JIMMA UNIVERSITY

COLLEGE OF AGRICULTURE AND VETERINARY MEDICINE

SERO EPIDEMIOLOGICAL STUDY OF INFECTIOUS BURSAL DISEASE IN BACKY ARD CHICKEN PRODUCTION IN WALISO DISTRICT, SOUTH WESTERN SHOA ZONE, OROMIA, ETHIOPIA

MSC THESIS

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JANUARY, 2020

JIMMA, ETHIOPIA

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DEDICATION

I dedicate this Msc Thesis work to my father Bedasa Muleta, my Loving Wife Mekdes Getacho and to my beloved childrens: Saba'if, Nathan and Kaku Chala for all the love they have given me, their dedicated partnership in the success of my life and for their prayers and support.

STATEMENT OF AUTHOR

First, I declare that this Thesis is my original work and all sources or materials used for this thesis have fully acknowledged. This thesis having been submitted in partial fulfillment of the requirements for Msc in Veterinary Epidemiology at Jimma University, College of Agriculture and Veterinary Medicine and is deposited in the University Library to be made available under the rules of the library. I declare that this thesis not submitted to any other institutions anywhere for the award of any academic degree, diploma or certificate. Brief quotations from this thesis are allowable without special permission if the source accurately acknowledged. Requests for permission for comprehensive citation from, duplicate of this manuscript in whole, or in part may grant by the School of Veterinary Medicine and or the School of Graduate Studies of Jimma University. In all other instances, however, permission should obtain from the author.

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BIOGRAPHICAL SKETCH

The author, Chala Bedasa Muleta was born on 22 August 1986 in Chobi District, West Shoa Zone of Oromia Regional State. He attended his elementary education (Grade 1-8) at Chobi elementary School from 1993-2001, secondary school and preparatory at Ginch Senior high school from 2002-2006. Chala Bedasa has attended undergraduate study at JUCAVM and received DVM degree from Jimma University in June 2010. Chala Bedasa had worked at Goro District Animal Health and Development Office in South West Shoa Zone, Southwestern Ethiopia as an owner process for past 7 years. He followed his MSc. study from September, 2017 to date at Jimma University, College of Agriculture and Veterinary Medicine.

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TABLE OF CONTENTS **PAGES**

LIST OF TABLES **PAGES**

LIST OF FIGERS **PAGES**

LIST OF ABBREVIATIONS

AGID	Agar gel Immuno-diffussion
BF	Bursa of Fabricius
CAM	Chlorioallantoic Membrane
CSA	Central Statistical Agency
C- ELISA	Competitive Enzyme-Linked Immunosorbent Assay
dsRNA	double stranded RNA
DXV	*Drosophila* X virus
In- ELISA	Indirect Enzyme-Linked Immunosorbent Assay
IBD	Infectious bursal disease
IBDV	Infectious bursal disease Virus
IBV	Infectious Brinchitis Virus
IFN-γ	Inflamatory cytokines -γ
IgG	Immunoglobulin G
IgM+	Immunoglobulin M positive
IPNV	Infectious pancreatic necrosis virus
MDA	Maternally Derived Antibody
NAHDIC	National Animal health Diagnosis and Investigation Center
NC	Negative Control
NO	Nitric Oxide
OR	Odd Ratio
OIE	Office of International Des Epizooties
OD	Optimal Density
PC	Positive Control
PI	Post Inoculation
RE	Restriction endonucleases
RFLP	Restriction fragment length polymorphism
RT-PCR	Reverse transcriptase- polymerase chain reaction
SPF	Specific-pathogen-free
SP	Sample to Positive
USA	United State of America
VN	Virus neutralization
vVP	Very Viral protein
VP	Viral protein
vv IBD	Very virulent Infectious bursal disease
X^2	Chi-square

LIST OF APPENDIXES

ABSTRACT

Infectious bursal disease (IBD) has been a great challenge to the poultry industry world-wide for a long time and has a major setback to productivity and profitability in the poultry industries of developing nations including Ethiopia. A cross sectional study on infectious bursal disease was conducted in apparently healthy back yard chickens of 6 selected kebeles located at Waliso district from November, 2018 to October, 2019. A total of 282 serum samples were collected from randomly selected village chickens to estimate seroprevalence of IBD infection for household respondents were used to determine chickens management practices associated with IBD. Simple random sampling method was used to t individual chicken at household level. Household respondent perception on IBD was gathered using a semi-structured questionnaire. Serological task was conducted at the laboratory of NAHDIC Sebeta. Out of 282 serum samples tested 224 were positive for Ab against IBDV using indirect ELISA technique and the overall prevalence of IBD in the study area was found to be 79.43 (%). Relatively Higher prevalence was observed in chickens farms kept by illiterate persons 88(%) than in educated one.A statistically difference was found in the prevalence of IBD among study sites ($P<0.01$), household chicken keeping experience ($p<0.05$), flock size, age chicken age ($p<0.01$) and some hygienic management practice ($p<0.01$). Majority study kebeles Except Bedesa Koricha Chicken keeping Experience less than 4 years, village chickens with large flock size, chickens aged less than 4 weeks and poor hygienic managements were associated with increased risk of IBD exposure. However, the association between seroprevalence of IBD and frequency of chicken house cleaning, household Age and education level, sick chicken isolation practice and the sex of chicken was not significant ($p>0.05$). Therefore, community training should be given on improved chicken health management practices. A further study is important on the characterization of virus strains.

Key words: *Backyard chickens, Ethiopia, Infectious bursal disease virus, Management, Risk factors, Seroprevalence.*

1. INTRODUCTION

Chickens are the most important species, adapted globally to different ecological conditions where human beings live and are important to subsistence, economic and social livelihoods of a large human population (Bettridge, 2014). Ethiopia has 50.38 million chickens population from which 96.9% of the chicken populations are indigenous chickens, while the remaining 2.56% and 0.54% consists of exotic and hybrid breeds (CSA, 2015). This indigenous poultry production contributes 98.5 and 99.2% of the national egg and poultry meat production, respectively (Taddele *et al.*, 2003). Chickens are especially important to women, children and aged individuals, who are the most vulnerable member of the society in terms of under nutrition and poverty; contribute a significant role in supplying animal origin protein to improve human nutrition (Gezali, 2017).

Despite, Ethiopia owned huge chicken flock; there are different constraints like poor nutrition, po or management and prevalent diseases that hinder the productivity of the chicken in most area of the country (Dessie and Ogle, 2001). Among the above obstacles, poultry diseases are the main constraints incriminated for reduction of total numbers and compromised productivity (Ashenafi, 2000). Infectious bursal disease, Newcastle, Coccidiosis, Salmonellosis and nutritional deficiency have been considered the major diseases inflicting heavy losses in Ethiopia. Infectiou s bursal disease (IBD) has been a great challenge to the poultry industry world-wide for a long time, but particularly for the past two decades following emergence of new pathotypes; variant and very virulent strains (Mazengia,2008).

The disease has a major setback to productivity and profitability in the poultry industries of both developing and industrialized nations (Rahman *et al.*, 2010). Until 1987, strains of the virus were of low virulence, causing less than 2% specific mortality, and were satisfactorily controlled by vaccination (van den Berg, 2007). But in 1986 and 1987, vaccination failures were described in different parts of the world (Kegne and Chanie, 2014).

The clinical form of the disease is less importance now a day, occurs in chickens over weeks of age when the bursa is well developed. The greatest economic losses are due to sub clinical disease in chicks from one to twenty-one days of age. At this stage the virus impairs the immune response and renders the chick susceptible to various infections. The disease is transmitted through orally via contaminated feed and water (Sharma *et al*., 2000; Sun and Gao, 2001).

In Ethiopia the occurrence of IBD was first reported in 2002 at privately owned Commercial p oultry farm in which from 45-50% mortality rate was documented (Zeleke *et al*., 2003). In addition Mariam and Abebe (2007) the reported seropositivity of 98.9% by Agar Gel Immuno deffusion test in Amhara region (Andasa farm), Hailu *et al*. (2010) and Zeleke *et al*. (2005) documented the incidence rates of 38.4% and 17.4in an outbreak of IBD in two localities namely Bahirdar and Farta, and Dabre zeit, respectively. 89.78% was reported in Waliso (Hailu *et al*., 2010), 72.7% in Gondor (Kassa and Molla, 2012), 38.39% in Bahirdar (Sinidu *et al*., 2015), and 38.3% in Sebeta district (Asamenew *et al*., 2016).

Several factors like vaccination status, biosecurity measure and management practice may play an important role in the prevalence of IBD. Furthermore, vaccine failure may be encountered partly due to the emergency of field strains that are antigenetically different to the strains used in available vaccine, there by offering very limited immunity or no immunity at all. Non-adherence to cold chain requirements of the vaccine and limited knowledge of farmers about the disease are also responsible factors for IBD prevalence (Mbuko *et al*., 2010). Epidemiological study on IBD is necessary for the successful prevention and control program at backyard chicken production system.There is a lack of information on the epidemiology of IBD in and around Waliso district, southwest shoa zone, Oromia, Ethiopia there is a need to determine the epidemiology and to identify IBD Virus in the area.

1.1. Research questions

1. What will be the seroprevalence and associated risk factors of infectious bursal disease in backyard chickens production systems in the study area?

1.2. Objectives

- To estimate seroprevalence of IBDV infection and Assess the potential risk factors in backyard chickens production systems and
- To determine chickens management practices of the community and its association with IBD prevalence in Waliso district of south western Shoa of Oromia, Ethiopia.

