help in poverty alleviation and ensure food and nutrition security for the majority of the people living in rural areas especially women and the young who rear indigenous poultry as integral part of family livelihood.

CHAPTER 2: LITERATURE REVIEW

2.1 BACKGROUND INFORMATION

Sonaiya, (2003) described indigenous poultry as any genetic stock improved or unimproved that are raised in relatively small numbers with least investment on inputs, most of which are generated in the homestead and reproduction is fully based on uncontrolled natural mating and hatching of eggs using broody hens. Indigenous chickens adapt in diverse environments and can survive on inadequate feed resources that vary in quality according to season (Kingori *et al.*, 2010). They play a major part in the economy of developing countries including poverty alleviation as alternate source of income and household food security, especially for women and the youth (FAO, 1997; Bebora *et al.*, 2002; Guèye, 2005).

The free range systems of production and mixed rearing of chickens with other different species of birds like ducks and turkeys exposes them to microorganisms which may end-up being resident in these birds without producing disease; some, such as infectious bursal disease viruses (IBDV), are highly pathogenic to chickens (Olwande, 2014). Sule *et al.* (2013) reported IBDV antibodies prevalence of 63% in non-vaccinated indigenous chickens in Yobe state, Nigeria, and recommended routine surveillance for IBDV antibodies and investigations of risk factors involved in the maintenance of the IBDV in indigenous poultry to aid in the development of acceptable control program. A serological survey carried out by Swai *et al.* (2011) in indigenous village chickens in Northern Tanzania indicated endemicity of the disease in the country and the authors recommended investigation of IBDV antibodies in other domestic birds like as ducks and turkeys to establish carrier status of these birds. This study was conducted to investigate IBDV sero-prevalence in chickens, ducks and turkeys in Kenya.

According to Swai *et al*. (2011) and Kebede *et al.* (2017), the relative increase in the overall sero-prevalence rate of IBDV antibodies in indigenous village chickens is due to many factors.

These are: inappropriate sanitary conditions, nutritional deficiencies, continuous exposure of the indigenous village poultry to wild birds, rearing of different species of bird together, absence of routine vaccination, ease of contact at local open-air markets among chickens from different areas which are taken back to various localities, and the free range conditions that favour contact of chickens from different villages. These involve various poultry value chain actors. Therefore, for the IBD control measures to be effective, this group of actors needs to be included in the control program; the starting point being establishment of their knowledge, attitudes and practices with regard to the disease maintenance and spread. This study was, therefore, carried out in Embu County, Kenya, to address this gap.

Infectious bursal disease was first identified and reported by Cosgrove in 1962, in the area of Gumboro, Delaware in the United States of America, hence the name Gumboro (AU-IBAR, 2013). It was later named "Infectious bursal disease" (IBD) by Edgar (Lukert and Saif, 2003) because of its effect on the bursa of Fabricius. Infectious bursal disease has since spread throughout the world as an economically important disease causing 100% morbidity and up to 100% mortality in susceptible flock (Kibenge, 1988; Mutinda *et al.,* 2013; Mutinda, 2016). In Africa, IBD appears to be endemic in some countries (Hailu *et al*., 2010; Swai *et al.,* 2011; Mutinda *et al.,* 2013; Sule *et al.,* 2013; Tsegaye and Mersha, 2014; Kebede *et al.*, 2017). In Kenya, the disease was first documented in the coast region by Mbuthia and Karaba (2000) and has since been diagnosed in other parts of the country. Ndanyi (2005) reported IBD as most prevalent (49.3%) cause of mortality in Newcastle disease vaccinated chicken flocks in Taita Taveta County, Kenya. Infectious bursal disease outbreaks, causing high mortality rates in indigenous village chickens, were reported by Mutinda *et al*. (2013) and Mutinda (2016). They also established the risk factors involved in IBD vaccination failures in broiler farms and cases of low immune response to IBD vaccine in Kwale, Kenya (Mutinda *et al*., 2014; Mutinda, 2016). Olwande (2014), in a study in Nyanza, Kenya, reported IBD as the second most

important disease of indigenous chickens and recommended further studies to generate data on the disease prevention and control measures. However, studies to establish the serological status of unvaccinated indigenous chickens, ducks and turkeys for IBDV have not been done in Kenya, neither is there any documented evidence on knowledge, attitudes and practises by farmers and other value chain actors on IBD in these birds in Embu County.

2.2 INFECTIOUS BURSAL DISEASE

2.2.1 Aetiology

Infectious bursal disease virus causes IBD. The virus has double stranded RNA with bi-segmented genome enclosed in a non-enveloped icosahedral capsid with a diameter ranging from 55 to 65 nm (Ashraf, 2005; Teshager, 2015). It belongs to the *Avibirnavius* genus and *Birnaviridae* family (Singh *et al*., 2010; AU-IBAR, 2013). It has two serotypes; serotype 1 strains that are pathogenic to chicken (Van Den Berg *et al*., 2004; Muller *et al.,* 2003; Eterradossi and Saif, 2008) and serotype 2 strains that are non-pathogenic to chicken (Eterradossi and Saif, 2008; Teshager, 2015). Both serotypes 1 and 2 infect ducks and turkeys but cause no disease (Eterradossi and Saif, 2008, Jackwood *et al.,* 2011; AU-IBAR, 2013). Serotype 1 has three strains: variant, classical (also referred as standard or virulent), and very virulent IBDV; their pathogenicity levels vary significantly in chicken (Jackwood *et al.*, 2011). Variant strains have antigenic differences from the classical strain; cause immunodepression but not clinical disease (Eterradossi and Saif, 2008; Teshager, 2015). Classical strains leads to bursal inflammation, lymphoid necrosis, immunodeficiency and fair mortality of 20-30% in specific pathogen free (SPF) chickens (Teshager, 2015). Very virulent IBDV (vvIBDV) strains cause major clinical signs and high mortality rates ranging from 60 - 100% in susceptible näive chicken flock (Muller *et al.,* 2003). The vvIBDV strains can break through the immunity from maternal antibodies and produce similar signs as standard strains (Van den Berg, 2000).

Infectious bursal disease virus is stable, survives for long periods (122 days in a chicken house and for 52 days in feed and water) outside the host (Benton *et al.,* 1967; Lukert and Saif, 1997) and can be difficult to eradicate from premises housing infected chickens (Eterradossi and Saif, 2008). The virus is inactivated by a pH of 12 and above (Eterradossi and Saif, 2008). It is sensitive to sodium hydroxide (Eterradossi and Saif, 2008), halogens derivatives and aldehydes (formaldehyde, glutaraldehyde) (Shirai *et al*., 1994).

2.2.2 Epidemiology

Chickens are the only birds known to form clinical disease and discrete lesions when exposed to IBDV (Lukert and Saif, 2003; AU-IBAR, 2013). However, IBDV antibodies have been observed in eider ducks, herring gulls (Hollomen *et al.,* 2000) and indigenous ducks in Nigeria (Oluwayelu *et al.,* 2007) in field surveys. Antibodies to IBDV have been reported in turkeys by Barnes *et al.* (1982) in Lowa. Infectious bursal disease virus has been isolated from faeces of healthy turkeys in India by McFerran *et al.* (1980) and in farmed commercial turkeys in Canada by Reddy and Silim (1991). Wild birds including ostriches, Antarctic penguins, crows and falcons have been shown to be carriers of IBDV (Eterradossi and Saif, 2008; OIE, 2016). Presence of IBDV antibodies in these bird types indicates that they may be involved in maintenance of IBDV and its resultant transmission to chickens. This study, therefore, included a sero survey in ducks and turkeys to determine their exposure to the virus; indicating their possible role in the epidemiology of IBD.

The optimum age of susceptibility to IBDV in chicken is 3- 6 weeks, which is the period of maximal bursal development; it is usually characterized by acute clinical signs (Lukert and Saif, 2004). Infections occurring before the age of 3 weeks are usually subclinical and immunosuppressive (Ashraf, 2005; Eterradossi and Saif, 2008; AU-IBAR, 2013). Clinical cases can be observed up to the age of 15 to 20 weeks (AU-IBAR, 2013). Light strains of laying

stock are more vulnerable to disease as compared to the heavy broiler strains (Van den Berg 2000; AU-IBAR, 2013).

Infectious bursal disease virus is directly transmitted when diseased chickens shed it in faeces; this contaminates water, feed and poultry house litter from which susceptible chickens are infected (Eterradossi and Saif, 2008). It can also be indirectly transmitted through contact with contaminated vehicles such as equipment, clothing, shoes and cars (Murphy, 1999). The lesser mealworm (*Alphitobus diaperinus*) and litter mites have been shown to carry the virus for up to 8 weeks (Eterradossi and Saif, 2008). Dogs have also been demonstrated by Albert *et al.* (2004) to excrete IBD virus in their faeces after being fed chicken tissues infected with IBDV.

2.2.3 Clinical signs

Severity of disease can be due to virulence of the strain, age, breed of the birds, and level of the bird's specific antibodies to IBDV (Van den Berg, 2000; Teshager, 2015; Mutinda, 2016). Incubation period ranges from 2 to 3 days after contact of susceptible bird to IBDV (Eterradossi and Saif, 2008). In general infected birds are exhausted, anorexic, prostrated, depressed, dehydrated and manifest white watery diarrhoea, soiled vents, closed eyes, ruffled feathers and sudden death (Teshager, 2015). High morbidity rate up to 100% is experienced in infected flock (Eterradossi and Saif, 2008; Mutinda *et al.*, 2013; Mutinda, 2016). Mortality rate varies, depending on: strain: vvIBD mortality ranges from 40-100% in susceptible naive chickens, 30% and 60% in vaccinated broilers and layers respectively (Jackwood *et al.*, 2009; Mutinda *et al.*, 2013; Mutinda, 2016); classical strains mortality rarely exceeds 20-30% and variant strains do not kill (AU-IBAR, 2013). Mutinda *et al.* (2013) and Mutinda (2016) found mortality rate to occur highest in indigenous chickens followed by layers and less in broilers. Mortality rate is usually highest in 3-6 weeks of age as compared to birds less than 3 weeks and more than 6 weeks of age (Eterradossi and Saif, 2008; AU-IBAR, 2013). By day four of infection

the virus tends to reduce drastically and after 5-7 days the chicken normally recovers (Van den Berg, 2000).

2.2.4 Pathology

2.2.4.1 Gross lesions

Degree of lesions depends on strain and pathogenicity of the virus (Eterradossi and Saif, 2008). Major pathological changes are observed in the bursa of *Fabricius* (AU-IBAR, 2013; Teshager, 2015; Mutinda *et al.*, 2015). Initially bursa of *Fabricius* enlarges and increases in weight due to oedema and hyperaemia. By day two to three post exposure, it contains yellowish gelatinous fluid, haemorrhagic longitudinal striations on outer and inner surface and necrotic lesions on surface of bursal folds (Eterradossi and Saif, 2008; Teshager, 2015; Mutinda, 2016). On fifth day, bursa returns to its normal size as fluid disappears, and on eighth day it atrophies and turns gray (Eterradossi and Saif, 2008; Teshager, 2015). Carcasses are generally dehydrated and as a result renal tubules and ureters appear white, distended and crammed with urates; and there is also increased mucus in intestines (Ashraf, 2005; Eterradossi and Saif, 2008; AU-IBAR, 2013). Haemorrhages are seen on thigh, pectoral and breast muscles, at the proventriculus-ventriculus junction mucosa as well as the serosa and plicae of the bursa (Teshager, 2015; Mutinda, 2016). Haemorrhages and enlargement of other lymphoid organs (caecal tonsils, thymus, Harderian glands, spleen, Peyer's patches and bone marrow) occur in cases of hyper virulent IBDV (Van den Berg, 2000).

2.2.4.2 Microscopic lesions

Microscopic lesions of IBD occur mainly in the lymphoid tissues (bursa, spleen, thymus, Harderian gland, and cecal tonsils (Ashraf, 2005; Eterradossi and Saif, 2008; Mutinda, 2011;

Teshager, 2015; Mutinda, 2016); lesions being mostly severe in the bursa. From day one of infection, individual lymphocyte degeneration and necrosis start occurring in medullary region of the bursa follicles. On day 3 to 4 post infection, lymphocytes are replaced by heterophils, hyperplastic reticulo-endothelial cells and pyknotic debris; bursa enlarges due to marked accumulation of heterophils, oedema and hyperaemia (Van den Berg, 2000; Teshager, 2015). In day five, inflammatory reaction reduces and cystic cavities form in medullary areas of follicles. Necrosis and phagocytosis of heterophils; plasma cells occur later leading to fibroplasia in inter-follicular connective tissue (Eterradossi and Saif, 2008). Later, lymphocytes start regenerating and fill the follicles (AU-IBAR, 2013). Other lymphoid organ involvement results in necrosis, hyperplasia of the epithelial components and the reticular cells in the medullary region of the follicles (Ashraf, 2005; Eterradossi and Saif, 2008). In spleen, hyperplasia of reticulo-endothelial cells around the adenoid arteries is seen. In caecal tonsils and thymus, damage of the lymphoid tissue can be present but is less severe. Kidney lesions are non -specific, and are a result of severe dehydration (Eterradossi and Saif, 2008; AU-IBAR, 2013; Teshager, 2015). For very virulent IBDV strains, apart from the atrophy and inflammation (after 7 to 10 days) of the bursa of Fabricius, the kidney can be swollen and ecchymotic change in the muscle and the mucosa of proventriculus mighty be observed in the many of affected birds (Van den Berg, 2000; Teshager, 2015). Haemorrhages are there in the bursa of Fabricius and muscle tissue (AU-IBAR, 2013; Mutinda, 2011; Teshager, 201

2.3.5 Diagnosis

Infectious bursal disease is diagnosed by: consideration of the flock history, clinical signs and post-mortem lesions, viral isolation, serology and detection of the viral RNA genome (Ashraf, 2005; OIE, 2016; Mutinda 2011; Mutinda, 2016). For chicken less than 3 weeks of age where,

due to passive antibodies, infection is usually subclinical, diagnosis usually relies on histopathology of bursa of Fabricius, serology or viral isolation (Rajaonarison, 2006; OIE, 2016). Confirmation of clinical IBD is made by necropsy examination; mainly the haemorrhagic streaks on muscle and hypertrophy of the bursa of Fabricius at acute stages (Ashraf, 2005; Eterradossi and Saif, 2008) .

2.3.5.1 Virus isolation

Virus is normally isolated from bursa of Fabricius and spleen of infected bird (Lukert and Sarif, 2003; OIE, 2016; Mutinda, 2016). The respective tissues are ground to make suspension in peptone broth treated with penicillin and streptomycin (OIE, 2016).The suspension is then inoculated into embryonated eggs or tissue culture (Lukert and Sarif, 2003; Mutinda *et al.*, 2015). In embryonated eggs, virus is inoculated in 9-11 day old embryos via chorio-allantoic route. Most embryos die from day three to five depending on the virulence of IBDV strain inoculated; embryos which do not die are normally stunted (Lasher and Shane, 1994; Mutinda *et al.*, 2015). Lesions which are normally observed include: subcutaneous haemorrhages, vascular congestion and oedema, liver, kidneys and spleen which are enlarged and mottled, chorio-allantoic membrane which is edematous, with or without congestion or haemorrhages (Lasher and Shane, 1994; Mutinda *et al.*, 2015). Serotype 2 strains do not cause oedema in subcutaneous or haemorrhages in infected embryos, but embryos are dwarfed with a pale yellowish discolouration (OIE, 2016). Primary cell cultures used to propagate the virus include: chicken embryo fibroblasts (CEF), chicken embryo bursa (CEB) and chicken embryo kidney (CEK; Ashraf, 2005; OIE, 2016). Cytopathic effect (characterised by small round refractive cells) is seen within six days post inoculation (OIE, 2016).