2. LITERATURE REVIEW

2.1. Background

Infectious bursa disease is highly contagious and characterized by destruction of lymphocytes in the bursa of Fabricius (Rautenschlein and Alkie, 2016). Initially there was a misconception that the disease was caused by Infectious bronchitis virus (IBV); this was because of presence of similar gross changes in the kidneys (Lasher and Davis, 2004). However in subsequent studies, the causative agent for IBD was isolated in embryonated eggs and the disease given the respective name (Wang *et al*., 2009).

The first cases were seen in area of Gumboro, United States of America (USA), which is the name derived, even if the terms IBD (infectious bursitis) are more accurate descriptions. In the year of 1960 and 1964, the disease observed in most part of the USA (Lasher and Davis, 2004), and become devastating disease in Europe in the years of 1962 to 1971 (Faragher, 1972). Infectious bursal diseases currently become an international issue, 95 % of the 65 countries that responded to a survey conducted by the (OIE, 2013) that announced the presence of infection (Eterradossi, 2000). Infectious bursal disease virus has recently been isolated from a sparrow in China suggesting that wild birds could act as carriers (Wang *et al*., 2009), including New Zealand which had been free of disease until 1993 (Farooq, 2003).

2.2. Etiology

Infectious bursal disease virus, classified in *Avibirnavirus* genus under the family of viruses called *Birnaviridae* family, is the causative agent of Infectious bursal disease (Minalu, 2015, Brown, 1996). The family includes 3 genera: *Aquabirnavirus* whose type species is infectious pancreatic necrosis virus (IPNV), which infects fish, mollusks, and crustaceans; *Avibirnavirus* whose type species is infectious bursal disease virus (IBDV), which infects birds; and *Entomobirnavirus* whose type species is *Drosophila* X virus (DXV), which infects insects (Delmas *et al.*,2004).

Infectious bursal disease virus particles are bisegmented, double stranded RNA (dsRNA) geno mes, non enveloped virions, which are packaged into single shelled with diameter of 60 to 70 nm (Muller *et al.* 2003; Eterradossi and Saif, 2008). Infectious bursal disease virus replicates

in differentiating lymphocytes of the Bursa of Fabricius, causing the immunosuppressive and often fatal condition called infectious bursal disease (IBD) or Gumboro. The capsid shell exhi bits icosahedral symmetry composed of 32 capsomeres and a diameter ranging from 55 to 65 nm (Brown *et al*., 1996).

Two serotypes of the virus have been described; these are Serotype 1 IBDV strains, pathogenic to chickens (Muller *et al*,.2003; Van Den Berg *et al*,.2004; Kasanga *et al.*,2008), where as serotype 2 strains are non-pathogenic (Caston, 2008). Serotype 1 IBDV isolates comprise the variant, classical virulent and vvIBDV strains, which wide differ in their pathogenicity to chickens. Variant IBDVs do not cause mortality, whereas the classical strains cause up to 20% mortality (Muller *et al.*, 2003). Chickens, especially young chicks at the age of 3 to 6 weeks, are the selected hosts for the serotype I virus (Mahgoub, 2012). In the case of vvIBDV infection, the age susceptibility is extended which covers the entire growing period in broilers (Ingrao *et al.,* 2013). In addition, it was reported that chickens infected with IBDV at the age of 14 days suffered from greater bursal atrophy and had higher viral RNA copy numbers than those infected on the day of hatching (Jayasundara *et al.*,2016).

Serotype II IBDV strains isolated from turkeys and Peking ducks are a virulent to chickens (V an den Berg *et al.,* 2000; Kasanga *et al.,* 2008). The specific tropism of IBDV to developing B cells in the BF has been well-documented, and most of the target B cells are immunoglobulin M positive (IgM+) cells (Dobos *et al.,* 1979; Sharma *et al.,* 2000). Infectious bursal disease Vi rus also invades and replicates in the cells of monocyte macrophage lineage in a persistent ma nner (http://www.dpichicken.org/.Delmarva Poultry Industry, Inc. Jan. 2009), which impedes the phagocytic activity of macrophages and facilitates virus dissemination (Le *et al.,* 2012). Therefore, the control of this disease depends mainly on vaccination (Al-Natour *et al*., 2004), but in some cases vaccinations have been ineffective in protecting birds (Islam *et al*., 2003).

2.3. Epidemiology

Infectious bursal disease also known as (Gumboro Disease, Infectious Bursitis and Infectious Avian Nephrosis) is caused by an acute, highly contagious *Birnavirus* that results in mortality and immunosuppression of young chickens (Jackwood and Sommer, 2007). Infectious bursal

disease occurs worldwide in major poultry production area. Virulence of field strains of the virus varies considerably; and 80% ofOIE member countries of reported the occurrence of acute clinical case of very virulent Infectious Bursal Disease Virus (vv IBDV) that can result in high mortality (Entradossi, 2000; Sa *et al.*, 2016).

Devastating outbreaks of the disease have been reported in many parts of the world (Farooq *et al*., 2003) and recently the IBD is reported indifferent parts of Ethiopia (Woldemariam and Wossene, 2007; Hailu *et al.*, 2010*;* Jebberie *et al*., 2012; Zeryehun and Fekadu, 2012). The disease has spread to all investigated commercial farms and multiplication centers occurring at an average outbreak rate of 3-4 farms per year. The disease was encountered commonly in backyard poultry production systems as well (Minalu, 2015).

2.3.1. Host Susceptibility

Chicken is the only species of bird among the avian species known to be susceptible to IBDV where the virus induces clinical disease and causes IBD characteristic lesions (Lukert and Saif, 2004). Infectious bursal disease virus (IBDV) is host specific. Although serologic evidence of natural infection with the virus has been reported in turkeys, ducks, guinea fowl and ostriches may be infected, clinical disease occurs solely in chickens (OIE, 2008; Lukert and Saif, 2004). It is strongly believed that the serotype IBDV 1 is highly host specific to chickens which develop IBD after infection by serotype 1 viruses. Reports have shown that serotype 2 of IBDV is more prevalent in many species of wild birds, with the natural host considered to be turkeys (Okwor *et al.*, 2011). Antibodies to IBDV have been detected in wild birds and several rare avian species including Antartic penguins, ducks, gulls, crows and falcons (Eterradossi and Saif, 2008). All breeds of chicken are affected but there is variation in severity of the disease between breeds (Mutinda *et al.*, 2013). White Leghorns exhibit the most severe disease and have the highest mortality rate (Lukert and Saif, 2004; Caston, 2008).

The time where chickens are most susceptible to IBDV is between the age of 3 and 6 weeks, when the bursa of Fabricius is at its maximum rate of development and the bursa follicles are filled up with immature lymphocytes. This is because the IBD Virus replicates in and cytolytically affects the actively dividing B lymphocytes in the bursa of fabricius (Van den Berg *et al*., 2007). Infections occurring prior to the age of three weeks are generally subclinica

l and immunosuppressive. Clinical cases may be observed up to the age of fifteen to twenty weeks (Jackwood, 2014).

2.3.2. Transmission of IBD Virus

Chickens are the only known avian species to develop clinical disease and distinct lesions when exposed to IBDV. Infectious bursal disease is a highly contagious disease that may be transmitted through direct contact between infected and susceptible young chickens or indirectly through fomites. Infected chickens begin to shed IBDV in faeces one day after infection and can transmit the disease for at least 14 days post infection (Office International des Epizooties, 2004). Under natural conditions, the most common mode of infection is via the oral route (Sharma *et al.,* 2000). Transmission can also occur through airborne dissemination of virus-laden feathers or poultry house dust (Mazengia, 2010).

Indirect transmission of virus usually occurs via fomites such as feed, equipment, people's clothing and shoes (Benton *et al.,* 1967). The IBDV transmit with horizontal way only, with healthy subjects being infected by the oral or respiratory pathway. Conjunctival and respirator y routes may also be involved (Sharma et *al.,* 2000). The virus is not transmitted through the egg or transovarian route and there is no carrier state in recovered birds (Lukert and Saif, 2004; Eterradossi and Saif, 2008).

There is no data that suggest IBDV is transmitted by wild birds, however direct or indirect transmission of the virus between wild birds and domestic chickens probably occurs (Minalu *et al.,* 2015). Tadelle *et al.,* 2003) reported that there is the extreme resistance of the virus to the outside environment and its viral incubation period is about 2-3 days and can be shed as soon as 24 hours following infection and can last up to two weeks. In the absence of effective cleaning, disinfection and insect control; can enhances the potential for transmission when they are scavenging of dead chickens, ingestion of contaminated water, or exposure of respiratory or conjunctiva membranes to contaminated poultry dust .

2.3.3. Morbidity and Mortality

Infectious bursal disease is extremely contagious and in infected flocks, morbidity is high or with up to 100 % serological conversion, after infection, whilst mortality is variable (Tsegaye and Mersha, 2014). In Europe, Africa and subsequently in Japan, high mortality rates of 50 % to 60 % in laying hens and 25 % to 30 % in broilers were observed. While in Ethiopia the mortality rate of the disease in different poultry houses ranges from 45-50 %. The overall mortality rate was 49.89%. Broiler mortality was 56.09% while 25.08% for layer chickens (Zeleke *et al*., 2005).

These hyper virulent field strains caused up to 100 % mortality in specific pathogen free (SPF) chickens (Van den Berg *et al.,* 2007). Severity depends on the age and breed of the affected birds, the degree of passive immunity and the virulence of the strain of virus, and secondary infections associated with the immunosuppressive effects of the disease (Van den Berg *et al.,* 2007). The most significant economic losses resulted from sub clinical infections of this form of IBDV infection greatly enhances the chicken's susceptibility to sequel such as gangrenous dermatitis chicken anemia virus, inclusion body hepatitis, respiratory diseases and bacterial infections (Van den Berg *et al.,* 2007; Mazengia, 2009).

2.4. Pathogenesis and Clinical Signs

Pathogenesis is defined as the method used by the virus to cause injury to the host with mortality, disease or immunosuppression as a consequence. Chickens acquire IBDV infection orally or by inhalation. The virus is transferred from the gut to the other tissues by phagocytic cells like macrophages. In macrophages of the gut associated tissues it could be detected as early as 4 hours after oral inoculation using immunofluorescence (Muller *et al.,* 2003). The virus then reaches the bursa of Fabricius via the blood where the most extensive virus replication occurs. By 13 hours post inoculation (PI) most follicles are positive for virus and by 16 hours PI a second and pronounced viremia occurs accompanied by secondary replication in other organs resulting in disease and death (Van den Berg *et al.,* 2007).

Cells that produce IgM (IgM+ cells) are the target lymphocytes for the virus. The bursa undergoes atrophy as the bursal follicles get depleted of B cells. Virus replication causes extensive damage to lymphoid cells in medullary and cortical regions of the 14 follicle. Apoptosis of the neighboring non infected B cells augments the destruction of the bursa morphology. By this time an ample amount of viral antigen can be detected in other lymphoid organs like caecal tonsils and spleen (Sharma *et al.,* 2000; Lawal *et al.,* 2014). The destruction of lymphocyte populations associated to the infection causes immune suppression and hampers the immunological maturation of infected birds (Sharma *et al.,* 2000).

Although T cells are resistant to infection by IBDV (Boudarod and Alloui, 2008), they play a significant role in the pathogenesis of IBD (Muller *et al.,* 2003). Transient lesions appear in the thymus during the acute phase of the disease (Sharma *et al.,* 2000). The IBDV induced cytotoxic T cells limit the spread of the virus by destroying the cells expressing the viral antigen and thus initiate the recovery process (Muller *et al.,* 2003). At the same time IBDV induced T cells enhance the viral lesions by producing inflammatory cytokines; T helper cells produce inflammatory cytokines like IFN-γ which activate the macrophages to produce nitric oxide (NO) (Sharma *et al.,* 2000). The Nitric Oxide production after IBD virus infection exerts antiviral effect since it has been shown that immune-suppressed chickens which failed to induce NO have more severe disease and higher degree of virus replication. Production of NO does not however seem to correlate with the hemorrhagic lesions which result from the

reaction of host-factors (anticoagulant) and the determinants responsible for virus virulence and virus clearance (Poonia and Charan, 2000).

Humoral immunity is the primary mechanism of the protective immune response. Antibody production is stimulated at the primary site of viral replication in gut associated tissue and they can be detected as early as 3 days PI (Muller *et al.,* 2003). These antibodies prevent the spread of the virus to other tissues. Due to the rapid onset of antibodies, the necrotic foci that form in the bursa of Fabricius stop expanding and are completely eliminated (Okwor *et al*., 2011). The incubation period is very short which range from 2 to 3 days. In acute cases, the chickens become tired, prostrated, dehydrated, suffered from watery diarrhea, and feathers are ruffled (Mutinda *et al.,* 2016). Mortality commences on the third day of infection, reaches a peak by day four, then drops rapidly, and the surviving chickens recover a state of apparent health after five to seven days. Moreover, a primary infection may also be in apparent when the viral strain is of low pathogenicity or if maternal antibodies are present (Tsegaye and Marsha, 2014).

The clinical signs of IBD vary considerably from one farm, region, country or even continent to another. Schematically, the global situation can be divided into three principal clinical forms, these are: - The first is Classical form, caused by the classical virulent strains of IBDV. Specific mortality is relatively low, and the disease is most often subclinical, occurring after a decline in the level of passive antibodies (Faragher, 1972).

The second is immunosuppressive form, principally described in the USA, is caused by low pathogenicity strains of IBDV, as well as by variant strains, such as the Delaware variant E, which partially resist neutralization by antibodies against the 'classical' viruses (Jackwood and Saif, 1987; Snyder, 1990). The 3rd is hyper acute form which is the acute form, first descri bed in Europe, Africa and then in Asia, It is caused by hyper virulent strains of IBDV, and is c haracterized by an acute progressive clinical disease, leading to high mortality rates on affected farms (Van den Berg *et al*., 2007).

A variant IBDV strains do not produce overt clinical signs, but cause immunosuppression and may cause mortality due to secondary opportunistic infections in immune compromised birds. Susceptible chickens younger than three weeks of age may not exhibit clinical signs, but

develop subclinical infections. This results in a decreased humoral antibody response due to B lymphocyte depletion in the cloacal bursa and a severe and prolonged immunosuppression (Van den Berg *et al.*, 2007; Lawal *et al.*, 2014).

Figure 1: Ruffled feathers in a depressed indigenous chicken pullet suffering from infectious bursal disease (Mutinda *et al*., 2016)

Figure 2: Haemorrhages on thigh and leg muscles of an indigenous chicken from an outbreak of Infectious bursal disease (Mutinda *et al.*, 2016)

Infectious bursal disease produces gross lesion. The tissue distribution and severity of lesions is dependent on the subtype and pathogenicity of the virus (Musa *et al.*, 2012). The cloacal bursa is the target organ for the replication of IBDV and hence the most severely affected. The bursa usually show necrotic foci (area of dead tissue) and cheesy mass is found within its lumen from fallen cell of tissue. At time small large hemorrhage on its inner surface (mucosal surface) is also seen. Sometimes wide spread hemorrhage throughout the entire bursa are present in such case, bird may pass blood in their drooping (Herdt *et al.*, 2005). Moderate to severe splenomegaly with small gray foci uniformly distributed on the surface has been reported. Occasionally, petechial hemorrhages have been in the mucosa at the junction of Proventricules and gizzard (Ashraf *et al*., 2006).

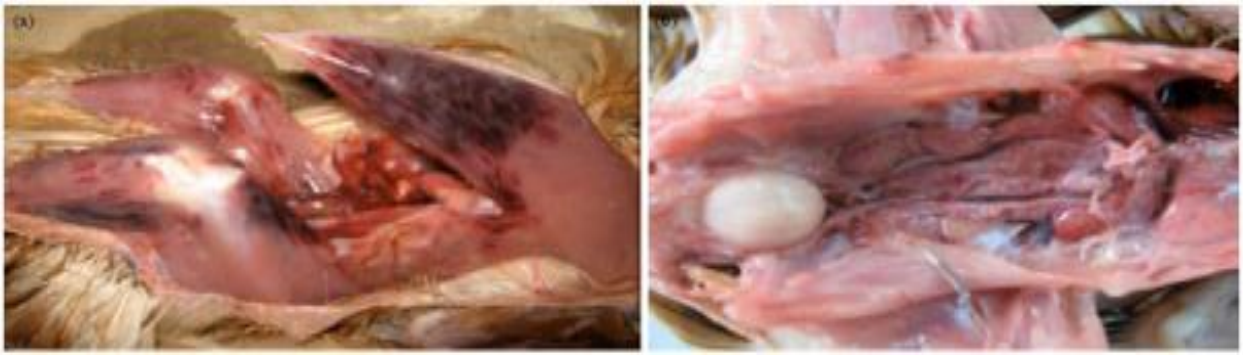


Figure 3: Gross pathology of (haemorrhagic muscles, inflamed bursa of fabricius) in the Gumboro infected bird (Jordan *et al.*, 2002).

2.5. Diagnosis

The clinical diagnosis of the acute forms of IBD is based on disease evolution of a mortality peak followed by recovery in five to seven days and relies on the observation of the symptoms and post-mortem examination of the pathognomonic lesions, in particular of the bursa of Fabricius (Rajaonarison *et al*., 2006). The diseases like avian coccidiosis, Newcastle disease in some visceral forms, stunting syndrome, mycotoxicoses, and chicken infectious anemia and nephropathogenic forms of infectious bronchitis are the differential diagnosis for IBDV. In all acute cases, the presence of bursal lesions allows for a diagnosis of IBD (OIE, 2012). In sub clinical cases, an atrophy of the bursa may be confused with other diseases such as

Marek's disease or infectious anemia. A histological examination of the bursa will allow differ entiation between these diseases (Lukert and Saif, 2004).

2.5.1. Symptomatology and Gross lesions

Hyper virulent IBDV infections are characterized by severe clinical signs and high mortality. Severe outbreaks are characterized by sudden onset of depression in susceptible flocks (OIE, 2008). Animals in the acute phase of the disease are prostrate and reluctant to move, with ruffled feathers and frequently watery or white diarrhea (Van den Berg *et al*., 2007). On post mortem examination of birds that died during the acute phase of vvIBDV, the bursa of fabricius is the principal diagnostic organ: it is turgid, edematous, and sometimes haemorrhagic and turns atrophic within 7 to 10 days. This atrophy might be more rapid, even 3 to 4 days after inoculation (Musa *et al.*, 2012).