2.3.5.2 Serology

Enzyme-linked immunosorbent assay (ELISA) is the most frequently used laboratory method to confirm IBD (OIE, 2016). An ELISA test is highly sensitive, quantitative and it enables handling of large amounts of samples; it can also be automated (Marquardt *et al*., 1980; OIE, 2016). These characteristics made it the best choice for this study to detect IBDV antibodies in the chicken, duck and turkey sera. Agar gel precipitation test (AGPT) is on the other hand, a semi-quantitative test mostly adapted to quantify antibodies against IBDV (OIE, 2016). The AGPT test has been used for screening of bursa of Fabricius samples prior to attempting isolation and more characterization of IBDV (Lukert and Saif, 2003; Mutinda, 2011; Mutinda, 2016). This was adapted in this study to screen bursae of Fabricius that showed signs of IBD obtained during postmortem of purchased Embu County chickens. Virus neutralization procedure is a sensitive test mostly used to differentiate IBDV serotypes and serotype 1 sub types (Ashraf 2005; OIE, 2016). Other serological tests that have been used to detect IBDV antibodies include; indirect haemagglutination test (IHA) which is considered to be less costly, easy and quick to perform (Aliev *et al.*, 1990; Rahman *et al*., 1994; Hussain *et al*., 2003) and counter immune-electrophoresis (Hussain *et al*., 2002) tests.

2.3.5.3 Molecular techniques

The most common molecular method used to detect IBDV genome is reverse-transcription polymerase chain reaction (RT-PCR) (Wu, 1992). This is the best to detect DNA of viruses that can't replicate in cell culture, since it is not crucial to grow the virus prior to amplification (OIE, 2016). Differentiation of the strains is possible if the RT-PCR products are further analyzed using restriction enzymes (Jackwood and Sommer, 1998; Jackwood and Jackwood 1997). Other molecular techniques include the use of DNA probes (Jackwood *et al.*, 1992).

2.3.6 Treatment, prevention and control

Currently, IBD has no specific treatment (Eterradossi and Saif, 2008; AU-IBAR, 2013). The most critical measures for optimal prevention and control are: biosecurity, appropriate vaccination program and monitoring chickens for signs of IBD (Block *et al*, 2007, Yannick *et al*, 2015; Mutinda *et al.,* 2014).

2.3.6.1 Vaccination

The high resistance of IBDV to disinfectants makes hygienic measures alone unproductive; vaccination, therefore, remains the main method to defend chicken against infection during the 1st weeks of age (Yannick *et al.,* 2015). Passive immunity protects the chicks (1 to 3 weeks of age) from earlier infections (Fahey *et al*., 1991). This is achieved when the breeder stock is vaccinated against IBD preceding the onset of lay (20 to 21 weeks) (Lasher and Shane, 1994). The main problem with active immunization of passive immunity to chicken is determination of suitable time of vaccination which will allow adequate replication of the vaccine virus to protect the young chicken from disease (Ashraf, 2005). The time of vaccination varies with the virulence of the vaccine virus, level of maternal antibodies and route of vaccination (Tizard, 1996). For a successful vaccination program, factors like management, environmental stresses, flock profiling for the presence of maternal antibodies, proper vaccine handling, good timing of vaccination and matching of disease agent and vaccine strains are to be considered (Mutinda *et al.,* 2014).

CHAPTER THREE: KNOWLEDGE, ATTITUDES AND PRACTICES OF VALUE CHAIN ACTORS ON INFECTIOUS BURSAL DISEASE IN INDIGENOUS VILLAGE CHICKEN, DUCKS AND TURKEYS IN EMBU COUNTY

3.1 INTRODUCTION

Majority of rural farmers in Kenya raise indigenous poultry; they are reared for meat and eggs thus contribute to the protein nutrition of different households in the country. Sale of poultry and products increases and diversifies revenue in the poultry sector (Nyaga, 2007; Kingori *et al.,* 2010). Poultry sub-sector provides employment and promotes overall economic development and involves various chain actors (Reddy *et al.,* 2016). The indigenous poultry chain is simple and involves few actors that include: producers (farmers), traders, consumers and sometimes slaughter personnel and animal health providers (Okello and Gitonga, 2010; Murekefu, 2013). Farmers are involved in all activities of raising the indigenous poultry and may pose risk of disease maintenance and spread during their day to day activities while attending to these birds (Kebede *et al*., 2017). Traders play a very modest role in the procuring and selling of poultry; they may pose a risk for the spread of disease as they normally collect poultry and products from a number of sources, bulking them with limited attention paid to bio-security measures (Reddy *et al.,* 2016). The interaction of farmers, traders, slaughter personnel and consumers for live bird markets where there are no bio-security measures in place increases risks of disease spread that can even lead to outbreaks (Reddy *et al.,* 2016). This study investigated poultry value chain actors (farmers, traders, slaughter personnel and animal health providers) current knowledge, altitude and practises, in respect to IBD so that proper intervention measures can be structured.

3.2 MATERIALS AND METHODS

3.2.1 Study area

The study was conducted in Embu County, Kenya. Embu County is located in the Eastern region of Mount Kenya and borders Kitui County to the East, Kirinyaga County to the West, Tharaka Nithi County to the North and Machakos County to the South (Ecob, 2015; Figure 3.1). Embu County has five sub counties namely: Embu West, Embu East, Embu North, Mbeere South, Mbeere North. The County occupies an area of 2,818 km^2 and human population estimated to be 543,221 by Kenya Population and Housing Census (KNBS, 2009). The County has different agro-ecological zones and rainfall varies, with respect to altitude, averaging about 1,067.5 mm annually. Temperature ranges from 20^oC to 30^oC (Ecob, 2015). Agriculture accounts for 70% of the total County incomes and is in form of livestock keeping (poultry, cattle, sheep and goats), small-scale food (maize, millet, green gram, sorghum and cotton) and cash crop production (coffee, tea) (Ecob, 2015). The area has a high population of indigenous village poultry, approximately 202,410 (KNBS, 2009), and rearing chickens is a major source of living in the County. The site was purposively selected based on the large population of free range poultry and previous studies which have unravelled several challenges in poultry production in the area (Sabuni, 2009; Njagi *et al.*, 2010; Kemboi *et al.*, 2013)

Figure 3.1 Map showing Embu County in Kenya and its detailed geography; showing the location and distribution of poultry farmers, traders and slaughter house personnel who participated in this study.

3.2.2 Study design

This was a cross-sectional study involving the use of semi-structured questionnaires and direct observations to collect data which was used to assess knowledge, attitudes and practices of selected poultry value chain actors, with respect to IBD in indigenous village chickens, ducks and turkeys. This design gave self-reported facts about respondents, their opinions, inner feelings, attitudes, and habits (Kombo and Tromp, 2006). Three different types of questionnaires were prepared for the selected three different groups of participants interviewed, namely: poultry farmers, traders and slaughter house personnel and animal health providers (**Appendices 1, 2 and 3).** The questionnaires were administered by face to face interviews by the researcher and enumerators. The answers were carefully recorded in the questionnaire as the interview proceeded and confirmed well filled before proceeding to the next person. Important household and flock-level data collected included: respondent's bio data, awareness of clinical presentation of IBD, knowledge, attitude and practices promoting maintenance and spread of IBD, methods they used to prevent and control the disease and knowledge on existence of IBD vaccine. Global position system (GPS) was used to locate the farms, traders and slaughter house personnel thus mapping the movement of the birds.

3.2.3 Sample size

This was done according to Martin *et al.* (1987)

$n = \hat{pq}\ [z\ /\mathrm{L}]^2$

Where:

n= required sample size for an infinite participants population

p= Anticipated prevalence

q= 1-p

Z= α at 95% confidence level

L=.required degree of precision or acceptable margin of error set at ± 10%

Substituting figures in the equation, this becomes: P=0.5, q=0.5, z =1.96, and L=0.1 i.e. 10% is the estimate of the required precision

n= [0.5 x 0.5] x $(1.96)^2/0.1^2$; where $(1.96)^2$ is replaced by 4,

n=100

This was adjusted for a finite participant's population using the following formula:

n'= 1/ [1/n +1/N], where: **n'**= Adjusted sample size, n=Original required sample size i.e. 100 and N=participants population size in study area taken to be 1400.

n'= 1/ [1/100 + 1/1400] **n'**=93

A total of 93 participants were recruited and interviewed at their specific operation. These included: poultry farmers (47), traders and slaughter house personnel (39; whom the farmers sell to) and animal health providers (7) (veterinarians, animal health assistants). Sampling was done conveniently based on farmers, traders, slaughter house personnel and animal health providers who were reachable and willing to be interviewed.

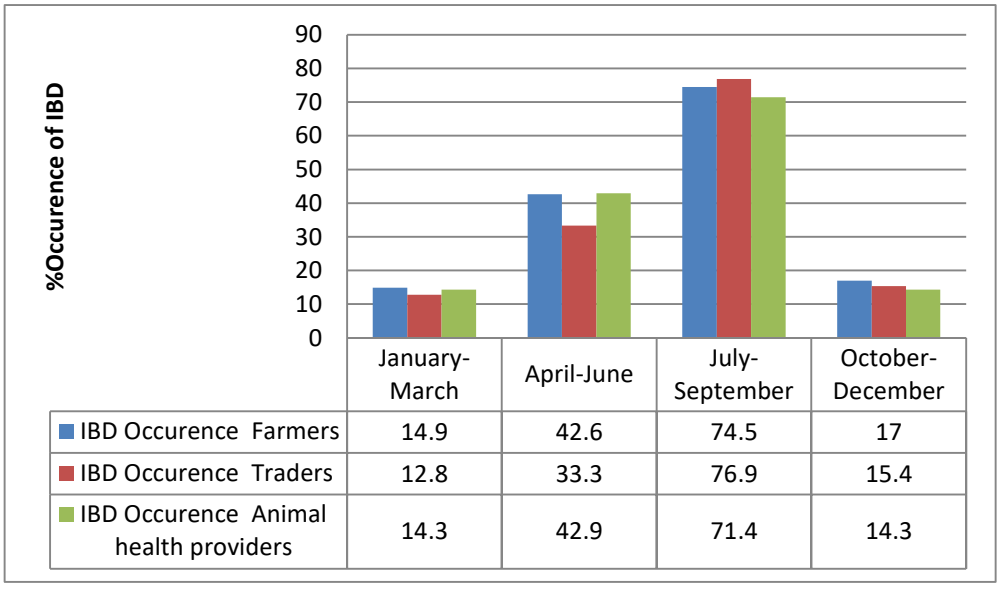
3.2.4 Data analysis

Data were entered in Microsoft Excel and analysed in R version 3.3.1. The proportions were used to calculate the percentages of responses per question per unit sampling group. Fisher's and Chi-square tests were used to test the association between risk factors and IBD transmissions. Odds ratio of greater than one (OR >1) and P-values of less than 0.05 ($p < 0.05$) were taken to be statistically significant.

3.3 RESULTS

3.3.1 Poultry value chain actors' knowledge and awareness of infectious bursal disease

Thirty nine (83%) indigenous village chicken farmers and thirty five (89.74%) traders reported to be aware of the clinical presentation of IBD. The signs they associated with IBD included: reduced feed and water consumption, whitish diarrhoea, matted feathers at the vent, ruffled feathers, closed eyes and tucked neck to shoulders. However the disease lacked a local name and majority of the farmers and traders associated it with "Kivuruto", the local name for Newcastle disease. Infectious bursal disease outbreaks occurred mostly in the months of July to September (farmers 75.5%; traders 76.9 %) as shown in **Figure 3.2.** Recent outbreaks were reported (51.1% farmers; 74.36% traders), that affected mostly chickens 2-3 months old (76.6% farmers; 64.1% traders). Most slaughter personnel didn't recognize both the ante-mortem and post-mortem signs and lesions of IBD but 38.46% reported having observed whitish diarrhoea.



	January-March	April-June	July-September	October-December
IBD Occurence Farmers	14.9	42.6	74.5	17
IBD Occurence Traders	12.8	33.3	76.9	15.4
IBD Occurence Animal health providers	14.3	42.9	71.4	14.3

Figure 3.2: Occurrence of infectious bursal disease in year 2016 as reported by farmers and traders in Embu County

3.3.2 Poultry value chain actors' attitudes that promoted maintenance and spread of infectious bursal disease in indigenous poultry in Embu County

Fifteen (38.5%) traders interviewed inquired about the vaccination and disease history of the farms from which they bought poultry, while twenty four (61.5%) did not bother to make this kind of inquiry. Almost half of slaughter house personnel (49%) had no idea about the frequency with which they encountered birds that suffered from IBD, while others reported low (28.2%), fair (20.5 1%) and high (2.1%) frequencies of occurrence. Nearly all slaughter house personnel had a negative attitude towards ante-mortem inspection with 67% having not carried out the inspection. **Figure 3.3** shows the author interviewing some of the personnel at a slaughter house in Embu town.

Figure 3.3: Author interviewing slaughter personnel in a slaughter house in Embu town

3.3.3 Poultry value chain actors' practices that promoted maintenance and spread of infectious bursal disease in indigenous poultry in Embu County

3.3.3.1 Farmer practises

All the forty seven (100%) indigenous village poultry farmers interviewed kept indigenous chickens and, in addition among them twelve (25.5 %) kept ducks, four (8.5 %) kept turkeys; only two (4.3 %) kept a mixture of chickens, ducks and turkeys. Thirty two (68.1%) sourced birds for restocking from other farmers, nine (19.1%) from markets, two (4.3%) inherited, one (2.1%) from government institution, one (2.1%) was given as a gift and two (4.3%) did not source rather they maintained their stock by breeding; none of them inquired about the disease or vaccination history from the source (**Figure 3.4)**.