In addition, dehydration and Nephrosis with swollen kidneys are common, and ecchymotic haemorrhages in the muscle and the mucosa of the proventriculous are observed in the majority of the affected birds (Sharma *et al*., 2000). In particular, atrophy of the thymus has been associated with the acute phase of the disease and might be indicative of the virulence of the isolate, although it is not associated with extensive viral replication in thymic cells (Tanimura *et al*., 1997,Tasfaye *et al*.,2008).

2.5.2. Isolation and characterization of the virus

Diagnosis depends on the isolation and characterization of the virus and its differentiation from endemic serotype 1 viruses; it can be made through following methods:

Histological diagnosis

Histological diagnosis is based on the detection of modifications occurring in the bursa. The ability to cause histological lesions in the non-bursal lymphoid organs, such as the thymus (sapats and Ignjatouic, 2000), spleen or bone marrow (Inoue *et al*., 1999) has been reported as a potential characteristic of hyper virulent IBDV strains. The histological diagnostic method has the advantage of allowing for diagnosis of both the acute and chronic or sub clinical forms of the disease. Detection of viral antigens: thin sections of the bursa of Fabricius prepared to

detect viral antigens specific to IBDV done by direct and indirect immunofluorescence (Allan *et al*, 1984; Meulemans *et al*., 1987) or by immune per oxidase staining (peters *et al*., 2005) in the bursal follicles of infected chickens between the fourth and sixth day after inoculation. No viral antigen is detectable from the tenth day. However, the virus can be isolated from bursae sampled from the second to the tenth day, with a maximum infectious titer after four days (Rahman *et al.,* 2010). The use of monoclonal antibodies in IHC techniques for detection of the virus enhances the specificity of the test (peters *et al.*, 2005).

Virological diagnosis

Infectious bursal disease virus may be detected in the bursa of Fabricius of chicks in the acute phase of infection, ideally within the first three days following the appearance of clinical signs (Muller *et al*., 2003). A filtered homogenate of the bursa of Fabricius is inoculated in nine- to eleven-day-old embryonated eggs originating from hens free of anti-IBDV antibodies. The most sensitive route of inoculation is the chorioallantoic membrane (CAM); the yolk sac route is also practicable, and the intra-allantoic route is the least sensitive. The specificity of the lesions observed must be demonstrated by neutralizing the effect of the virus with a mono specific anti-IBDV serum. Isolation in embryonated eggs does not require adaptation of the virus by serial passages, and is suitable for vvIBDV. In the absence of lesions, the embryos from the first passage should be homogenized in sterile conditions and clarified, and two additional serial passages should be performed (Lukert P and Saif, 2004).

Serological diagnosis

In areas contaminated by IBDV, most broiler flocks have anti-IBDV antibodies when leaving the farm. Current serological tests cannot distinguish between the antibodies induced by pathogenic IBDV and those induced by attenuated vaccine viruses, so serological diagnosis is of little interest in endemic zones. Nonetheless, the quantification of IBDV-induced antibodies is important for the medical prophylaxis of the disease in young animals, in order to measure the titer of passive antibodies and determine the appropriate date for vaccination (Dewit, 2001; Okwor *et al.,* 2011) or in laying hens to verify success of vaccination (Meulemans *et al.,*

1987). Serology is likewise essential to confirm the disease-free status of flocks. Each serological analysis must include a sufficient number (at least twenty) of individual serum samples representative of the flock under study. A kinetic study requires at least two serological analyses separated by an interval of three weeks (paired sera) (Le *et al*., 2012).

Molecular identification

Most efforts at molecular identification have focused on the characterization of the larger seg ment of IBDV (segment A) and especially of the vVP2 encoding region. Several protocols have been published on characterization using restriction endonucleases of RTPCR products (J ackwood and Sommer, 2007). These approaches are known as RTPCR/RE or RT-PCR-RFLP (restriction fragment length polymorphism) (Jackwood, 2014; Zierenberg *et al*, 2001, Jackwoo d and Sommer, 2007).

In most very virulent viruses, four typical amino acids are present (222 A, 256 I, 294 I and 299 S) (kasanga *et al.*, 2013). However, it is not yet known whether these amino acids play a role in virulence or if they are merely an indication of the clonal origin of most vvIBDV isolates. Several recent studies indicate that although VP2 is an important virulence determinant, it may not be the only one (Wu *et al*., 2007). It has been reported that segment A and B of IBDV mostly co-evolve (i.e. most significant IBDV clusters, such as vvIBDV-related strains, may be identified by analysis of both genome segments), however some potentially reassortant viruses have been identified (Lenouen *et al*., 2006).

2.6. Control and prevention

Infectious bursal disease virus is both highly contagious and very resistant to inactivation, which accounts for its persistent survival on poultry farms, despite disinfection (Van den Berg *et al.*, 2007). Therefore, even with strict biosecurity programs (e.g. ‘down time’ between broods, all-in/all-out production, cleaning and disinfection of the premises and equipment), vaccination is especially important to reduce the incidence and impact of IBDV in the poultry industry (Eterradossi Saif, 2008). Traditionally, breeder flocks are hyper immunized with live and killed vaccines in order to confer high titers of maternal antibodies to their progeny (Van den Berg *et al.*, 2007). This passive immunity protects chicks against early immunosuppressiv

e infections for 1 to 3 weeks; however, protection may be extended to 4 or 5 weeks by boosting the immunity in breeders with oil-adjuvanted vaccines (Besseboua et al., 2015).

Immunization of breeders is an important part of the IBDV control program. Antibodies produced by the hen are passed through the egg to the broiler chick. These maternal antibodies, if present in adequate levels, protect the chicks against sub clinical IBDV (Jackwood and Sommer, 2005).

Live vaccines are administered to achieve active immunity but interference of maternally derived antibody (MDA) is the crucial problem in determining a successful live IBDV vaccination schedule. Vaccinating chickens in the presence of high levels of maternally derived antibodies results in vaccine virus neutralization and no immunity (Besseboua *et al*., 2015). Currently as reported by (Shiferaw *et al.*, 2012) in Mekele, Tigray, Ethiopia, determining the proper time for administration of live intermediate IBDV vaccine important than giving IBDV vaccine to chickens whose parents that have taken IBDV vaccine without determining maternally derived antibodies (MDA) titer and age for vaccination (Okwor *et al.*,2011). Therefore, in order to have chickens protected from IBDV, it is crucial to determine the optimal timing for IBDV vaccine delivery. The optimal timing is often predicted based on serological data following detection of IBDV MDA by an ELISA system during the first week post hatch (De Wit, 2001; Besseboua *et al*., 2015).

The dramatic impact of a very virulent IBD virus can be reduced by proper clean-up and disinfection between flocks, and that traffic (people, equipment and vehicles) onto the farm be controlled. The development and enforcement of a comprehensive biosecurity program is the most important factor in limiting losses by IBD due to IBD virus is very resistant and can survive for more than 100 days in a contaminated area. Phenolic and formaldehyde compounds have been shown to be effective for disinfection of contaminated premises (Gary and Richard, 2015). Since the virus is very stable for months. It is largely excreted through feces hence contaminated litter, feed and water have to be burnt or buried deep under the lime cover (Besseboua *et al*., 2015).

Besides this other measures are; lower stocking densities, increasing intervals between flocks and complete removal of organic waste between batches. In areas where management

practices to reduce virus concentration are used, the disease trends to occur at a later age, and immunosuppressive form of infection is reduced (Bedaso *et al.*, 2017). Administration of inactivated vaccines to breeder hens induces long-standing and high levels of antibodies in the hatched chicks. But in some areas where very virulent IBD virus has caused significant losses the producers do not adopt inactivated vaccination. But intensive live virus vaccination program is used in the hatched chicks from the unvaccinated breeder hens. Such chicks escape the strong risk of immunosuppressive form of the disease (Wu *et al*., 2007). Use of antibiotics can sometimes be advisable to limit the impact of secondary infections (Zeleke *et al.*, 2005a).

2.7. Status of Infectious Bursal Disease in Ethiopia

The disease has spread to all investigated commercial farms and multiplication centers occurring at an average outbreak rate of 3-4 farms per year. The disease was encountered commonly in backyard poultry production systems as well (Ethiopia animal health year book, 2011). According to Ethiopia Animal health year book undertaken during the 2011 fiscal year, Gumboro disease surveillance/investigation was conducted by the NAHDIC in different Regions and they reported that the overall prevalence rates to be about 77.48 %.

There were also researches carried out by different authors where the disease is prevalent in different parts of our country. Zegaye *et al*., 2015, showed the overall seroprevalence of 45.05% of IBD in chicken reared under backyard poultry production systems in Tigray regional state of around Mekele town. Out of 552 serum samples tested, 458 (83%) where positive in backyard chickens at selected woredas of Eastern Ethiopia as described by Tadesse and Jenbere (2014); whereas 82.2% (227/276) reported on backyard chickens in both peasant associations and kebelles of DebreZeit that indicated the presence of field exposure of household chickens to the virus (Tesfaheywet and Getnet, 2012); and out of 27.8% cases, the fatality rates of 98.56% and 77.73% due to IBDV in chickens of Bahir Dar and Farta districts in Amhara region respectively (Hailu *et al*., 2009). Hailu *et al*., 2010), also reveals ov erall seroprevalence of 76.64% IBD on backyard local chicken on study conducted in south and west Showa zones of Oromia region. Jenberie *et al., (*2012) reported that phylogenetically,

Ethiopian IBDVs represented two genetic lineages: very virulent (vv) IBDVs or variants of the classical attenuated vaccine strain (D78).