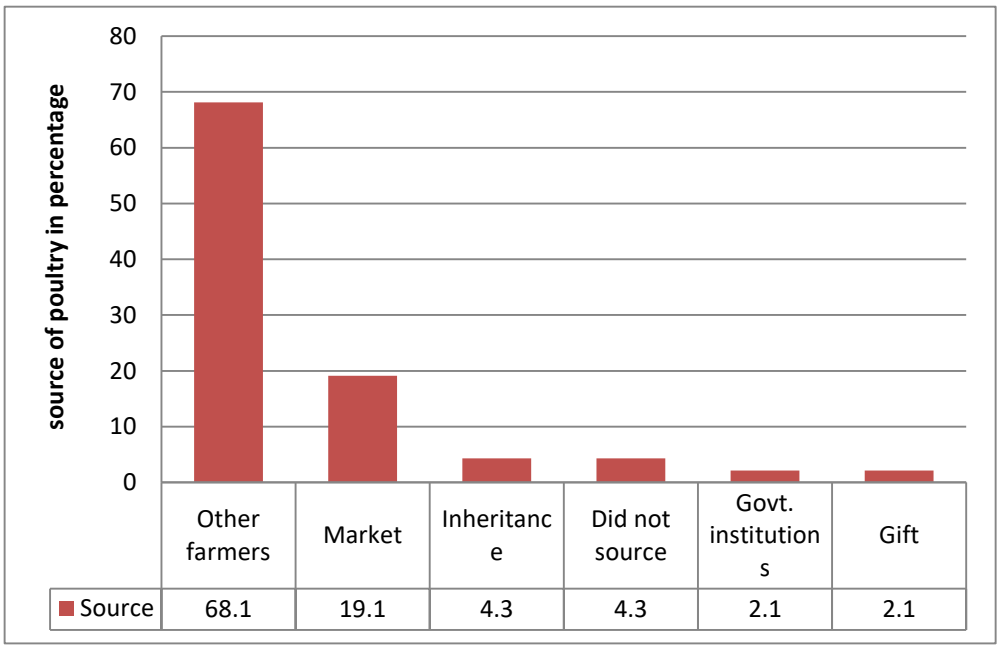


Figure 3.4: Sources of indigenous breeding stock for indigenous poultry farmers in Embu County, Kenya

Nineteen farmers (40.4%) did nothing to prevent their birds against IBD. Four (8.5%) vaccinated their indigenous village chickens against the disease while fifteen (31.9%) relied on

herbal plants consisting mainly of Aloe Vera and Neem. Two (4.3%) used disinfectants and seven (14.9 %) used other methods including; use of antibiotics (2/47; 4.3%), milk (1/47; 2.1%), confinement of birds (3/47; 6.4%); one (2.1%) sprayed poultry houses with pesticides **(Figure 3.5)**.

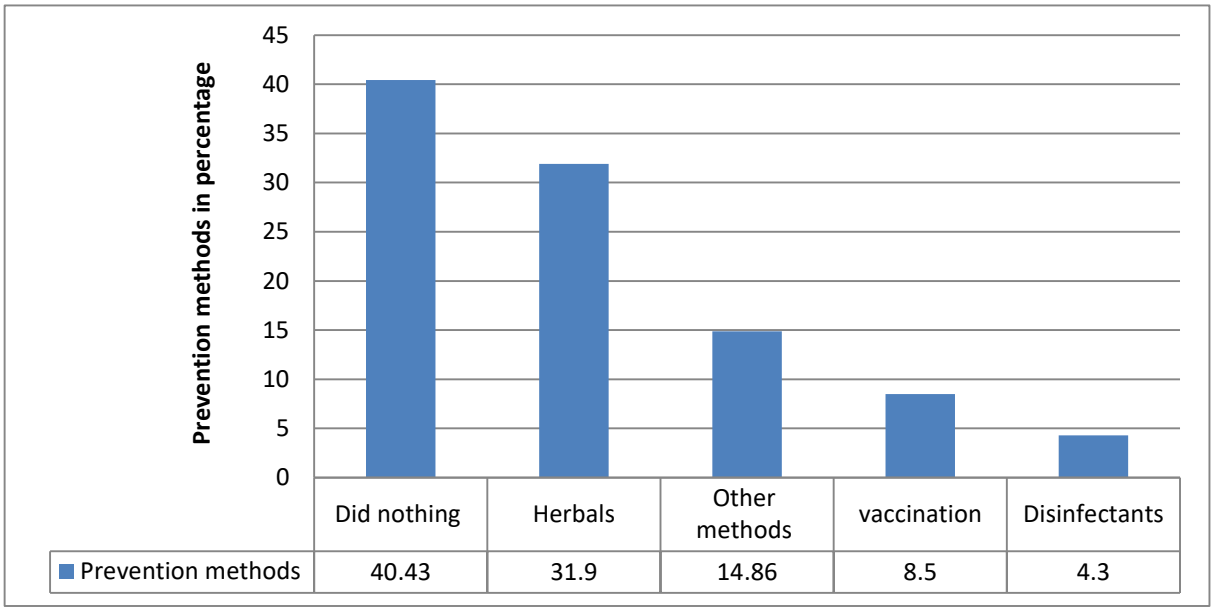


Figure 3.5: Control and prevention measures undertaken by indigenous poultry farmers in Embu County, Kenya

Five (10.6%) farmers sold their birds at the market when they suspected IBD outbreak in their farm, thirty two (68.09%) treated their birds, two (4.3%) vaccinated them, one (2.1%) sold them to neighbours, one (2.1%) gave them away and six (12.8%) did nothing (maintained them in their farms) as shown in **Figure 3.6**.

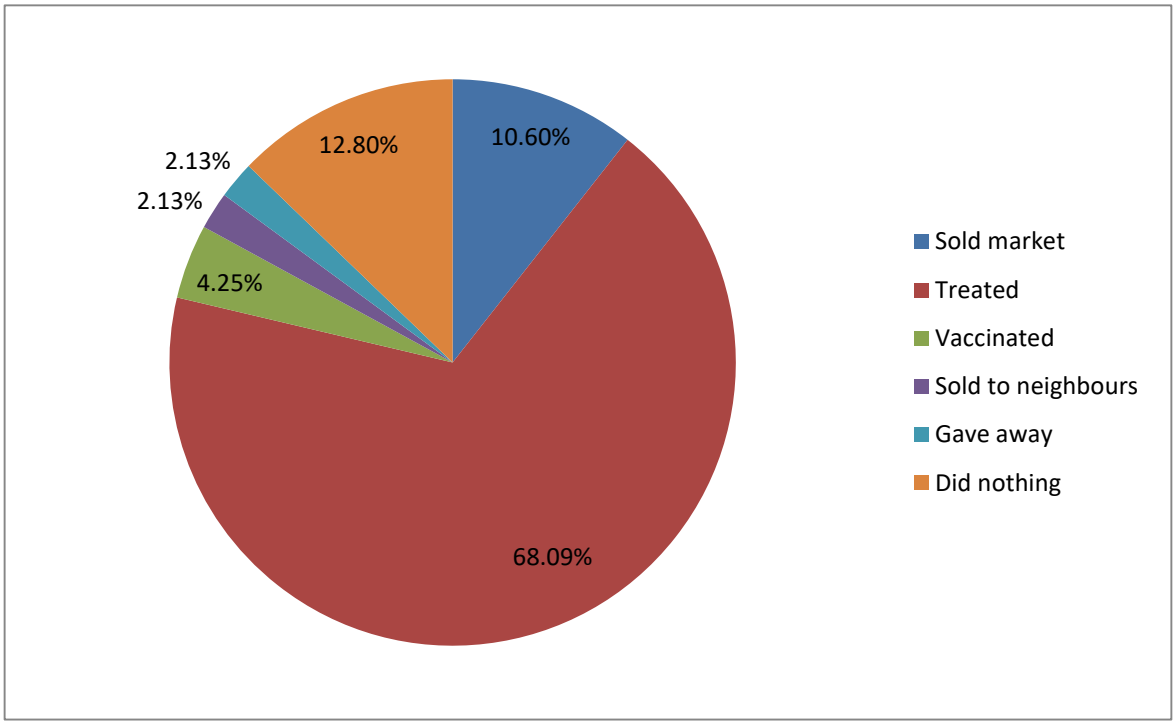


Figure 3.6: **Embu County farmers' practises when they suspected infectious bursal disease outbreaks on their farms**

Of the thirty two (68.09%) farmers who treated their birds, twenty four (75%) bought drugs from agro vets, twenty (62.5%) used local herbs, four (12.5%) got drugs from animal health providers, two (6.25%) borrowed drugs from other farmers, and one (3.1%) did nothing while two (6.25%) applied human Amoxicillin capsules and raw milk in drinking water. Twenty six farmers (55.3%) disposed sick and dead birds by burying, twelve (25.5%) fed the birds to dogs as shown in **Figure 3.7**. Forty five of the forty seven farmers (95.7%) disposed their poultry manure on their farms, one (2.1%) sold it and one (2.1%) threw it away in bushes.

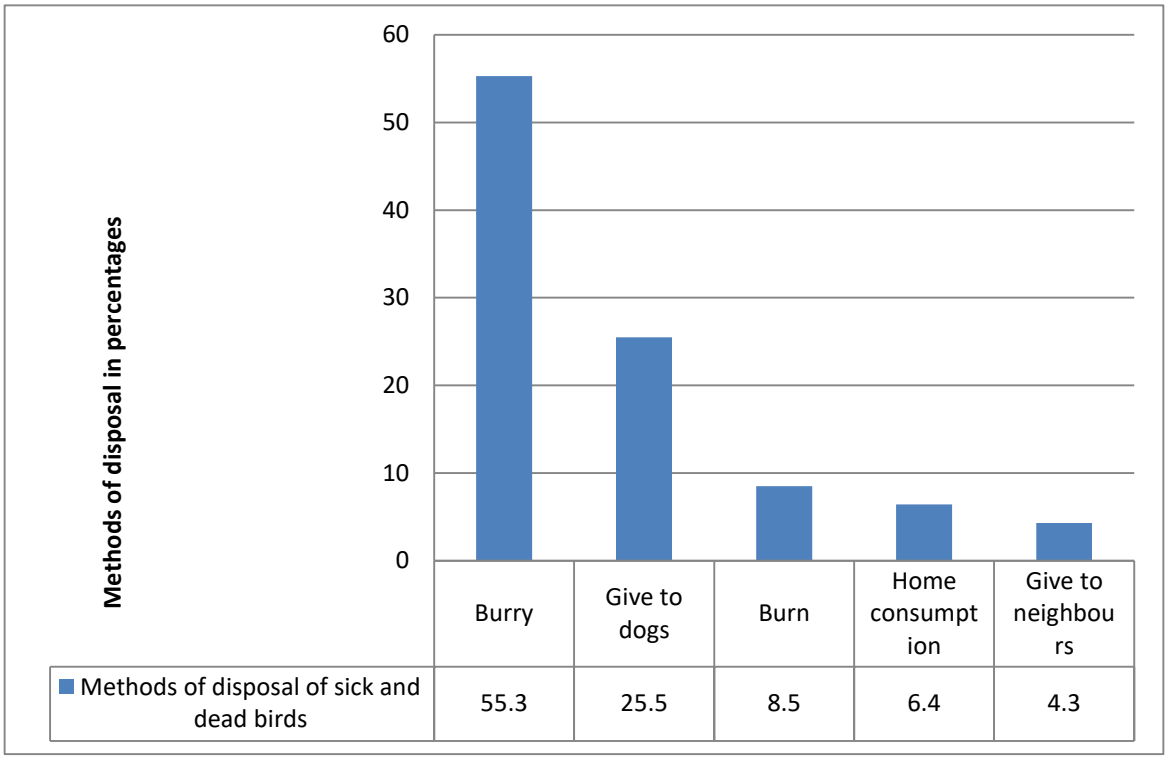


Figure 3.7: Disposal methods of dead and sick birds by indigenous poultry farmers in Embu County

Thirty seven (78.7%) farmers transported poultry to and from the market on foot as shown in **Figure 3.8**; nine used both motorbikes and on foot (19.1%) and one used cars (2.1%). Direct mixing of unsold birds from the market with home flocks was reported by twenty six (55.3%) of trading farmers while only one (2.1%) reported that he separated unsold birds once they were brought back from the market. Eighteen (38.3%) left them at the market for next sale and two (4.3%) did not respond.

Figure 3.8: Trading farmers (white arrow) in Embu County transporting indigenous chicken on foot to market

3.3.3.2 Traders' practises

Twenty two (56.41%) poultry traders sourced their poultry from local farmers, fifteen (46.15%) from other traders and eleven (28.2%) from farmers in other regions. majority of the chickens from outside the County originated from Tharaka Nithi County (35.9%) followed by Machakos (18.0%) and Kirinyaga (10%) counties respectively. All traders did not enquire disease history of the birds before purchase. Eighteen (46.2%) traders interviewed walked with purchased birds from one farm to another during procurement process. Fifteen (38.5%) had an isolated place where they gathered poultry from various farms and six (15.4%) farmers brought birds to them at their location within the market, that is, traders bought the birds directly from farmers at the market place and put them in their vehicle as one luggage. A large number of traders (35/39; 89.7%) did not disinfect themselves when moving from farm to farm during procurement process. Cars (20/39; 51.3%) and motorbikes (11/39 28.2%) were the most

popular means of transport of birds to the market by traders as shown in **Figure 3.9.** These vehicles and motorbikes were not disinfected between farms or farm and market.

Figure 3.9: A vehicle (A) and motorbike (B) transporting indigenous poultry to and from the market by traders in Embu County

Thirty three traders (84.6%) sold sick birds infected with IBD and other diseases at a discount to unsuspecting farmers. Eleven (28.2%) took them home for domestic consumption, four (10.26%) treated them for farm re-stock and three (7.69%) slaughtered and sold them to consumers.

Seventeen traders (43.6%) left their unsold birds in make-shift shelters at the market centre, eight (20.5 %) took them home and mixed with their home flocks before the next sale while six (15.4 %) separated market birds from their home flocks and eight (20.5%) kept them in a rented a house. Two (5.1 %) traders disinfected their premises in the local markets while most of them (37/39; 94.9 %) used other prevention measures such as hand cleaning using soap and putting Aloe vera in poultry drinking water. Sixteen (41.0 %) buried birds that died at the market centre either from IBD or any other disease, fourteen (35.9%) fed them to stray dogs and six (15.4%) took them home for domestic consumption (**Figure 3.10).**

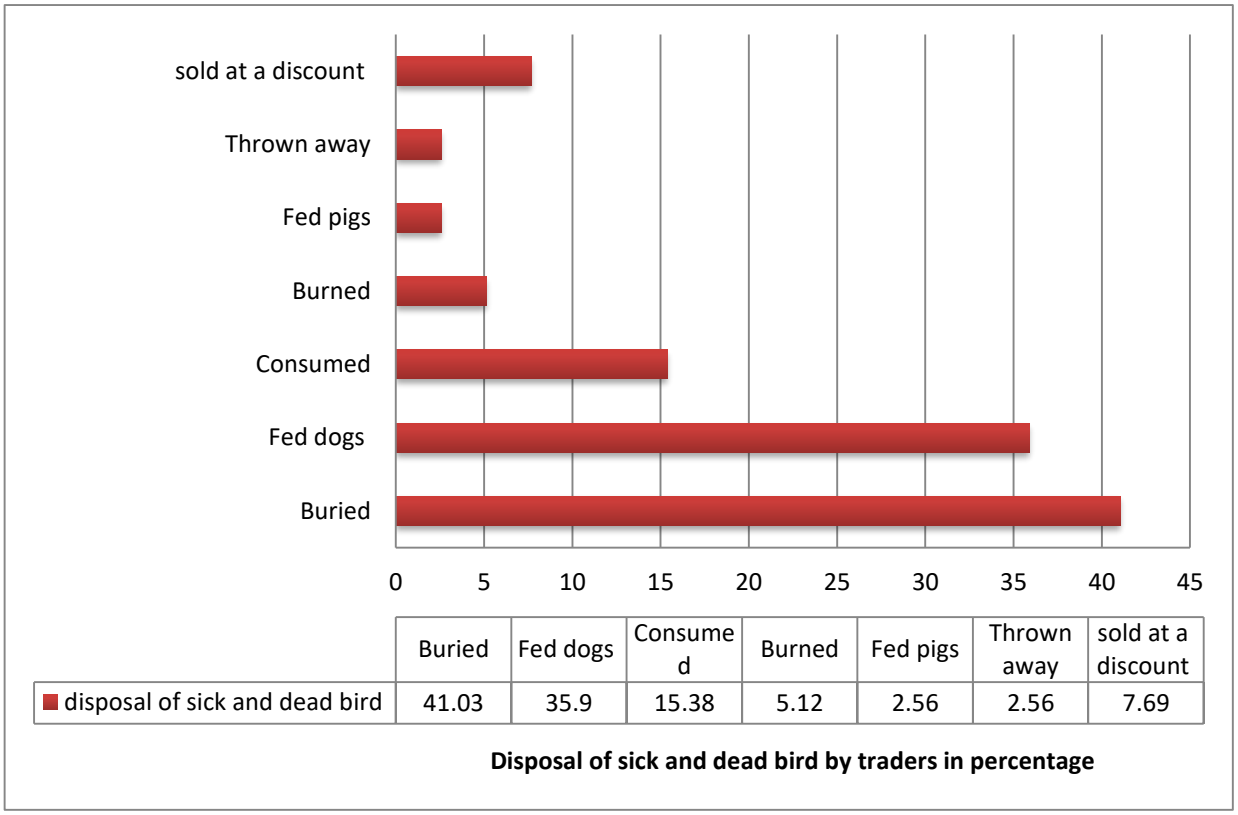


Figure 3.10: Methods of disposal of sick and dead birds by traders in Embu County

3.3.3.3 Slaughter personnel practises

Slaughter personnel handled sick and dead birds by burying them (33.3%), put in condemnation pits (17.9%), thrown in kitchen waste pits (10.3%) and feeding them to dogs (12.8%; **Figure 3.11).** Ninety two point three percent of slaughter personnel did not disinfect their slaughter houses; those who attempted cleaning mostly used washing powder ("omo") and bar soap.

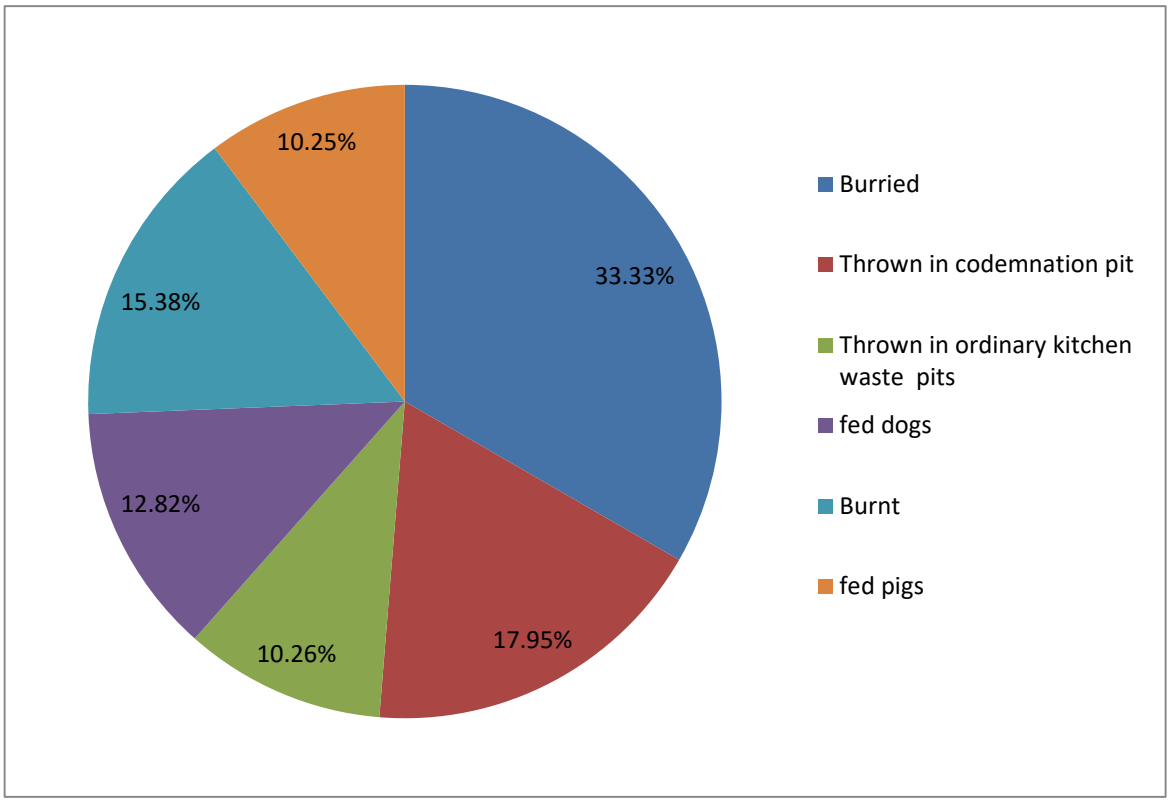


Figure 3.11: Methods of disposal of sick and dead birds by slaughter house personnel in Embu County

3.3.3.4 Animal health provider's practises

Four (57.1%) of the interviewed animal health providers did not disinfect themselves when moving from one farm to another and almost all (6/7) did not use protective equipment (gloves and gumboots), when attending to sick bird(s).

There were no significant associations ($p>0.05$, O.R<1) between risk factors and transmission of IBD in indigenous village chickens including: feeding sick and dead birds to dogs and farm manure disposal. However, a significant association ($p<0.05$) was found between traders sourcing of chickens from local farmers ($p= 0.0107$) and feeding sick and dead birds to dogs (O.R=2.75) by farmers; low IBD vaccine use by farmers (O.R=3.07) and maintenance of IBD in indigenous village chickens.

3.4 DISCUSSION

Infectious bursal disease is a highly contagious viral infection of chicken (Eterradossi and Saif, 2008). Control of the disease is often difficult because of the different poultry production and farming systems, virus characteristics, irregular antibody levels and vaccines types used, which usually results to only a inequitable control of the virus action on a farm (Mutinda *et al.*, 2014). Farmers, traders, slaughter house personnel and animal health providers (poultry value chain actors) lacked knowledge on some daily/ different poultry production and farming practises that lead to maintenance and spread of IBD in indigenous chicken.

Many farmers and traders in Embu County recognised the clinical presentations of IBD which were compatible with the clinical form of the disease (Eterradossi and Saif, 2008; Mutinda, 2016). However the disease lacked a local name and majority of the farmers, traders and slaughter personnel associated it with "Kivuruto", the local name for Newcastle disease. This coincides with the finding by Mutinda *et al.* (2014) and Olwande (2014) where the disease was associated with other diseases like Newcastle disease, infectious bronchitis and coccidiosis. Lack of IBD local name in Kenya and its association with other diseases has resulted to majority of poultry value chain actors treating and preventing the disease with wrong treatment and vaccines leaving the virus alive. This contributes to its continuous maintenance and spread among the indigenous village poultry (Mutinda *et al*., 2014).

There is lack of knowledge on IBD transmission methods and mechanism for most farmers (68.1%) and traders (100%, p=0.0107). The traders and farmers were reluctant to inquire about the disease nor vaccination history from the source farm when buying poultry. This finding is similar to Nyaga (2007) who found that farmers obtained initial breeding poultry as gifts from friends or neighbours or they purchased the birds from local live markets without inquiring disease history. This habit can result in transmission of virus beyond the respective farm, in case of disease outbreaks (Swai *et al.*, 2011; Kebede *et al.*, 2017).

The movement of 89.7 % of the traders and 57.1 % of the animal health providers, without disinfection, from farm to farm increased chances of IBD spread between farms. Infectious bursal disease virus can spread via fomites such as soles of shoes, vehicles and clothing (Zanella, 2007) from infected farms to others. Mixing of chicken by traders from different farms without isolation points (46.2 %) as found in this study, is a biosecurity issue as infected birds can spread IBDV to others; especially since some of these birds were bought restocking their farms. Rearing of different species of birds together in the open range environment encourages cross infection between birds (Swai, *et al.,* 2013).Ducks and turkeys are mostly raised together with chickens under free range systems (Nyaga, 2007). They are asymptomatic carriers and can be significant foci of infection to the free range indigenous chicken.

The practise of over-relying on herbal drugs such as Aloe vera (*Barbadensis miller*) and Neem (*Azadirachta indica*) for treatment of sick birds by 31.9% of the farmers and not vaccinating (91.5%), contributed to maintenance of IBDV among indigenous village poultry. Okello and Gitonga (2010) in a survey conducted in Kiambu, Kilifi, Vihiga and Nakuru Counties of Kenya, found that in case there was a disease outbreak; a number of farmers gave herbal concoctions as a treatment to their indigenous birds and most used herbs included juices made from the leaves of Aloe vera or the neem tree using water. Other farmers' pound African bird eye chilli, mixed it with water and administered to the birds, while others gave lemon juice. The herbal treatments are administered irrespective of disease symptoms. In another survey by Kebede *et al.* (2017) in North Shewa Zones of Oromia and Amhara Regions, Ethiopia, farmers reported treating sick birds using traditional herbs like “Tenadam”, Holly water “Tebel” and local “Areke”. Lack of appropriate vaccine use, due to lack of knowledge on the importance of vaccination for disease control, results in birds lacking immunity to IBD; which they would have acquired from direct vaccination (Yannick *et al.,* 2015) or maternal antibodies from vaccinated mother hens (Fahey *et al*., 1991; Zaheer and Saeed, 2003). The unvaccinated birds

will be susceptible to subsequent IBD outbreak(s) and will end up shedding the virus to the environment, perpetuating the infection.

Infectious bursal disease virus is shed in faeces (AU-IBAR, 2013); it is very stable and survives for long periods outside the host (Eterradossi and Saif, 2008). It remains viable for at least four months outside the host (Eterradossi and Saif, 2008; Zeryehun and Fekadu, 2012). Therefore the practice of disposing poultry manure on farms done by a majority of the farmers (95.7%) promotes maintenance and spread of IBDV to the freely-scavenging indigenous village poultry. A significant number of farmers (25.5%), traders (35.9%) and slaughter personnel (12.82%) disposed the sick and dead birds by feeding to dogs. This is in accordance to Nyaga (2007)' s review on poultry sector in Kenya and Kebede *et al.* (2017) survey in Ethiopia where farmers reported feeding sick and dead birds to carnivores, mostly dogs. Albert *et al.* (2004) showed that a dog fed chicken tissues contaminated with IBDV excreted that virus in its faeces from 24-48 hours post ingestion. The excreted virus remained infective and maintained its unique pathogenic characteristics. Majority of dogs are kept mostly inside and near poultry facilities for security purposes. If dogs are fed dead birds that are infected with IBDV, they can play a significant role as a carrier of IBDV inside the facility, or spread the virus from one poultry flock to another.

There was ease of contact at open-air markets between chicken from diverse areas, which are then taken back to different localities, where they are mixed with home flocks as reported by 55.3% of the farmers and 21.51% traders. This can undoubtedly facilitate the rapid spread and maintenance of IBD among indigenous poultry as suggested by others (Swai *et al*., 2011).

Lack of proper biosecurity measures and vaccination to prevent IBD can lead to heavy losses as a result of high morbidity and mortality rates as reported by Mutinda *et al.* (2013), Mutinda (2016) and Kebede *et al.* (2017); due to IBD or other diseases that may affect the birds due to

the resultant immunosuppression. Therefore, some of the poultry value chain actors' practises, which are done due to lack of knowledge of IBD and how it spreads, coupled by their attitude towards biosecurity measures on indigenous poultry, increase the maintenance and spread of IBD in the area. Understanding of the usual patterns of movements of poultry, poultry products and materials, people and vehicles would lead to better understanding of how disease can spread if introduced into the system at dissimilar places, which in turn allows for planning of strategies to minimize risks within the system.

In conclusion, this study has shown that the value chain actors of Embu County had low knowledge on IBD and were carrying out some practices that end-up maintaining and disseminating the disease-causing virus. The practices include: poor biosecurity, lack of vaccination, disposal of poultry manure on farms, feeding IBD sick and dead birds to dogs and lack of disinfection of slaughter house premises and market places. There is therefore a need for knowledge - creating campaigns and training on various aspects of IBD, for the value chain actors, as a measure towards control of the disease.

CHAPTER FOUR: STATUS OF INFECTIOUS BURSAL DISEASE IN UNVACCINATED INDIGENOUS VILLAGE CHICKEN, DUCKS AND TURKEYS IN EMBU COUNTY

4.1 INTRODUCTION

In Kenya, first case of infectious bursal disease was documented in Coast region (Mbuthia and Karaba, 2000) and the disease has since been diagnosed in other parts of the country. Infectious

bursal disease outbreaks, causing high mortality rates in indigenous chicken, have been reported by Mutinda *et al*. (2013) and Mutinda (2016) in Kenya. The epidemiology of the disease has however not been sufficiently studied in Kenya, especially among indigenous chickens that are often reared together with ducks and turkeys, which normally do not exhibit clinical disease in both natural and experimental infections. However, IBDV antibodies have been observed in these two types of bird (ducks and turkeys) (Eddy, 1990), indicating that these bird types may be involved in the maintenance of IBDV and its resultant transmission to chickens in mixed flocks. Relative increase in prevalence of IBDV antibodies in indigenous chicken has been documented by Swai *et al.* (2011), Sule *et al.* (2013) and Kebede *et al.* (2017) in Tanzania, Nigeria and Ethiopia respectively. However, no studies have been carried out in Embu County, Kenya, to determine whether smallholder indigenous chickens have been exposed to infectious bursal disease virus. Further investigation of anti-IBDV antibodies in other domestic poultry such as ducks and turkeys was recommended by Swai *et al.* (2011). The objective of this study was therefore to evaluate whether unvaccinated indigenous village chickens, ducks and turkeys had detectable IBDV antibodies in Embu County.

4.2 MATERIAL AND METHODS

4.2.1 Study area

This is as described in **section 3.3.1**

4.2.2 Study design

This was a cross sectional study. Enzyme-linked immune-sorbent assay (ELISA) was used to test antibodies against IBDV in the serum of the sampled birds. Agar gel precipitation test

(AGPT) was used to detect presence of the virus in homogenised bursa of *Fabricius* collected during post mortem from chickens that showed signs of IBD.

4.2.3 Sample size

This was done according to Martin *et al.* (1987)

$n = \hat{pq}\ [z\ /\mathrm{L}]^2$

Where:

n= required sample size for an infinite poultry population

p= Anticipated prevalence

q= 1-p

Z=α at 95% confidence level

L=.required degree of precision or acceptable margin of error set at ± 8%

Substituting figures in the equation, this becomes: P=0.5, q=0.5, z =1.96, and L=0.08 i.e. 8% is the estimate of the required precision

n= [0.5 x 0.5] x $(1.96)^2/0.08^2$; where $(1.96)^2$ is replaced by 4,

n=156

This was adjusted for a finite poultry population using the following formula:

n'= 1/[1/n +1/N], where: **n'**= Adjusted sample size, n=Original required sample size i.e. 156 and N=Poultry population size in study area taken to be 1600.

n'= 1/[1/156 + 1/1600] **n'=**142

NB/. The estimate is at 95% confidence level

Sampling was done conveniently based on owners being reachable, willing to sell or the bird being bled, kept the birds on free range system and that they had no history of IBD vaccination in their flocks. A total of 142 small holder indigenous village birds comprising: (97) chickens,

(32) ducks and (13) turkeys were sampled. The sample comprised birds of all age groups and of both sexes. Poultry per farm were determined by minimum number of birds in the flock where systematic random sampling was applied. All chickens purchased were wing tagged and transported alive in disinfected cages to the University of Nairobi (UoN), Department of Veterinary Pathology, Microbiology and Parasitology (VPMP), Kabete for post-mortem examination and blood sampling.