The nucleotide identity between Ethiopian vvIBDV ranged between 0% and 2.6%. Ethiopian vvIBDV are clustered phylogenetically with the African IBDV genetic lineage, independent of the Asian/European lineage. This report demonstrates the circulation of vvIBDV in commercial and breeding poultry farms in Ethiopia. In addition to IBDV strains included in their study for phylogenetic comparison of VP2 nucleotide sequences, Ethiopian strains form a cluster within the vvIBDV lineage. Ethiopian IBDV strains have shown mutations in the VP1 region. This report could help to select the most appropriate vaccination program for the genomic sequences of field strains through diagnostic testing (Tamiru *et al.,* 2012).

2.8. Economic Importance of Infectious Bursal Disease

Infectious Bursal disease virus is worldwide in distribution and is an important virus in the poultry industry as it causes immune suppression and mortality in infected chickens (Jackwood *et al.,* 2007). The disease is a major set-back to productivity and profitability in the poultry industries of both developing and industrialized nationals. Direct losses linked to specific mortality depend on the dose and virulence of infecting IBDV strain, age and breed of the chicken and presence or absence of immunity (van den Berg *et al.,* 2007). Indirect economic impact of the disease, when quantified, is considerably significant (Musa *et al.,* 2012). It occurs due to virus induced immune-suppression and the interactions of IBDV and other viruses, bacteria or parasites (Farooq *et al.,* 2003).

Losses occur due to secondary infections, growth retardation and condemnation of carcasses at the slaughter houses (Farooq *et al.,* 2003). A decrease of 10% in profit margin was attributed to relative depression in body mass and feed conversion efficiency due to IBDV in a study on economic effect of sub clinical IBDV (Mcllroy *et al.,* 1989). In studies on vvIBDV, it was shown that infection with strain 849VB in 38-day-old hybrid Leghorn pullets, resulted in 60% losses in form of mortality rate, whereas, broiler chicks infected at the same age showed 17% mortality (van den Berg and Meulemans, 2007). Even if birds survive, the resulting immunosuppression and effect on egg production in layer birds is significant (Muller *et al.,*

2003). The virus does not affect man and has no direct public health significance (Lukert and Saif, 2004).

In Ethiopia outbreak investigation was carried out for the first time in 2002 on suspected IBDV case which was reported from a commercial poultry farm in DebreZeit town. At the time of investigation there was mortality of 22,437 Broiler chicken and 2508 layer chicken, and 40, 000 Hubbard broiler chickens and 10, 000 Lohman Brown layer chicks were at risk in 4 weeks time of an outbreak in the farm (Zeleke *et al*. 2005). The overall mortality rate was 49.89%. Broiler mortality was 56.09% while 25.08% for layer chickens. In addition to, the case report study under taken in Andassa poultry farm indicated that the overall mortality of chicken due to IBDV was 12% in young (1-70 day old) and 7% in adult (>70 day old) and 98.9% seroprevalence has been recorded in non-vaccinated flock (Mariam and Abebe 2007). While, economic losses associated with outbreaks and or occurrences of IBDV in the studied farms as mentioned above, back yard village chickens and small scale poultry owned farmers of different region reported in our country may appeared unimaginable to the farm owners and farmers as the owner did not relent efforts to restock his farm. IBDV has been reported earlier to be an important cause of economic losses in the poultry industry. These compounded losses are often high, unimaginable and alarming if properly quantified (Jackwoo d *et al.,* 2007).

3. MATERIALS AND METHODS

3.1. Study area

This study was carried out between November, 2018 and October, 2019 in Waliso district of S outh Western Shoa Zone of Oromia Regional state, Ethiopia. The district is located at 116 km South West of Addis Ababa on the highway leading to Jimma at an altitude of 2063 m above sea level. The area is located at longitude of 37°58'16.3"E and latitude of 8°32'23.0"N. It was characterized by mild sub-tropical weather, with average minimum and maximum temperatures of 5.5°C and 23°C respectively. This area experiences a binomial rainfall pattern with along rain season from June-September and short rain season from march-April (CSA, 2015).

The livestock resource of the study district comprises of 224,334 cattle, 39,543 sheep, 51,042 goats, 7,625 horses, 2101 mules, and 16,320 donkeys, 147,679 chickens (115,814 local and 31 ,865 hybrids). The area has 35 rural and 2 urban administrative Kebeles. The total human population of the district was 165,391, of which 50% was reported to be male (Waliso Finance and Economic Development Office, 2018).

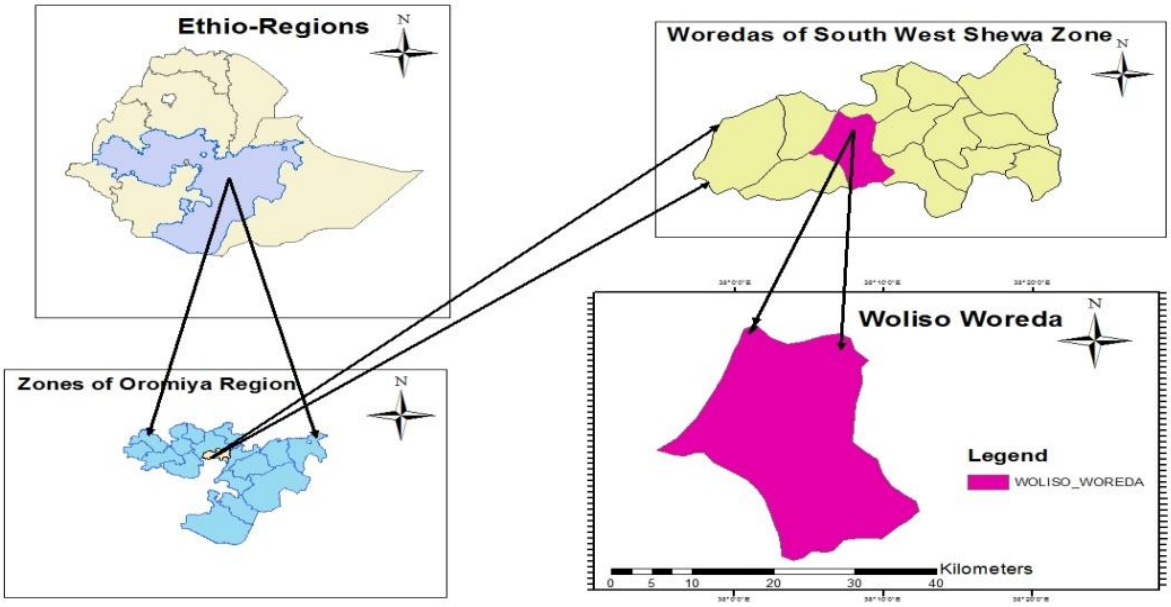


Figure 4: Map of the study Area

3.2. Study Population and Management

The study was conducted in chickens raised under backyard production system. Feeding systems in the backyard poultry production system was not purposeful and scavenging was almost the only source of diet. Different feeds were present for scavenging including seeds, plant materials, worms, insects and unidentified materials.

Housing management in the backyard poultry production system is rudimentary and mostly built with locally available materials, if any. The biosecurity of the backyard poultry production system was very poor, as scavenging birds live together with people and other species of livestock. Cleaning of chicken houses usually included manual removal of manure and bedding, which was subsequently used as fertilizer. Isolate sick birds from the household flocks and most of the time dead birds were left for either domestic or wild predators.

3.3. Study Design

A cross sectional study was implemented between November, 2018 and October, 2019 to asse ss seroprevalence of IBDV with its associated risk factors in backyard chickens production of selected kebeles. Semi structured questionnaire was used to collect information from selected household owners on managemental practices associated to the occurrence of IBD.

3.3.1. Sample size and sampling technique

From a total of 37 kebeles in the study district 6 kebelles, which include Dire duleti, Bedese k oricha, Fodu gora, Obi koji, Gurura Beka and Tombe Anchabi were selected purposively based on easy of accessibility and chickens population per household. The desired sample size for this study was calculated as described by Thrusfield (2005) with 95% confidence interval at 5% precision. The expected prevalence of infectious Bursal disease 89.78% reported was used for sample size determination (Hailu *et al.*, 2010).

The required sample size was calculated using the formula:

$$n = \frac{z^2 \, Pexp \, (1\text{-}Pexp)}{d^2}$$

Where, n = sample size; d = Desired absolute precision at 95% confidence interval = 5%; z =1.96; Expected prevalence =89.78%. Therefore, the required sample size was calculated as 141.The final sample size became [141x2] = 282 to increase accuracy of the outcome results.

The sample was collected from apparently health chickens from the age of 3 weeks up to 6 months. The selection of household and samples was proportionally allocated between selected kebeles of study area. Flock size in the study animals were range from 9-15 were only local breeds were included in the study animals. About 10-13 household per kebeles and 3-6 chickens per households were selected randomly for sampling.

3.3.2. Sample collection

Information data on potential risk factors for the occurrence of IBDV was collected during the time blood samples were collected. Semi-structured questionnaire was conducted on 70 selected households the first round of the questionnaire format. Housing system, kebeles, flock size, sex, age in weeks and hygienic level of house were emphasized as risk factors of the disease.

In the second phase of the questionnaire different aspects of the backyard poultry production system and owners characteristics such as educational Level, experience of keeping chicken (year), frequency of cleaning house, use of disinfectant, isolation practice of sick chickens, disposal of dead birds and source of replacements were considered to identify risk factors for the occurrence of IBDV infection in the study area.