4.2.4 Animal welfare

Permission to use chicken, ducks and turkeys in the experiment was granted by the Biosecurity, Animal use and Ethic Committee of the Faculty of the Veterinary Medicine University of Nairobi. The birds were handled according to the internally accepted regulation and ethical consideration in animal experiment.

4.2.5 Blood collection, processing and storage of serum

4.2.5.1 Ducks and turkeys

Ducks and turkeys were bled in the field. Three millilitres (3 ml) of blood was collected from the brachial vein of healthy indigenous village ducks and turkeys using 5 ml sterile syringe with 22 gauge needle. Harvested blood was transferred to sterile labelled universal bottles (without anticoagulant) and placed on a rack in a slanted position for 4 hours to allow blood clotting (Singh, 2010). After four hours, the universal bottles were kept in a cool box (4^{o}C) and transported to laboratory where they were centrifuged at 3000 revolutions per minute (rpm) for 10 minutes. Separated serum was harvested and transferred to sterile labelled bijou bottles and stored at -20^{o} C until tested.

4.2.5.2 Chickens

All selected indigenous village chickens were in good condition after transportation to the laboratory at the University of Nairobi. They were clinically examined and euthanized (manual cervical dislocation at atlanto-occipital joint) and bled by severing the jugular veins and carotid arteries using a sterile scalpel blade as reported by Kemboi *et al.* (2013) during post mortem. Collected blood was transferred into sterile labelled universal bottles, without anticoagulant. The universal bottles were placed in a rack and slanted for 4 hours for clot to form; they were then centrifuged at 3000 rpm for 10 minutes. Separated serum was harvested and transferred to sterile labelled bijou bottles and stored at -20^{o} C until tested.

4.2.6 Serology

Indirect enzyme linked immunosorbent assay was performed on all serum samples as described by the manufacturer (IDEXX IBD-XR from IDEXX Laboratories, Inc. Westbrook, Maine 04092 USA). The kit consisted, IBD coated plates; IBD positive and negative control sera, goat anti-chicken (IgG) horseradish peroxidase conjugate, sample diluent buffer, TMB substrate and stop solution.

4.2.6.1 Procedure for enzyme linked immunosorbent assay.

Briefly, samples and reagents were allowed to gain room temperature; then samples were mixed using vortex mixer and reagents gently mixed by inverting and swirling. Dilutions of test serum were made in the sample diluents buffer at five hundred folds (1:500). One hundred microliters (100 µl) of each sample, including the positive and negative control serum samples were dispensed in duplicates into appropriate microtitre wells in the already IBD antigen coated plates and incubated for 30 minutes at room temperature. If there were antibodies against IBDV in the serum, they would bind to the pre-coated antigen and form antigen –antibody complex. The plate wells were then emptied and washed with 350 µl of distilled water three times. This was to remove unbound antibodies together with other serum components that did not react.

One hundred microliters (100 µl) of conjugate were added to the washed wells, incubated for 30 minutes at room temperature followed by another washing as described above. Finally, 100 µl of substrate were added to the wells at room temperature for colour development and stopped after 15 minutes by 100 µl of stop solution dispensed into each well (OIE, 2016). The optical densities of colour change were then measured and recorded using ELISA plate reader (Titertek Multiskan Plus, Finland) at 620 nm absorbency wavelength (IDEXX IBD-XR Ab. Test Kit technical guide).

4.2.6.1.1 Enzyme linked immunosorbent assay validity test

The validity of the test was determined when the difference between positive control mean and negative control mean was greater than 0.075 and the negative control mean absorbance was less than or equal to 0.150 (IDEXX IBD-XR Ab. Test Kit technical guide).

4.2.6.1.2 Interpretation of enzyme linked immunosorbent assay results

The relative level of antibody in the sample was determined by calculating the sample to positive (S/P) ratio (OIE, 2016).

$$\frac{\text{s}}{\text{p}}\ \text{ratios} = \frac{\text{sample mean} - \text{Negative control mean}}{\text{Positive control mean} - \text{Negative control mean}}$$

Serum samples with S/P ratios of less than or equal to 0.20 were considered negative. Sample to positive ratios greater than 0.20 were considered positive and indicated vaccination or exposure to IBD virus (IDEXX IBD-XR Ab Test Kit technical guide; appendix 4)

4.2.7 Virus detection

4.2.7.1 Bursa of Fabricius collection and processing

Post-mortem examination of the birds was done in the Department of Veterinary Pathology Microbiology and Parasitology, University of Nairobi, using the method described by Charlton *et al***.** (2006) (Appendix 5). In cases where bursae of Fabricius showed signs of IBD (Mutinda, 2016) they were aseptically collected, placed in sterile labelled universal bottles and chilled, in the virology laboratory, for preparation of respective bursal suspensions.

Each of the collected bursal tissue was then sliced and homogenized in sterilized mortar and pestle to make 50% (w/v) suspension in phosphate buffered saline (**appendix 6-**)containing streptomycin (1000 µg/ml) and penicillin (1000 units; OIE, 2016). The homogenate was centrifuged at 3000 rpm for 10 minutes and the supernatant was harvested and stored at -20^{o}C for AGPT (Mutinda, 2016; OIE, 2016). Agar gel precipitation test was used to test presence of viral antigen in the bursae of Fabricius samples using known IBDV positive serum. Standardized antigen, Cat number RAA0123 (IBDV antigen), and standardized antiserum, Cat number RAB0124 (IBDV type 1 positive serum) used had been imported from Animal Health and Veterinary Laboratories Agency, United Kingdom for a previous study (**Mutinda *et al.*, 2015)**.

4.2.7.2 Procedure for agar gel precipitation test

Plates were prepared one day prior to use and agar was dissolved by standing in a steamer (**Appendix 7.** Dissolved agar (20 ml) in the universal bottle was poured into petri dishes, placed on a flat surface. Plates covered with the molten agar were then stored at 4° C overnight. Four sets of hexagonally arranged wells 6 mm in diameter and 3 mm apart on the agar plate were cut using a template and tubular cutter. The agar plug was removed from the wells using a hooked needle. Central well was filled with 50 µl of the reference antiserum. Four peripheral wells were filled with 50 µl of test homogenised bursa using a pipette; the other two peripheral

wells were filled with 50 µl of the negative and positive control reagents. The Petri dishes were incubated at room temperature for 48 hours in a humid chamber to prevent drying of the agar. The observations in the test sample wells were compared with those in the positive and negative controls. The petri dishes were examined after an incubation period of 24 and 48 hours for precipitation line between the central well and peripheral wells by placing them in an unlit background against an oblique light source (OIE, 2008).This precipitation line is expected to occur in places where homologous antigens and antibodies meet in optimal concentration.

4.2.8 Data analysis

Data were entered in Microsoft Excel and analysed in R version 3.3.1. The prevalence of antibodies to infectious bursal disease virus was calculated using the formula outlined by Bennette *et al*. (1991):

Prevalence (%) = number of serum positive/total number of serum examined× 100

Chi-square was used to test association between IBD seroprevalence and age groups of individual species of bird (chicken, ducks and turkeys) and P-value less than 0.05 (P-value < 0.05) was considered significant.

4.3 RESULTS

4.3.1. Sero prevalence of chicken, ducks and turkeys to infectious bursal disease

Turkeys had the highest sero-prevalence of 92% (12/13) followed by chickens 65% (63/97), then ducks 6% (2/32) as shown in **Figure 4.1**.

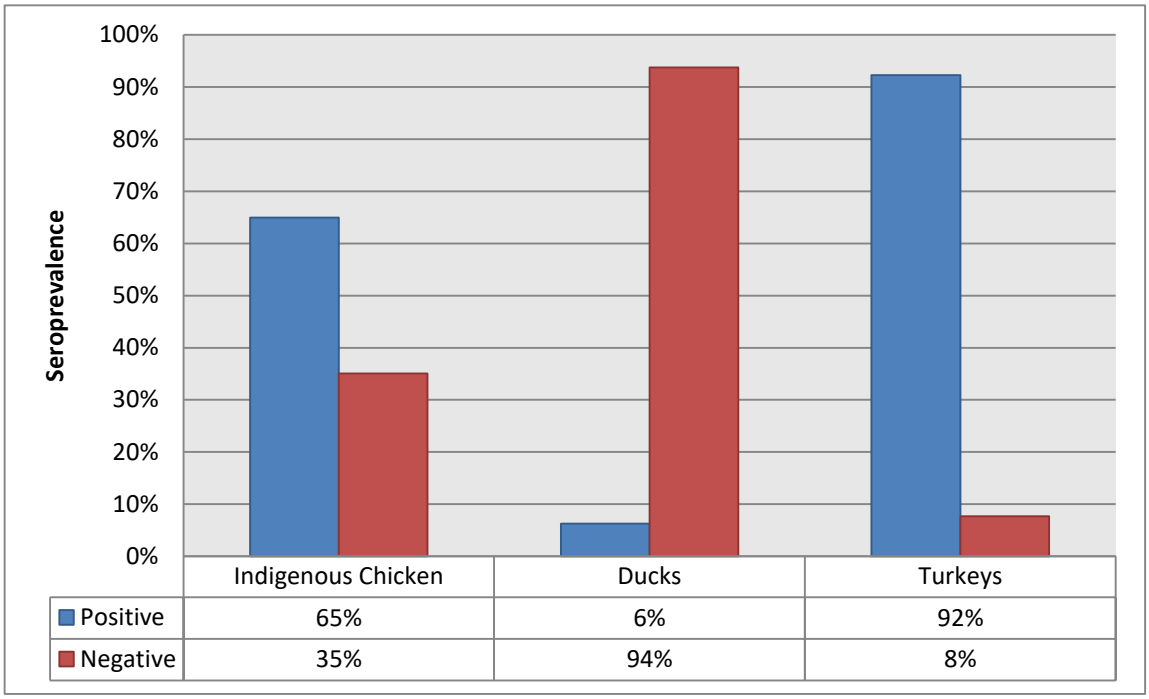


Figure 4.1: Sero prevalence of infectious bursal disease in chicken, ducks and turkeys

4.3.2 Sero prevalence in different age groups of indigenous village chicken, ducks and turkeys

When indigenous village chickens were considered with respect to age, sero prevalence rate of 51.16%, 69.70% and 85.71% were found in chicks, growers and adults respectively. For ducks sero prevalence rates of 0%, 7.14% and 6.67% were found in duckling, growers and adults respectively. For turkeys 0%, 50% and 90.9% sero prevalence was found in poults, growers and adults respectively.

There was no significant difference (P>0.05) between age groups and individual species of bird (indigenous village chicken, ducks and turkeys; **Table 4.1**).

Table 4.1: Sero prevalence in different age groups of indigenous village chickens, ducks and turkeys

Variable	Number examined	Percentage positive	p-value
Chicken age groups			**0.25**
Chick	43	51.16% (22/43)	
Grower	33	69.70% (23/33)	
Adult	21	85.71% (18/21)	
Ducks age groups			**1.0**
Duckling	3	0% (0/3)	
Grower	14	7.14% (1/14)	
Adult	15	6.67% (1/15)	
Turkeys age groups			**1.0**
Poult	0	0%	
Grower	2	50% (1/2)	
Adult	11	90.9% (10/11)	

NB: Chicks, duckling and poults were less than 2 months old; growers were between 2 to 8 months; and adults, above 8 months of age (Sabuni, 2009).

4.3.2 Infectious bursal disease virus detection

Agar gel precipitation test was done on bursa of *Fabricius* (that showed IBD signs) homogenates collected from three healthy indigenous chickens. The results were all negative for IBDV antigen as shown in **Figure 4.2**.

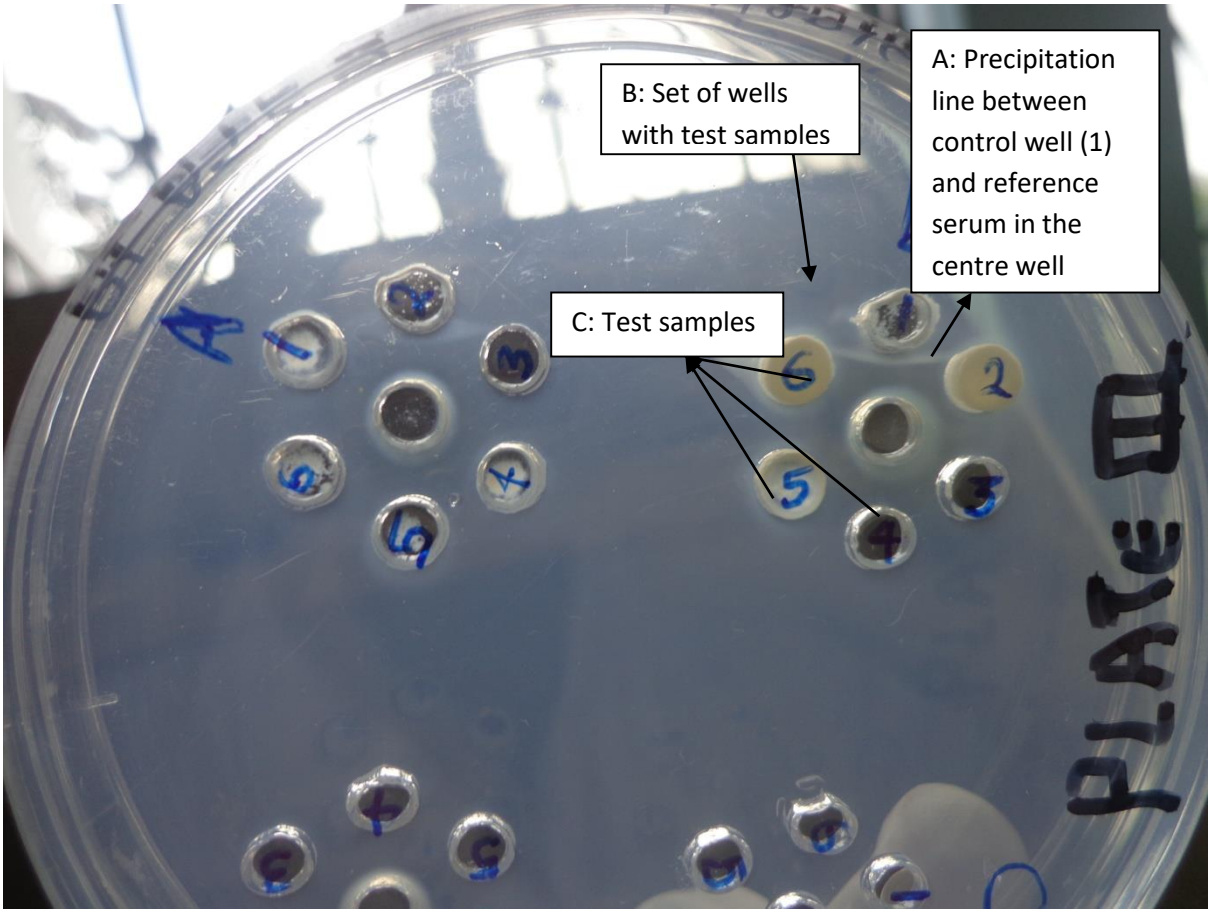


Figure 4.2: Agar gel precipitation test plate with B set of six wells with; (A) precipitation line between central well with standard positive infectious bursal disease serum and the peripheral well (well number 1) with standard positive control reagents; no precipitation line between central well with standard positive infectious bursal disease serum and peripheral well (well numbers 4, 5 and 6) with test homogenised bursa (C).