Serum collected by plucking few feathers from the ventral surface of the humeral region of the wing and wiping the site with cotton damped with alcohol. About 1.5-3ml of blood samples were collected from brachial veins using 3g and 21 inch needle and syringes. The blood samples was allowed to clot overnight (24 hrs) in the syringe and then serum was separated, kept in ice box and transported to the Laboratory of NAHDIC Sebeta.

3.3.3. Serological test

Samples were tested using a commercial ELISA kit (ProFLOK® PLUS, IBD Coated ELISA, Symbiotic Corporation, San Diego, USA) at National Animal Health Diagnosis and Investigation Center (NAHDIC), Sebeta Ethiopia. This commercial ELISA kit specially detects IBD antibody and demonstrates excellent correlation with the virus neutralization (VN) test. All conditions were standardized according to the kit manufacturer and conditions described for poultry disease monitoring using ELISA. Briefly samples were tested for IBDV specific antibodies using a commercial IBDV ELISA kit (Proflok plus IBD, Sybiotic Corporation, Frotera San Giego) following manufacture's direction. Serum was prediluted to1:500 in dilution buffer, added to an antigen coated plate. Specific IBD antibodies in the serum form antigen -antibody complex with antigen bounded to the plate. After washing the plate, anti- chicken horse radish peroxidase conjugate is added to each well and the formed antigen- antibody bind to the conjugate. After incubation period unbounded conjugate is removed by washing and substrate which contains chromogenis added which form a clear to green blue color in the presence of enzyme, after incubation for 15 minute stop solution is added to terminate reaction and plate is read using ELISA reader at 450nm.Row absorbance data was transferred to a personal computer for further calculation and analysis.

ELISA test validation and Interpretation

After reading of the ELISA results, the test validity was checked for each plate based on two criteria set by the kit manufacturer; the mean optical density (OD) of the positive controls and normal controls on each plate. The test is considered valid of when the mean OD_{405} of the positive control value range between 0.250 and 0.900 and when the mean OD_{405} of the normal (negative) control serum is less than 0.250. The sample to positive (SP) ratio of each test serum was calculated as:

$$\textbf{SP} = \frac{\textbf{Sample absorbance – Average normal control}}{\textbf{Corrected positive control absorbance}}$$

Hence, SP value ≤ 0.299 is Negative while SP value > 0.299 is considered Positive

Antibody titters of test samples were calculated according to Snyder and Marquardt (1989) by

applying a linear equation (LOG_{10} TITER = (1.172* LOG_{10} SP) +3.614) generated by the kit manufacturer to define the relationship between LOG10 SP of a single serum dilution and the LOG10 of observed antibody titters. Hence, geometric mean titter calculation was according to Villegas and Purchase (1989) as LOG_{10} titter = (1.172* LOG_{10} SP) +3.614. So, Titter = $10^{log10\ titer}$ or (AntiLOG_{10}).

3.4. Data management and Analysis

Data obtained from questionnaire and laboratory test (Indirect ELISA) were inserted into Microsoft Excel for Windows 2007. Analyzes were performed using STATA software version 12.Chicken serum is positive for IBDV antibodies when the value is > 0.20 and negative when the value is ≤ 0.20. Descriptive statistical methods were used to summarize prevalence of IBDV and population characteristics of the study animals. Odd ratio (OR) was used to examine the strength of association between risk factors and outcome. Unavailable and Multivariable logistic regression was conducted to examine the association of the risk factors with occurrence of IBDV. A 95 % confidence intervals were calculated and P-value <0.05 was used for significance.

4. RESULTS

Out of 282 serum samples tested 224 were positive for Ab against IBDV antigen. In questionnaire survey 70 respondents were interviewed on management practice and owner characteristic in rearing village chickens in the study area. The sources of replacement of chicken are from market (57.14%) and their surrounding neighbors (42.86%). Concerning frequency of house cleaning, irregular and daily cleaning activities were equally practiced; whereas the use of disinfectants and Isolation of sick chicken and its importance were less understood in the study area (Table 1).

Table 1: Village chicken managemental practices in selected kebeles of the Waliso district.

Management practice	**Number (%) (n=70)**
Source of replacement	
Market	40 (57.14)
Neighbors	30 (42.86)
Housing System	
Cage	17(24.29)
Separate	21(30.00)
With family	32(45.71)
Frequency of cleaning house	
Irregular	20(28.57)
Every day	20(28.57)
Two days interval	8(11.43)
3-4 days interval	22(31.44)
Use of disinfectant	
Used	19(27.14)
Not used	51(72.86)
Isolation practice	
Practiced	48(68.57)
Not practiced	22(31.43)
Disposal of dead birds	
Buried	35(50)
Thrown on the field	35(50)

Table 2: Seroprevalence of IBD associated with village chicken household characteristics and chicken keeping.

Factors	Number of respondents	Number of Positive flock (%)	P-value
Owner age			
<25	31	23(74.19)	0.655
25-30	19	15(78.95)	
>30	20	17(85.00)	
Education level			
Illiterate	17	15(88.24)	0.403
Grade 1-4	33	26 (78.79)	
Grade 5-9	20	14(70)	
Experience of keeping chicken (year)			
≤ 1	16	15(93.75)	0.030
2	15	14(93.33)	
3	17	12(70.59)	
≥ 4	22	14(63.64)	

Majority of village chicken households are younger and has education level of grade 1 to 4. Experience of keeping chicken was associated to seroprevalence of IBDV ($p<0.05$).In experiences of rearing chickens have inverse relationships with seroprevalence of IBDV ($p<0.05$) as prevalence decreases with increasing experiences of owners with (table 2).

The factors effects the chicken rearing like experience of keeping chicken (year) has statistical significant effect on IBDV seroprevalence ($P< 0.05$). The persons rearing backyard chickens was indicated in illiterate group was significantly higher seroprevalence of IBDV (88.24%) than those educated persons rearing backyard chickens or grade 5-9 (70) with $p= 0.0403$ and experience of keeping chicken ≥ 4 years was significantly Lower seroprevalence of IBDV (63.64%) than those $\leq$ 1 year experience (93.75%) with $p=0.030$ (table 2).

The effect of difference in managements like use of disinfectant ($p<0.01$) and disposal of dead birds has a statistically significant effect on IBDV seroprevalence ($p<0.05$). Properly disposal of dead carcass (Buried) (65.71%) indicated lower prevalence of IBDV than that group practicing to thrown dead carcass on the field (91.43%) with $p<0.05$ (Table 3).

Table 3: Seroprevalence of IBDV associated with biosecurity and management systems

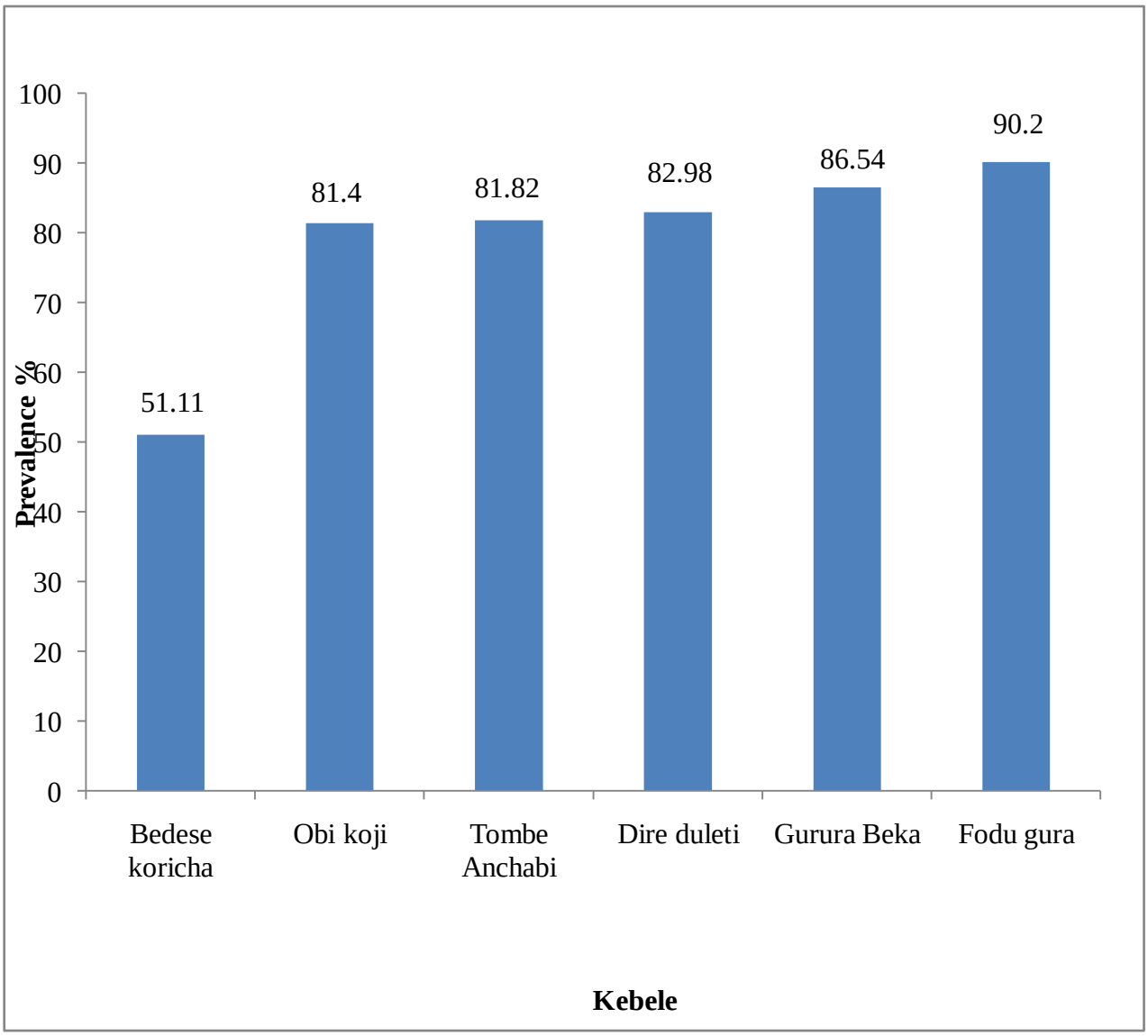
Factors	**Number of respondents**	**Number /percent/ of positive flock**	**P-value**
Source of replacement			
Market	40	32(80)	0.737
Neighbors	30	23(76.67)	
Frequency of cleaning house			
Irregular	20	16(80)	0.915
Every day	20	15(75)	
Two days interval	8	7(87.5)	
3-4 days interval	22	17(77.27)	
Use of disinfectant (detergents)			
Used	19	12(63.16)	0.000
Not used	51	43(84.31)	
Isolation practice (sick chicken)			
Practiced	48	35(72.92)	0.089
Not practiced	22	20(90.91)	
Disposal of dead birds			
Buried	35	23(65.71)	0.009
Thrown on the field	35	32(91.43)	