4.4 DISCUSSION

Chicken are the only birds known to develop clinical signs and marked lesions when exp 0osed to IBDV (AU-IBAR, 2013) while ducks and turkeys are asymptomatic reservoirs for IBDV (Barnes *et al*., 1982; Hollomen *et al.,* 2000). The findings of this study indicated the existence of IBDV among indigenous village chickens, ducks and turkeys, Oladele (2008) in an experiment detected IBD virus using immunoperoxidase technique in the three bird types.

Indigenous village chickens are rarely vaccinated against infectious bursal disease and are raised in poor management and bio-security measures (Swai *et al.,* 2011; Tadesse and Jenbere, 2014; Mutinda, 2016; Kebede *et al.*, 2017). This is one of the possible explanations for the high mortality rates of 39.2% compared to layers (31.1%) and broiler (13.4%) in Kenya, reported by Mutinda (2016) and 57.14 % compared to 34.69% in semi-intensive chickens and 21.05% in intensive system in Ethiopia, as reported by Teshager (2015). The detection of anti-IBDV antibodies in indigenous village chickens shown by this study can therefore, be associated with natural infection as reported elsewhere (Oni *et al.,* 2008; Mushi *et al.,* 1999; Zaheer and Saeed, 2003; Yannick *et al.*, 2015; Mutinda, 2016); this is backed by the exclusive inclusion of reported non vaccinated birds of all ages in this study. Detection of the IBDV antibodies in the young birds may be linked to maternal antibodies which have been reported to persist in unvaccinated chicks up to 21 days; expected to disappear by 28 and 35 days post infection (Zaheer and Saeed, 2003).

The sero prevalence of chickens (65%), ducks (6%) and turkeys (92%) found in this study confirmed observations made by the farmers in Embu County on IBD occurrence despite them being unable to give it a local name. The sero prevalence (65%) found in chicken was similar to the one found in other studies conducted in non vaccinated free range indigenous chicken

(Sule *et al.*, 2013; Lawal *et al.*, 2014). However this finding was lower than those of Oni *et al.* (2003) of 89.7% in Nigeria, Degefu *et al.* (2010) of 76.64% in Western Ethiopia, Kassa and Mola (2012) of 75% in Northern Ethiopia, Zeryehun and Fekadu (2012) of 82% in Central Oromia, Tadesse and Jenbere (2014) of 83% in Eastern Ethiopia and Kebede *et al.* (2017) of 84.2% in six districts of North Shewa Zones of Oromia and Amhara Regions, Ethiopia; all of which used indirect ELISA as the diagnostic tool. The finding was however, higher than those of Mushi *et al.*(1999) of 30% in Gaborone, Botswana, Mahasin and Rahaman (1998) of 30.7% in Sudan, Ndanyi (2005) of 49.3% from Taita Taveta, Kenya who used AGPT as diagnostic tool; Swai *et al*. (2011) of 58.8% in Northern Tanzania, Sule *et al.*(2013) of 63% prevalence in Yobe state Nigeria and Zegeye *et al.* (2015) finding of 45.05% around Mekele in Northern Ethiopia who used indirect ELISA as the diagnostic tool. Lawal *et al.* (2014) found prevalence of 63.5% in cloacal swab samples of village chicken growers using the Antigen Rapid IBDV Antigen Test Kit as diagnostic tool in Gombe State, North Eastern Nigeria.

Generally ELISA test is considered as the most ideal serological test as described by OIE Terrestrial manual (2016) for diagnosis of viral antibodies due to: sensitivity, specificity and simplicity. This study was conducted in month of August and September (dry season); the sero prevalence finding (65%) in chickens and 92% in turkeys correlated with the farmers' and traders' response; that there was high IBD occurrence in months of July to September. However, Lawal *et al*. (2013) reported high prevalence (70.8%) of IBD in indigenous chickens during the wet season as compared to dry season (40.3%); the 65% finding of this study during dry season giving a higher prevalence level. This could be due to stress, as a result of change in climate that lowered resistance at the beginning of dry season, due to inadequate feed and parasitism.

Oladele *et al.* (2008) using immunohistochemistry found that the turkeys and ducks are infected by IBD virus, but normally do not manifest clinical disease. Out of the 32 ducks sampled in

this study, only 2(6%) were sero positive; this was significantly lower compared to the findings of Hollomen *et al.* (2000) who detected 75% in the sera of Eider ducks in Finland, and Oluwayelu *et al*. (2007) 19.1% (24/126) in indigenous Nigerian ducks. However the findings were higher than those reported by Geetha *et al.* (2008) 1.09% in domestic ducks in Asia. Infectious bursal disease virus has been isolated from the faeces of healthy ducks and from bursae of 5-16 day old duckling by McFerran *et al.* (1980) and Karunakaran *et al.* (1992) who reported disease in India respectively. Weisman and Hitchner (1978) experimentally infected turkeys with IBDV and later found they developed antibodies against IBDV. Almost all turkeys 12/13 (92%) in this study were sero-positive suggesting a subclinical infection of the turkeys with serotype 1 IBDV resulting in development of detectable antibodies as reported in Lowa (Barnes *et al*., 1982) but no disease (Weiss and Kaufer, 1994).

There was no significant difference (P>0.05) between IBD prevalence and age groups and individual species of bird (indigenous village chicken, ducks and turkeys). The reason could be that young, growers and adults in the study area shared common house, and feed in the free range production system. This facilitates same exposure of IBDV to all age groups, which agrees with the prevalence reported by Zeryehun and Fekadu (2012) from central Oromia. Highest prevalence rate was recorded in adults (chickens, 85.71% and turkeys, 90.9%). This finding was same as Zegeye *et al.* (2015) finding of highest IBDV prevalence in chickens aged more than >12 months old (64.57%) and contrary to finding reported by Saif *et al.* (2000) showing higher prevalence of IBD in chickens aged below 12 weeks.

In an African rural setting like Embu, ducks and turkeys are mostly raised together with chickens under free range systems. The detection of IBDV antibodies in non-vaccinated ducks and turkeys in this study indicates these birds were exposed to the virus at some point of their life. This is of epidemiological importance as it suggests that these birds play an important role in the natural maintenance and spread of IBDV. They are asymptomatic carriers and can be

significant foci of infection to the free range indigenous and commercial exotic chickens. With turkeys showing such high levels of antibodies, they could be a major risk to other free ranging birds in the village set up especially chicken. More research is needed to find out the role of turkeys in the spread of IBDV in chicken.

Only three bursae from the apparently health indigenous chickens studied showed signs of IBD and they tested negative for IBDV; using AGPT as diagnostic tool. Sera of the -respective three chickens tested positive for anti IBDV antibodies using ELISA. Three samples were a small number and having been isolated from apparently healthy chickens indicated low IBDV titres that could hardly be detected by AGPT; this could be the reason for the negative results.

In conclusion, anti-IBDV antibodies have been detected in indigenous village chickens, ducks and turkeys in Embu County indicating endemicity of IBD in the area. Vaccination of indigenous chickens, ducks and turkeys against IBD is therefore highly recommended to prevent spread of disease in indigenous chickens and exotic chickens that are reared in close proximity with indigenous birds.

CHAPTER FIVE: OVERALL DISCUSSION, CONCLUSION AND RECOMMENDATION

5.1 OVERALL DISCUSSION

Infectious bursal disease is endemic in Kenya (Mbuthia and Karaba, 2002; Ndanyi, 2005; Mutinda *et al.,* 2013; Mutinda, 2016). This was confirmed by the sero positivity obtained from chickens, ducks and turkeys in Embu County despite farmers and traders lacking the local name for it and associating it mostly with Newcastle disease. Infectious bursal disease sero positivity in indigenous chickens, ducks and turkeys may be due to the husbandly systems that favour easier and faster spread and maintenance of this economically important infectious disease (Swai *et al.,* 2011). These conditions include: inappropriate sanitary conditions, nutritional deficiencies, continuous exposure to carrier wild birds, absence of routine vaccination, rearing of different species of birds together, and mixing of chickens during transit and at points-of-sale in markets (Kebede *et al.,* 2017). This was commonly observed in the study area: the free range indigenous chickens freely scavenged and mixed with other species like ducks, turkeys, neighbouring village chickens while searching for feed and points-of-sale in markets. This facilitated transmission of IBD from one flock to another. The IBD virus is resistant to many environmental changes hence it can survive for long in the environment; thus facilitating its transmission (Mutinda *et al.,* 2014).

Currently, IBD has no specific treatment. The most critical measures for optimal prevention and control of IBD is vaccination coupled with biosecurity measures (Swai *et al.,* 2011; Sule *et al.,* 2013; Mutinda *et al.,* 2013).Vaccination using locally developed vaccines to maximize maternally derived antibody is recommended (Yannick *et al.,* 2015; Mutinda, 2016). Vaccination using imported classical live attenuated IBD vaccines has been widely employed in the control and prevention of IBD with limited success in Kenya since new cases of the disease are still reported even among vaccinated flocks (Mutinda *et al.,* 2013; Mutinda *et al.,* 2014; Mutinda, 2016). Cleaning and disinfection of backyard poultry facilities, poultry selling facilities in open live-bird markets and poultry transport equipment and vehicles after each use to reduce and prevent maintenance and spread of IBDV (Block *et al.,* 2007). Infectious bursal

disease virus is inactivated by a pH of 12 and above (Eterradossi and Saif, 2008). It is sensitive to sodium hydroxide (Eterradossi and Saif, 2008), halogens derivatives and aldehydes (formaldehyde, glutaraldehyde) (Shirai *et al*., 1994). Avoid raising mixed species of poultry together to prevent transmissions of IBDV to naive birds from asymptomatic carrier birds like ducks and turkeys to indigenous and exotic chickens (Eterradossi and Saif, 2008). Training on proper disposal (burying, burning) of sick and dead birds that to control and prevent dissemination of IBDV; confinement of pets like dogs and cats in the household area to prevent them from roaming continually among the poultry and avoiding feeding them with sick and dead birds from any disease; this can reduce dissemination of diseases (Albert *et al.,* 2004).

This study recognised that: 1 poultry diseases are extend by movements of input materials, poultry and poultry products and vehicles; 2 these movements are driven and guarded by people; 3 understanding people's motivations is a answer element in determining the risks of poultry disease maintenance and spread. Assessment of the indigenous village chicken, ducks and turkeys status and knowledge, attitude and practices of poultry value chain actors' regarding IBD maintenance and spread in indigenous village poultry as part of a long term action plan, is certainly a source of useful information for programs aiming for a real control of the disease.

5.2 CONCLUSIONS

1. Some of the poultry value chain actors' practises, which are done due to lack of knowledge on IBD and how it spreads, coupled by their attitude towards biosecurity

measures on indigenous poultry, increase the maintenance and spread of IBD in the area

2. Presence of IBDV antibodies in unvaccinated indigenous chicken, ducks and turkey indicates endemicity of IBD in Embu County, Kenya
3. The sero-positivity found confirmed observations made by the farmers on IBD occurrence despite them being unable to give it a local name.
4. This study reports for the first the presence IBDV antibodies in ducks and turkeys in Kenya.
5. Presence of IBD antibodies in ducks and turkey that do not show clinical signs of IBD, indicates their probability of acting as carriers of the disease to the indigenous chicken that are reared together in the rural setting

5.3 RECOMMENDATIONS

1. There is a need for knowledge - creating campaigns and training on various aspects of IBD, for the value chain actors, as a measure towards control of the disease
2. There is need for routine surveillance coupled with vaccination against IBD in indigenous village chickens, ducks and turkeys to control the disease.
3. More research is needed to find out the role of turkeys and ducks in the spread of IBDV.

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CHAPTER 7: APPENDICES

APPENDIX 1: POULTRY FARMERS QUESTIONNAIRE

QUESTIONNAIRE TO ASSESS INFECTIOUS BURSAL DISEASE STATUS, KNOWLEDGE, ATTITUDES AND PRACTICES OF VALUE CHAIN ACTORS IN SMALLHOLDER INDIGENOUS VILLAGE POULTRY IN EMBU COUNTY, KENYA

Questionnaire no..... Name of interviewer.....

Date...../...../..... Tel no. of respondent.....

BACKGROUND INFORMATION

1. Village..... Sub-location.....

   Location.....

GPS Reading: Eastings……. Northings ……. Elevations.....

2. Name of person interviewed (respondent).....

   Gender; [1] Male [2] Female

3. Occupation of the respondent:

   [1] Farmer [2] business person [3] employee [4] others, specify……..

4. Age of respondent?

   [1] 21-30 years [2] 31-40 years [3] 41-50 years [4] > 50 years

5. What is the relationship of respondent to the household head?

   [1] Self [2] spouse [3] son [4] daughter [5] employee [6] relative [7] Others specify

Poultry information on Gumboro disease

1. How long have you been keeping poultry?

   [1] 1-5 years [2] 6-10 years [3] 11-15 years [4] 16-20 years

   [5] 20 years and above

2. Why keep poultry?

[1] Business [2] Hobby [3] Food [4] Others specify…….

3. Where do you source/obtain your poultry from?

Other farmers	Government institution	Research institutions	Gift	Inheritance	Others (Specify)

4. What category of birds do you keep (tick appropriately)

[1]chicken	[2]ducks	[3] turkeys	[4]Others - specify

5. Have you come across a disease with the following signs: whitish diarrhoea, matted feathers at the vent, ruffled feathers, closed eyes, tucked neck to shoulders, no coughing, no sneezing, no mucous discharge and with constant mortality rate?

   [1]Yes [2] No.

If Yes:

6. What is the local name of the disease?.....
7. When does it occur mostly?

[1]January -March [2] April-June [3]July-September [4]October-December.

8. What age group is mostly affected?

   [1] 0-1 month [2] 2-3 month [3] 4-5 months [4] 5-6 months [5]6 months and above

9. The disease with the signs give earlier is called Gumboro disease. Are you aware of any recent outbreaks of Gumboro disease?

   [1] Yes [2] No

10. If yes, when did it occur last:

    a. in your farm? [1] year-……...[2] month-……..

    b. in nearby farm [1] year-…….[2] month-……..

11. When you suspect Gumboro disease in your flock what do you do?(circle the correct ones)

    [1]Vaccinate [2]Treat [3] Sell the birds at the market [4] Sell the birds to a neighbour [5] Give away the birds [6] Do nothing.

12. If you treat the birds, how is this done?

[1] Use local herbals	
[2] Use drugs from other farmers	
[3] Use drugs from Agro-vets	
[4] Use drugs from Veterinary officers	
[5] Do nothing	
[6] Others: specify	

13. How do you prevent or control the disease?

[1] Vaccination	[2] Disinfectants	[3] Herbals	[4] Others, specify

14. How do you dispose dead birds?

    [1] Burry carcasses [2] Burn carcasses [3]Home consumption [4]Give to dogs [5]Others, specify .....….

15. How do you dispose of manure/litter?

    [1] Use on the Farm [2] Sell it [5] feed cows [3] other - specify ……

16. What loses do you suffer in case of Gumboro disease outbreak?

    1a] Number dead……. [1b] Number in the flock……

    [2] Stunted growth …….

    [3] a. Number of eggs laid before disease outbreak……[3]b. Number of eggs laid during disease outbreak…….

    [4] Cost of drugs used…….

    [5] Veterinary Costs per bird treated……

    [6] Others, specify……..