From a total of 282 serum samples examined 224 were positive to IBDV using indirect ELISA test with an overall seroprevalence of 79.43% in the study area. The present study showed that sex of study animals have not effect on the prevalence of IBDV ($p>0.05$). The prevalence of IBD virus significantly ($p<0.01$) decreases with increasing age of chicken as shown in (table 4).

Table 4: Seroprevalence of IBD associated with Age and Sex in the study area.

Factors	**Number of sample**	**Number of Positive (%)**	**Uni-variable**		
			OR	**P-value**	**(95% CI)**
Sex					
Female	156	128(82.05)	0.70	0.227	(0.28-1.45)
Male	126	96(76.19)			
Total	**282**	**224 (79.43)**			
Age/week					
≤4	207	173(83.57)	0.95	0.001	(0.299- 1.003)
>4	75	51(68)			
Total	**282**	**224 (79.43)**			

A slightly higher prevalence was recorded in female chickens (82.05%) than males (76.19%); however, there was no statistically significant difference was recorded between sexes ($p>0.05$). The highest (83.57%) and the lowest (69.98) prevalence of IBD was found in age groups of less than or equal to 4 weeks and greater than 4 weeks respectively. The difference in the prevalence of IBD among different age group were statistically significant ($p<0.05$) as shown in Table 4.



P=0.001

Figure 5: Prevalence of IBD in selected kebeles of Waliso District.

On the other hand the highest seroprevalence of IBD was found at Fodu gora (90.2 %) and the lowest was recorded at Bedese Qoricha (51.11%). The difference in the seroprevalence of IBD among kebeles was statistically significant ($p<0.001$) as shown in figure 5.

Table 5: Sero-prevalence of IBD associated with health Multivariable logistic regression.

Factors	Number of sample	Number of Positive (%)	Multivariable logistic regression.			
			OR	SE	P-Value	95% CI
Hygienic level of house						
Good	61	14(22.95)				
Fair	107	94(87.85)	29.3	12.91	0.000	12.36-69.47
Poor	114	112(98.25)	188	145.82	0.000	41.11-859.8
Housing system						
Cage	75	67(89.33)	0.801	0.365	0.626	0.33-1.96
With family	131	114(87.02)	0.156	0.068	0.000	0.07-0.37
Separate (traditional house)	76	43(56.58)				
Flock size						
≤ 9	76	50(65.76)				
10-14	123	103(83.74)	2.68	0.920	0.004	1.36-5.25
>14	83	71(85.54)	3.08	1.214	0.004	1.42- 6.67

Hygienic condition was significantly associated with the occurrence of IBD when Multivariable logistic regression analysis was carried out (Table 5). Hygienic condition the environment was observed highest seroprevalence in flocks maintained poor hygienic conditions (98.25%) than those maintained under fair (87.85%) and good hygienic condition (22.95%).

Housing system was associated with the occurrence of IBD when Multivariable logistic regres sion analysis was carried out. Housing system indicated a difference in seroprevalence of IBDV variety could be due to risk factors such as, Cage system was highest 89.33% and Lowest was separate housing system 56.58%.Household flock size had significant effect on the seroprevalence of IBD in the study area. Flocks of chicken with size of≥14 chickens per flock had an odd of having IBD higher seropositivity than flocks with size less than or equal

to 9 chickens per flock. This difference was statistically significant ($p<0.05$). Flocks of chicken with size greater than or equal to 14 animals per flock had an odd of having IBD higher than that of those with flock size 10-14 this difference was statistically significant ($p<0.05$).

5. DISCUSSION

The present study was conducted on backyard chickens in Waliso District of 6 Kebelles in apparently health chicken, which indicates the presence of field exposure of household chickens to the virus. According to this study a questionnaire survey on the occurrence of IBDV was assessed via farmers' interview and serum samples also collected and laboratory analysis was made for seroprevalence determination.

Majority of the respondents 87 % in Waliso district responded that chicken are kept in the same house with family. Around 56% of respondents constructed a separate traditional chicken house, which is higher than those reported by Moges *et al*. (2010) and Mengesha *et al.*(2011) 22.1% and 21.2% village chicken owners provided separate traditional chicken house in Bure district and South Wollo, respectively.In the current study most of the respondents 75 % Reported that they clean their house /shelter every day. This results was lower than the survey undertaken by Gezali, (2017) in district of Jimma Zone who reported (76.6%) and Halima, (2007) in Northern Ethiopia reported that (74.02%) of the households cleaned their chickens house once a day respectively. About 28.57% of the owners cleaned it irregular while 11.43% of the households clean their chicken house every two days interval in the present study area. The variation between the study areas might be due to farmers "awareness" to the importance of poultry housing and frequency of cleaning in respect to health management and production improvement (Animal health Australia, 2009).

Serological study was conducted to evaluate the prevalence of IBDV in apparently health chickens. The study indicated that an overall seroprevalence of 79.43% of IBDV in chickens kept under backyard poultry production system in the study areas. This report is comparable with findings of Hailu *et al*. (2010) who reported 76.64% seroprevalence from three districts of West and South West Shoa, 72.7% in Gonder (kassa and Molla, 2012), Tesfaheywet *et al.*(2012) reports of 82.2% from Central Ethiopia. The overall seroprevalence in the current study finding was lower than the serological studies conducted in the different parts of the country 100% in DebreZeit (Woldemariam and Wossene, 2007), 93.3% in DebreZeit (Zeleke *et al.,* 2005b) and 90.3% in Mekele (Shiferaw *et al.,* 2012). However, lower seroprevalence of 38.39% was reported by Sinidu *et al.* (2015) in Bahirdar and 38.3% by Asamenew *et al.* (2016) in Sebeta hawas Ethiopia. The variation in prevalence of IBDV in

these studies attributed to the difference in poultry management systems in backyard poultry production such as poor management practice, poor sanitary condition, nutritional deficiencies or frequent contact of wild birds.

In this study relatively higher prevalence of IBD was recorded in female chickens (82.05%) than male (76.19%). This finding was agreed with the report of Sinidu *et al.* (2015) in Bahirdar. The lower seroprevalence of IBDV was recorded in male chickens with by Shiferaw *et al*. (2012) and Tadesse and Jenbere (2014) in different parts of Ethiopia. However, the difference was not statistically significant (P>0.05) between male and female chickens.

The seroprevalence of IBD was found high (83.46%) in age group ≤4 weeks, than >4 age groups. A comparable result of seroprevalence 86.6% and 87.26% of IBDV in young chicken was reported by Shiferaw *et al*. (2012) and Hailu *et al*. (2010) respectively. This is due to the fact that, at early age the virus impairs the immune response and renders the chick susceptible to various infections and different genetic backgrounds of chicken breeds also may have different impacts on the early immune responses to IBDV infection (Aricibasi *et al*,.2010;Tippenhauer *et al*.,2013).

A statistical significant difference in prevalence of IBD was observed in different flock size of the present study. The highest seroprevalence of IBDV (85.54%) were found in largest flock size ≥14 than the lowest flock size ≤ 9 with (65.76%). This might be due to the fact that increased chicken population number is a factor for stress, transmission and widely occurring of the diseases (Farooq *et al*,. 2003).

Among A statistical significant difference was found in the prevalence of IBD in study sites . The highest prevalence of IBDV 90.2% and the lowest 51.11% were recorded in Fodu gora and Bedesa Qoricha kebele, respectively. Similar findings were reported by Hailu *et al*. (2010) in backyard Local chicken of the three district of Oromia region and Hailu *et al*. (2010) in village chicken of different district of the Amhara national regional state of Ethiopia. The higher prevalence of IBD generally attributed to the poor chicken management systems exercised in back yard poultry production such as poor management practice, poor sanitary condition, nutritional deficiencies, frequent contact with wild birds and the flourishing commercial poultry farms in the area (Asamenew *et al.* 2016).