17. Where do you sell your Chicken?

[1]To other farmers [2] At the open air market [3] Supermarkets

[4] Restaurant and hotels [5] others, specify ……..

18. How do you transport your birds to the market?

[1] On foot [2] Motorbikes [3] Bicycles [4] Cars

[5] others – specify……...

19. Do you mix your birds with others at the trading point?

[1] Yes [2] No

20. How do you handle birds that are not sold?

[1] Bring them back and mix with others

[2] Bring them back and separate them from others for some time

[3] Leave them at market for next sale

[4] Other – specify ……

APPENDIX 2: TRADERS AND SLAUGHTER PERSONNEL QUESTIONNAIRE

QUESTIONNAIRE TO ASSESS INFECTIOUS BURSAL DISEASE STATUS, KNOWLEDGE, ATTITUDES AND PRACTICES OF VALUE CHAIN ACTORS IN SMALLHOLDER INDIGENOUS VILLAGE POULTRY IN EMBU COUNTY, KENYA

Questionnaire no..... Date...../...../.....

Name of interview..... Tel no. Of respondent

1. Village..... Sub-location.....

   location.....

2. GPS Reading: Eastings……. Northings ……..…Elevations.....
3. Name of person interviewed (respondent).....

   Gender; [1] Male [2] Female

4. Age of respondent?

[1] 21-30 years [2] 31-40 years [3] 41-50 years [4] > 50years

5. Have you come across a disease with the following signs: whitish diarrhoea, matted feathers at the vent, ruffled feathers, closed eyes, tucked neck to shoulders, no coughing, no sneezing, no mucous discharge and with constant mortality rate?

   [1]Yes [2] No.

If Yes:

6. What is the local name of the disease? .....
7. When does it occur mostly?

[1]January -March [2] April-June [3] July-September [4] October-December

8. What age is most affected?

   [1] 0-1 Month [2] 2-3 month [3] 4-6 month [4] 6 month and above

9. Are you aware of any recent Gumboro disease outbreaks in this region?

   [1] Yes [2] No

10. Where do you source your poultry?

    [1] Local Farmers [2] farmers from other region [3] other traders [4] institutions [5] others, specify

11. If bought from other regions, which county mostly supplies you?

    .....

    .....

12. Do you enquire Gumboro disease history or vaccination when purchasing the poultry?

    [1] Yes [2] No

13. How do you gather chickens from different farms before transporting them to market?

[1] Walk with them from farm to anther farm

[2] Have an isolated place to gather them

[3] Other, specify

14. Do you disinfect yourself when moving from one farm to anther?

[1] Yes [2] No

15. How do you transport the chicken to the market?

[1]On foot [2] Motorbikes [3] Bicycles [4] Cars [5] others - specify

16. If chicken get sick with Gumboro in the market how do you do to them?

[1] Treat them [2] sell at discount [3] others, specify.....

17. How do you handle chickens that are not sold?

[1] Take them back to the farmer

[2] Keep them at your home [a] with other birds [b] separately

[3] Leave them at market for next sale

[4] Other - specify

18. How do you prevent or control the disease at the market?

[1] disinfectants	[2]Other, specify

19. What do you do if a chicken die out of Gumboro?

[1] Burry carcass [2] burn carcass [3] give to dogs [5] others - specify

Slaughter Personnel

20. How frequent do you encounter poultry with Gumboro disease?

[1] Low [2] fair [3] high

21. Do you do ante mortem inspection?

[1] Yes [2] No

22. If yes what are the major finding to poultry with Gumboro disease?

[1] Whitish diarrhoea

[2] Matted feathers at the vent,

[3] Ruffled feathers,

[4]Closed eyes and tucked neck to shoulders

[5] others, specify .....

23. Do you observe the following lesion in a poultry carcass suffering from Gumboro disease during post mortem inspection?

[1] Dehydration

[2] Bursal highly enlarged.

[3] On opening the bursae has yellow gelatinous transduate.

[4] Haemorrhages that create longitudinal striations.

[5] Necrotic lesion on surface of bursae fold.

[6] Echymotic haemorrhages on thigh and breast muscles.

[7] Atrophying bursa

24. How do you dispose the sick/dead birds?

[1] Burying [2] incineration [3] condemnation pit [4] throwing to ordinary pits [3] other, specify.....

25. Which disinfectant do you use to clean the premises? .....

26. Where do you sell the poultry meat?

    [1] Traders [2] restaurants [3] Locals consumers [4] Other, specify.

27. Other comments on Gumboro disease? .....

APPENDIX 3: ANIMAL HEALTH OFFICER QUESTIONNAIRE

QUESTIONNAIRE TO ASSESS INFECTIOUS BURSAL DISEASE STATUS, KNOWLEDGE, ATTITUDES AND PRACTICES OF VALUE CHAIN ACTORS IN SMALLHOLDER INDIGENOUS VILLAGE POULTRY IN EMBU COUNTY, KENYA

Questionnaire no..... Date...../...../.....

Name of interview.....

1. Name of person interviewed (respondent).....

   Gender; [1] Male [2] Female

2. Village..... Sub-location.....

   location.....

3. GPS Reading: Eastings……. Northings ……Elevations.....

4. Respondent's age group:

   [1] 15 to 25 years [2] 26 to 35 years [3] 36-45 years

   [4] 46 years and above

5. What is the general poultry production situation in this locality?

.....

.....

6. What are the major productions constraints reported/ experienced by farmers in this area?

[1] Diseases	
[2] Predation	
[3] Accidents	
[4] Lack of feed	
[5] Lack of water	
[6] Lack of market	
[7] Lack of medication/vaccines	
[8] Parasites	
[9] Others - specify	

7. Do poultry farmers seek advice or consult your input?

   [1] Yes [2] No

8. If yes what type of consultation do they make? Tick appropriately

[1]Diagnosis	[2] Treatments	[3] Vaccinations	[4]Managements	[5]Others - specify

9. What is the status of Gumboro disease in this area?

.....

.....

.....

10. What prevention or control measures are being used for the disease in this area?

[1] Vaccination	[2] Disinfectants	[3] All in all out method	[4] Others - specify

11. Which vaccines do you use to prevent Gumboro disease?

.....

12. Any recent Gumboro disease outbreaks in this area?

[1] Yes [2] No

13. If yes, how was it managed? .....

.....

14. What are the significant impacts of Gumboro disease outbreak in this area?

.....

.....

.....

.....

15. Give comments on general local indigenous poultry production in this area?

.....

.....

.....

16. Do you know you can contract disease from chickens?

[1] Yes [2] No

17. If Yes, How do you protect yourself?

.....

1. Do you know you can transfer disease from one farm/household to another?

[1] Yes [2] No

19. If yes, how do you minimise it.....

APPENDIX 4: ENZYME LINKED IMMUNOSORBENT ASSAY RAW DATA

ELISA: Plate 1

			Positive Cntl	**Negative Cntl**			
			0.354	0.065			
			0.355	0.073			
			0.3545	**0.069**	**0.2855**	**0.29**	
						>0.2 positive	
Well No	**Sample ID**	**Species**	**Sample A 1**	**Sample A 2**	**Mean**	**S/P Ratio**	**Status**
1	253	Chicken	0.449	0.457	0.453	1.32	Positive
2	258	Chicken	0.075	0.075	0.075	0.02	Negative
3	N2	Chicken	0.1	0.119	0.1095	0.14	Negative
4	210	Chicken	0.092	0.094	0.093	0.08	Negative
5	252	Chicken	0.093	0.093	0.093	0.08	Negative
6	234	Chicken	0.084	0.096	0.09	0.07	Negative
7	290	Chicken	0.119	0.107	0.113	0.15	Negative
8	201	Chicken	0.734	0.773	0.7535	2.36	Positive
9	254	Chicken	0.108	0.106	0.107	0.13	Negative
10	N1	Chicken	0.098	0.087	0.0925	0.08	Negative
11	211	Chicken	0.474	0.486	0.48	1.42	Positive
12	235	Chicken	0.602	0.581	0.5915	1.8	Positive
13	255	Chicken	0.166	0.16	0.163	0.32	Positive

14	229	Chicken	1.48	1.517	1.4985	4.93	Positive
15	258	Chicken	0.07	0.077	0.0735	0.02	Negative
16	244	Chicken	0.101	0.101	0.101	0.11	Negative
17	241	Chicken	0.146	0.143	0.1445	0.26	Positive
18	259	Chicken	0.082	0.081	0.0815	0.043	Negative
19	204	Chicken	1.097	1.109	1.103	3.57	Positive
20	274	Chicken	0.105	0.107	0.106	0.13	Negative
21	230	Chicken	1.264	1.258	1.261	4.11	Positive
22	288	Chicken	1.149	1.074	1.1115	3.59	Positive
23	248	Chicken	0.189	0.178	0.1835	0.4	Positive
24	266	Chicken	0.081	0.081	0.081	0.041	Negative
25	232	Chicken	0.622	0.611	0.6165	1.89	Positive
26	281	Chicken	0.229	0.225	0.227	0.55	Positive
27	271	Chicken	0.329	0.353	0.341	0.94	Positive
28	203	Chicken	1.155	1.145	1.15	3.73	Positive
29	278	Chicken	1.216	1.164	1.19	3.87	Positive
30	252	Chicken	1.349	1.384	1.3665	4.47	Positive
31	N11	Chicken	0.127	0.106	0.1165	0.16	Negative
32	213	Ducks	0.317	0.326	0.3215	0.87	Positive
33	287	Ducks	0.073	0.072	0.0725	0.012	Negative
34	284	Chicken	1.367	1.361	1.364	4.45	Positive
35	263	Chicken	1.349	1.358	1.3535	4.43	Positive
36	233	Chicken	1.462	1.371	1.4165	4.65	Positive
37	279	Chicken	1.09	0.998	1.044	3.36	Positive
38	218	Chicken	0.177	0.138	0.1575	0.31	Positive
39	249	Chicken	0.144	0.12	0.132	0.22	Positive
40	245	Chicken	0.102	0.092	0.097	0.097	Negative
41	235	Chicken	0.093	0.095	0.094	0.086	Negative
42	231	Chicken	1.121	1.133	1.127	3.65	Positive
43	205	Chicken	1.144	1.159	1.1515	3.73	Positive
44	269	Chicken	0.966	0.92	0.943	3.01	Positive
45	246	Chicken	0.141	0.137	0.139	0.24	Positive
46	206	Chicken	0.176	0.167	0.1715	0.35	Positive

ELISA: Plate 2

			Positive Cntl	**Negative Cntl**			
			0.342	0.066			
			0.365	0.071			
			0.3535	**0.0685**	**0.285**	**0.29**	
						>0.2 positive	
Well No	**Sample ID**	**species**	**Sample A 1**	**Sample A 2**	**Mean**	**S/P Ratio**	**Status**
1	DK4	Ducks	0.106	0.108	0.107	0.13	Negative

2	D3	Ducks	0.068	0.073	0.0705	0.005	Negative
3	D8	Ducks	0.166	0.16	0.163	0.32	Positive
4	D2	Ducks	0.088	0.104	0.096	0.09	Negative
5	D9	Ducks	0.062	0.071	0.0665	-0.009	Negative
6	T4	Turkeys	0.327	0.33	0.3285	0.9	Positive
7	D5	Ducks	0.109	0.092	0.1005	0.11	Negative
8	D3	Ducks	0.091	0.096	0.0935	0.084	Negative
9	D1	Ducks	0.083	0.073	0.078	0.031	Negative
10	D6	Ducks	0.106	0.107	0.1065	0.13	Negative
11	DV6	Ducks	0.089	0.091	0.09	0.072	Negative
12	DK1	Ducks	0.096	0.097	0.0965	0.095	Negative
13	DC1	Ducks	0.094	0.099	0.0965	0.095	Negative
14	DC4	Ducks	0.101	0.115	0.108	0.13	Negative
15	DK2	Ducks	0.077	0.075	0.076	0.024	Negative
16	D6	Ducks	0.073	0.074	0.0735	0.016	Negative
17	DK5	Ducks	0.083	0.068	0.0755	0.022	Negative
18	D5	Ducks	0.072	0.073	0.0725	0.012	Negative
19	D7	Ducks	0.069	0.075	0.072	0.01	Negative
20	DC3	Ducks	0.107	0.104	0.1055	0.13	Negative
21	D4	Ducks	0.075	0.087	0.081	0.041	Negative
22	D10	Ducks	0.073	0.076	0.0745	0.019	Negative
23	DV5	Ducks	0.087	0.088	0.0875	0.064	Negative
24	DK1	Ducks	0.079	0.076	0.0775	0.029	Negative
25	DU3	Ducks	0.079	0.079	0.079	0.034	Negative
26	TK3	Turkeys	0.268	0.266	0.267	0.68	Positive
27	TK1	Turkeys	0.151	0.143	0.147	0.27	Positive
28	TK5	Turkeys	0.337	0.345	0.341	0.94	Positive
29	D2	Ducks	0.082	0.094	0.088	0.066	Negative
30	TK4	Turkeys	0.231	0.235	0.233	0.57	Positive
31	DC4	Ducks	0.095	0.101	0.098	0.1	Negative
32	DC2	Ducks	0.067	0.069	0.068	-0.003	Negative
33	DK3	Ducks	0.082	0.083	0.0825	0.047	Negative
34	T5	Turkeys	0.296	0.281	0.2885	0.76	Positive

35	T1	Turkeys	0.191	0.193	0.192	0.42	Positive
36	DU1	Ducks	0.093	0.09	0.0915	0.078	Negative
37	T6	Turkeys	1.107	1.079	1.093	3.53	Positive
38	T3	Turkeys	0.329	0.336	0.3325	0.91	Positive
39	T2	Turkeys	0.199	0.184	0.1915	0.42	Positive
40	T8	Turkeys	0.263	0.313	0.288	0.76	Positive
41	TKU	Turkeys	0.147	0.145	0.146	0.27	Positive
42	219	Chicken	1.116	1.126	1.121	3.63	Positive
43	265	Chicken	0.1	0.095	0.0975	0.098	Negative
44	261	Chicken	0.914	0.903	0.9085	2.9	Positive
45	T7	Turkeys	0.078	0.094	0.086	0.059	Negative
46	N7	Chicken	0.323	0.323	0.323	0.88	Positive
47	N13	Chicken	0.143	0.143	0.143	0.26	Positive

ELISA: Plate 3

Positive Cntl			**Negative Cntl**				
0.333			0.062				
0.333			0.055				
0.333			0.0585	0.2745	0.28		
					>0.2 positive		
Well No	**Sample ID**	**Species**	**Sample A 1**	**Sample A 2**	**Mean**	**S/P Ratio**	**Status**
1	75	Chicken	0.285	0.31	0.2975	0.85	Positive
2	240	Chicken	0.107	0.101	0.104	0.16	Negative
3	N10	Chicken	0.173	0.174	0.1735	0.41	Positive
4	251	Chicken	1.254	1.255	1.2545	4.27	Positive
5	277	Chicken	1.109	1.187	1.148	3.89	Positive
6	267	Chicken	0.1	0.087	0.0935	0.12	Negative
7	N9	Chicken	0.125	0.12	0.1225	0.23	Positive
8	270	Chicken	0.774	0.701	0.7375	2.42	Positive
9	257	Chicken	0.069	0.068	0.0685	0.033	Negative
10	239	Chicken	0.1	0.095	0.0975	0.14	Negative
11	208	Chicken	0.313	0.299	0.306	0.88	Positive
12	280	Chicken	0.053	0.054	0.0535	-0.01	Negative
13	N4	Chicken	1.221	1.219	1.22	4.15	Positive
14	236	Chicken	0.111	0.109	0.11	0.18	Negative
15	212	Chicken	0.349	0.34	0.3445	1.02	Positive
16	N3	Chicken	0.112	0.126	0.119	0.21	Positive

17	276	Chicken	1.158	1.204	1.181	4.01	Positive
18	298	Chicken	1.435	1.386	1.4105	4.66	Positive
19	228	Chicken	0.564	0.585	0.5745	1.78	Positive
20	272	Chicken	0.101	0.096	0.0985	0.14	Negative
21	244	Chicken	0.337	0.353	0.345	1.02	Positive
22	260	Chicken	0.17	0.165	0.1675	0.39	Positive
23	300	Chicken	1.15	1.139	1.1445	3.88	Positive
24	298	Chicken	0.941	0.932	0.9365	3.03	Positive
25	217	Chicken	0.135	0.138	0.1365	0.28	Positive
26	250	Chicken	1.013	1.027	1.02	3.43	Positive
27	292	Chicken	0.139	0.129	0.134	0.27	Positive
28	268	Chicken	0.858	0.805	0.8315	2.76	Positive
29	273	Chicken	0.097	0.087	0.092	0.12	Negative
30	215	Chicken	0.656	0.668	0.662	2.15	Positive
31	216	Chicken	0.048	0.049	0.0485	-0.038	Negative
32	286	Duck	0.074	0.07	0.072	0.046	Negative
33	N6	Chicken	0.138	0.133	0.1355	0.27	Positive
34	289	Chicken	0.084	0.092	0.088	0.1	Negative
35	209	Chicken	0.143	0.132	0.1375	0.28	Positive
36	226	Chicken	0.276	0.262	0.269	0.75	Positive
37	237	Chicken	0.091	0.078	0.0845	0.091	Negative
38	291	Chicken	0.478	0.493	0.4855	1.52	Positive
39	243	Chicken	0.105	0.108	0.1065	0.17	Negative
40	N5	Chicken	0.086	0.086	0.086	0.096	Negative
41	294	Chicken	0.089	0.088	0.0885	0.11	Negative
42	207	Chicken	0.159	0.143	0.151	0.33	Positive
43	256	Chicken	0.15	0.14	0.145	0.31	Positive
44	299	Chicken	0.088	0.086	0.087	0.1	Negative
45	242	Chicken	0.093	0.093	0.093	0.12	Negative
46	N2	Chicken	0.049	0.049	0.049	-0.036	Negative

ELISA: Plate 4

Positive Cntl	**Negative Cntl**
0.34	0.062
0.316	0.051
0.339	0.053
0.33	0.053

		0.33125	**0.05475**	**0.2765**	**0.28**		
				>0.2 positive			
Well No	**Wing Tag No.**	**Species**	**Sample A 1**	**Sample A 2**	**Mean**	**S/P Ratio**	**Status**
42	**227**	Chicken	**0.391**	**0.383**	**0.387**	**1.19**	**Positive**
43	**269**	Chicken	**0.931**	**0.968**	**0.9495**	**3.19**	**Positive**
44	**N8**	Chicken	**0.131**	**0.126**	**0.1285**	**0.26**	**Positive**

APPENDIX 5: POST MORTEM EXAMINATION FINDINGs

No.	Wing tag No.	Age	Sex	Sample	Post mortem finding
1.	275	Grower	Female	Whole blood for serum	Haemorrhages on the thymus
					Tapeworms and Heterakis species

2.	288	Grower	Female	Whole blood for serum	Heterakis and tapeworms
3.	291	chick	Male	Whole blood for serum	Tapeworms and Heterakis
					Hyperaemia of proventriculus mucosa
4.	243	Adult	Female	Whole blood for serum	Worms
5.	296	Chick	Male	Whole blood for serum	Haemorrhage spot on breast muscle
					Mottled liver
					Caseous necrosis /debris in the bursa of fabricious.
					Worms
6.	219	Chick	Female	Whole blood for serum	No significant finding
					Tapeworms
					Small atrophying bursa of Fabricius
					Fleas
7.	284	Chick	Male	Whole blood for serum	Fleas
					Atrophying bursa of Fabricius
					Mottled liver
8.	242	Chick	Female	Whole blood for serum	No significant finding
9.	245	Chick	Female	Whole blood for serum	No significant finding
10.	246	Chick	Male	Whole blood for serum	Mixed worm infection
					Haemorrhagic caecal tonsils
11.	215	Chick	Female	Whole blood for serum	Fleas
12.	248	Grower	Male	Whole blood for serum	Tapeworms
13.	280	Grower	Male	Whole blood for serum	Atrophying bursa of Fabricius
					Dilated proventriculus
					Tetrameres and tapeworms
14.	262	Grower	Female	Whole blood for serum	Atrophying bursa of Fabricius and thymus
					Spot haemorrhage on duodenum serosa
					Tapeworms
15.	203	Grower	Female	Whole blood for serum	Spot haemorrhage in the ileum
16.	265	Grower	Male	Whole blood for serum	Ecchymotic haemorrhage on thigh muscles
					Dilated proventriculus
					Haemorrhage cecal tonsil
					Tapeworms
17.	241	Grower	Female	Whole blood for serum	Worms
					Haemorrhage cecal tonsil
18.	216	Grower	Female	Whole blood for serum	Fleas
					Scaly leg (mange)
					Tapeworms
19.	251	Grower	Female	Whole blood for serum	Swollen spleen and bursa of Fabricius
				Bursal of Fabricius	
					Haemorrhage and swollen cecal tonsil

20.	210	Grower	Male	Whole blood for serum	Thymus and bursa of Fabricius atrophied
					Tapeworms
21.	260	Adult	Female	Whole blood for serum	No significant finding
22.	230	Adult	Female	Whole blood for serum	Worms
					Scaly leg
23.	232	Grower	Male	Whole blood for serum	No significant finding
24.	233	Grower	Male	Whole blood for serum	Heterakis worms
25.	226	Grower	Male	Whole blood for serum	Spot haemorrhage at the serosa of the small intestines
26.	255	Grower	Male	Whole blood for serum	No significant finding
27.	209	Grower	Male	Whole blood for serum	Worms
					Congested proventriculus
					Scaly leg
28.	270	Grower	Female	Whole blood for serum	Swollen spleen
					Nodular worms on the intestine serosa
29.	244	Grower	Male	Whole blood for serum	Multifocal nodules on serosa and mucosal of intestines
30.	206	Grower	Female	Whole blood for serum	Scaly leg
					Nodules on intestines and worms
31.	298	Adult	Female	Whole blood for serum	No significant finding
32.	227	Grower	Male	Whole blood for serum	No significant finding
33.	287	Adult	Male	Whole blood for serum	No significant finding
34.	214	Adult	Female	Whole blood for serum	No significant finding
35.	213	Adult	Female	Whole blood for serum	No significant finding
36.	263	Chicks	Male	Whole blood for serum	Two pinpoint haemorrhages on the left thigh
					Pale liver
					Tetrameres cyst on proventriculus
					Enlarged cecal tonsil
					Tapeworms
37.	272	Chick	Female	Whole blood for serum	Few haemorrhage spot on the right thigh
					Tapeworms nodules on intestines serosa
38.	240	Chick	Male	Whole blood for serum	Few spot haemorrhage on breast muscle
					Hyperemia cecal tonsils
39.	273	Chick	Male	Whole blood for serum	Hyperemia of duodenum
					Tapeworms and nodules on the walls of the intestines
40.	208	Chick	Female	Whole blood for serum	Few focal haemorrhages on thigh muscles
41.	207	Chick	Male	Whole blood for serum	Spot haemorrhage on thigh muscle

					Ejected blood vessels at duodenum serosa
					Tapeworms
42.	N1	Chick	Male	Whole blood for serum	*Argus persicus*
					Multiple petechiae haemorrhage at breast muscles
					Focal haemorrhage on thymus gland
					Congested duodenum
					Tapeworms
43.	297	Chick	Female	Whole blood for serum	Atrophied bursa of Fabricius
					Tapeworms
					Congested duodenum
44.	253	Chick	Male	Whole blood for serum	Heavily infested with *Argus persicus*
					Mottled liver
					Atrophied bursa of Fabricius
45.	264	Chick	Male	Whole blood for serum	Tetrameres embedded in proventriculus
					Tapeworms
					Congested duodenum
46.	254	chick	Female	Whole blood for serum	No significant finding
47.	299		Female	Whole blood for serum	Linear congestion on the left thigh muscle
					Tapeworms
48.	290	Chick	Male	Whole blood for serum	No significant finding
49.	259	Chick	Male	Whole blood for serum	Calcified necrotic lesion on the proventriculus serosa
50.	247	Chick	Female	Whole blood for serum	No significant finding
51.	N2	Chick	Female	Whole blood for serum	Tetrameres worms
					Tapeworms
52.	234	Chick	Male	Whole blood for serum	Slight congestion of pancreas
53.	N3	Chick	Female	Whole blood for serum bursal of fabricious	Enlarged congested bursa Enlarged spleen with a focal grey spot
54.	249	Chick	Female	Whole blood for serum	Scaly leg
					Tapeworms and tapeworms nodules
55.	N4	Chick	Female	Whole blood for serum	Tetrameres on the proventriculus
56.	294	Chick	Female	Whole blood for serum	Tetrameres on the proventriculus
					Slight enlarged spleen
57.	N5	Chick	Male	Whole blood for serum	Tapeworms
58.	281	Chick	Female	Whole blood for serum	*Argus persicus*
59.	N6	Adult	Male	Whole blood for serum	Few spot haemorrhages on the left thigh
					Enlarged spleen

					Tetrameres
					Tapeworms
60.	289	Grower	Male	Whole blood for serum	Brush like haemorrhages on thigh muscles
61.	292	Grower	Female	Whole blood for serum	Enlarged spleen
62.	293	Chick	Male	Whole blood for serum	Tapeworms in the whole intestines
63.	N7	Chick	Male	Whole blood for serum	Small spleen
					Tapeworms
64.	N8	Chick	Female	Whole blood for serum	Enlarged bursal with haemorrhages in on the outer surface
				Bursal of Fabricius	Congested duodenum
					Tapeworms and tapeworms nodules
65.	148	Grower	Female	Whole blood for serum	*Argus persicus*
66.	204	Chick	Female	Whole blood for serum	Tetrameres on the proventriculus
67.	238	Grower	Female	Whole blood for serum	Tapeworms
68.	267	Chick	Female	Whole blood for serum	Tetrameres on the proventriculus
					Tapeworms
69.	257	Grower	Male	Whole blood for serum	Focal haemorrhages on thigh muscles
					Tapeworms
70.	235	Chick	Male	Whole blood for serum	*Argus persicus*
					Tapeworms
71.	266	Chick	Female	Whole blood for serum	Petechiae haemorrhages on thigh muscles
					Pale liver
					Multifocal haemorrhages on small intestines
					Tapeworms
72.	N9	Grower	Male	Whole blood for serum	Focal haemorrhages on breast muscles
73.	220	Grower	Male	Whole blood for serum	Congested lungs
74.	239	Chick	Female	Whole blood for serum	Focal haemorrhages on the right thigh
					Small spleen
75.	256	Chick	Male	Whole blood for serum	No significant finding
76.	N10	Grower	Female	Whole blood for serum	Pale liver
					Tapeworms
77.	218	Grower	Male	Whole blood for serum	No significant finding
78.	202	Grower	Male	Whole blood for serum	No significant finding
79.	N11	Grower	Female	Whole blood for serum	Small spleen
					Tapeworms
80.	274	Chick	Male	Whole blood for serum	Multifocal haemorrhages on thigh muscles
					Tetrameres

					Tapeworms
81.	258	Chick	Female	Whole blood for serum	*Argus persicus*
					Focal haemorrhage on the left thigh
82.	211	Grower	Female	Whole blood for serum	Brush like haemorrhages on thigh muscle
83.	237	Grower	Female	Whole blood for serum	Slight congestion of the duodenum
84.	N12	Chick	Male	Whole blood for serum	*Argus persicus*
					Pale liver
					Tapeworms
85.	212	Chick	Female	Whole blood for serum	Yellowish liver
86.	295	Adult	Female	Whole blood for serum	No significant finding
87.	205	Adult	Male	Whole blood for serum	Tapeworms
88.	269	Adult	Female	Whole blood for serum	No significant finding
89.	277	Adult	Female	Whole blood for serum	No significant finding
90.	252	Adult	Male	Whole blood for serum	No significant finding
91.	286	Adult	Male	Whole blood for serum	No significant finding
92.	300	Adult	Male	Whole blood for serum	No significant finding
93.	261	Adult	Female	Whole blood for serum	*Argus persicus*
94.	236	Adult	Female	Whole blood for serum	No significant finding
95.	217	Adult	Male	Whole blood for serum	Tapeworms
96.	278	Adult	Female	Whole blood for serum	No significant finding
97.	201	Adult	Female	Whole blood for serum	*Argus persicus*
98.	229	Adult	Male	Whole blood for serum	No significant finding
99.	228	Adult	Female	Whole blood for serum	Tapeworms
100.	276	Grower	Female	Whole blood for serum	*Argus persicus*
101.	231	Grower	Male	Whole blood for serum	Tapeworms
102.	250	Chick	Male	Whole blood for serum	Tetrameres
					Tapeworms
103.	268	Adult	Female	Whole blood for serum	No significant finding
104.	271	Adult	Male	Whole blood for serum	No significant finding
105.	279	Adult	Male	Whole blood for serum	No significant finding

APPENDIX 6: PREPARATION OF AGAR GELS AND PLATES

Sodium chloride 80 g

Phenol	5 g
Agarose	12.5 g
Distilled water	1 litre

Sodium chloride and phenol were measured in the above quantities and dissolved in 1 litre of distilled water. The agar indicated above was measured and added then the whole mixture was steamed till the agar dissolved. While still hot it was dispensed into 20 ml volumes in universal bottle and refrigerated until required. To prepare agar plates the contents of one universal bottle were dissolved and poured into a petri dish on a level surface. When set the plates were refrigerated overnight before use.

Four set of hexagonally arranged well were cut on the agar plate using a templates and tubular cutter. The agar plug from cut wells was removed using a hooked needle. The wells were 3 mm in diameter and up to 6 mm apart. The plates so prepared were used for the detection of the antigen and the antibody as the quantitative antibody test.

APPENDIX 7: PHOSPHATE BUFFERED SALINE

Solution A

Sodium chloride (NaCl) 8.00 g

Potassium chloride (KCl) 0.20 g

Sodium phosphate ($NaPO_4$) 1.15 g

Potassium hydrogen phosphate (KH_2PO_4) 0.20 g

0.4% Phenol red 2.00 ml

Dissolve in de – ionised water. Add 2 ml of 0.4% phenol red. Make up to 800 ml and autoclave at 10 lbs for 15 minutes.

Solution B

Magnesium chloride ($MgCl_2.6H_2O$) 0.1 g

Dissolve in 100 ml de – ionised water. Autoclave at 10 1bs for 15 minutes

Solution C

Calcium chloride ($CaCl_2$) 0.1 g

Dissolve in 100 ml de – ionised water. Autoclave at 15 lbs for 15 minutes

Working solution of PBS

Add 8 parts of solution A to 1 part of solution B and 1 part of C.