Experience in village chicken has an importance in controlling chicken health management and to easily understand problems that occurring in the flocks of chicken. In this study the level of IBDV infection is decreasing with increasing experiences of owners. This implies that owner might be able for early identification of the problems, take measures for its control and provide good management to prevent the disease as become more experienced. Hygienic condition of the environment has a significant effect ($p < 0.05$) on prevalence of IBDV. Higher prevalence was observed in flocks maintained under poor hygienic conditions (98.25%) than good hygienic condition (22.95%). The lower prevalence in good hygienic conditions could probably be due to the favorable and healthy environmental condition. This might be due to the various predisposing factors such as improper cleaning, keeping used litter, poor ventilation and crowding as these factors influence spread of the infection from house to house and from flock to flock (Animal health Australia, 2009).

Higher prevalence of 84.31% of IBDV occurred in flocks that don't use cleaning material (detergent) than those using cleaning material (detergent) 63.16% in chicken house cleaning activities. Hailu *et al*. (2010) reported the status of hygienic condition of the chicken shed highly associated with the occurrence of IBD as they observed the importance of some management practice such as fumigation of the shed with formalin and potassium permanganate were essentials to prevent IBDV infection.

Even different housing system (Cage (traditional) and separate (transitional) and house shared with family) used in the study area has no significant effect on the prevalence of IBDV. However, they significantly differ from a traditional house which shared with family. This might be due to poor handling practice and bio-security of the backyard poultry production system as a whole (Asamenew *et al.,* 2016).

A significant variation in practicing disposal of dead carcass was reported in the present study. A 91.43% prevalence of IBDV occurred in those groups thrown dead carcass on the field and 65.71% in flocks where owners dispose dead carcass and waste products by burning or buried it. This can associated with frequent movement of backyard chicken and constant contact with infected environments.

6. CONCLUSSION AND RECOMMENDATIONS

In this study higher seroprevalence of IBDV was indicated using indirect ELISA test. The magnitude of Sero-positivity of IBDV in backyard chicken was influenced by different factors. Such as flock size, chicken keeping experience, chicken age, chicken housing system living with family and condition in poor and fair housing managemental practices have a significant effect on prevalence of the disease. The current study revealed that the seroprevalence of IBD among the study sites was higher in large flock size, < 4 years chicken keeping experience, chicken aged with < 4 weeks and poor hygienic managements. Infectious Bursal disease prevalence was not affected by chicken house cleaning, house hold ,educational level, the age of rearing chicken, sick chicken (Isolation practice), sex and traditional housing in cage. Experiences of rearing chickens improve ability of owners to provide proper management that lowers the prevalence of IBDV. A lower Sero-prevalence of IBDV was obtained in good hygienic level of houses than fair and poor level of chicken house hygiene. Based on the above conclusion, the following recommendations were forwarded;

- Attention should be given in improvement of village chicken production and managem ent practices such as disease monitory program, for appropriate prevention and control measure.
- .Community training should be given on good management of chickens including hygienic conditions of houses, flock sizes and importance of vaccination.
- Further studies is warranted for better understanding and characterize virus strains circulating in the study area in order to properly aid control of IBDV and to estimate their impact on the backyard poultry production system.

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APPENDIXES

Date _____

Appendixes 1: Semi-structured Questionnaires for data collection on awareness of farm owners about IBD of chicken in Waliso district, South-East Shoa, Oromia, Ethiopia

Owner name_____ sex_____Age_____

kebele_____ Address _____Education Level_____

1. How long you experienced in chicken rearing?
2. Do you encounter health problem in your chicken/Farm? Yes/No
3. Is IBD occurring in your farm? Yes/ No can you tell me the Signs and frequency of its occurrence per year? _____
4. Which age group is more affected by this disease (IBD)? <2weeks, 2-4weeks, 1-2 months, 2-4 months, 4-6months, 6months- 1year
5. Sex group commonly affected? Male/Female
6. Which breed will be affected ?Local or Cross chicken
7. What is your source of replacement flock? Market/neighbors/commercial farm/
8. What do you expect as source of IBD in your farm? Chicken return from market / neighbors/air or soil/don't know
9. In which season of the year IBD is commonly occur? Autumn/winter/spring/summer
10. Frequency of chicken house cleaning? Once per day/ two days/ three days/week/irregularl y
11. Do you use disinfectants? Yes /No, If used what are common disinfectant used_____
12. Disposal of dead carcass? Buried/burned/throw on the field
13. Vaccination of chicken in previous 1 year vaccinated /not vaccinated
14. If vaccinated type of vaccine given _____ length of time_____

Format for data collection during sample collection from individual chicken

S.no.	Owner name	Kebele	Sex and Age of owner	Flock size	Sex(Male/Fem.	Age in weeks	Hygienic cond. (Good, Mediurn. and poor	Housing syste m (Separate or Living with human)
1								
2								
3								
4								
5								
6								
7								
8								
9								
10								
11								
12								
13								
14								

Appendixes 2: Test Procedure of Infectious Bursal Disease

All reagents must be allowed to come to 18-26% ℃ before use. Reagents should be mixed by gentle inverting or swirling.

1. Obtain antigen –coated plate (s) and record the sample position.
2. Dispense 100µL of UNDILUTED Negative control (NC) into duplicate wells.
3. Dispense 100µL of UNDILUTED Positive control (PC) into duplicate wells.
4. Dispense 100µL of DILUTED sample into appropriate wells. Samples may be tested in duplicate, but a single well is acceptable.
5. Incubate for 30 minutes (±2 minutes) at 18-26%℃.
6. Remove the solution and wash each well with approximately 350µL of distilled or deionized water 3-5 times. Avoid plate drying between plate washings and prior to the addition of the next reagent. Tap each plate onto absorbent material after the final wash to remove any residual wash fluid.
7. Dispense 100µL of Conjugate into each well.
8. Incubate for 30 minutes (±2 minutes) at 18-26%℃.
9. Repeat step 6.
10. Dispense 100µL of TMB substrate into each well.
11. Incubate for 15 minutes (±1 minute) at 18-26%℃.
12. Dispense 100µL of Stop solution into each well.
13. Measure and record absorbance values at 650nm, A(650)
14. Calculation :- Controls $NCX = \frac{NC1\ A(650 + NC2A(650)}{2}$ $PCX = \frac{PC1\ A(650 + PC2A(650)}{2}$

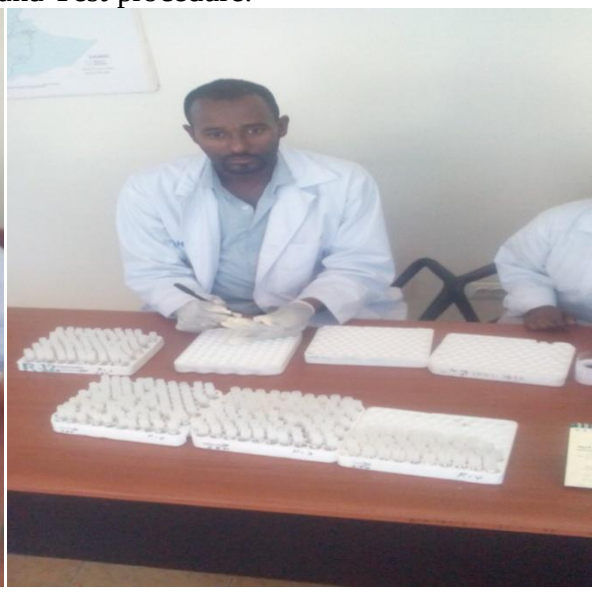
    Validity criteria.

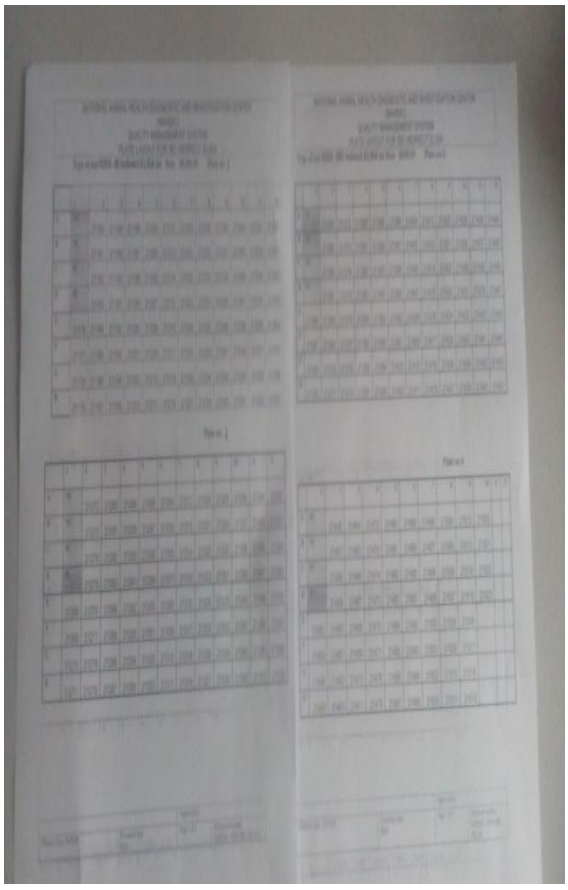
    PCX-NCX > 0.075

    NCX≤ 0.150
15. Interpretation $S/P = \frac{\text{sample mean} - \text{NCX}}{\text{PCX} - \text{NCX}}$

Hence, SP value ≤ 0.20 is Negative while SP value > 0.20 is considered Positive

Appendixes 3: Serum Collection submission and Test procedure.



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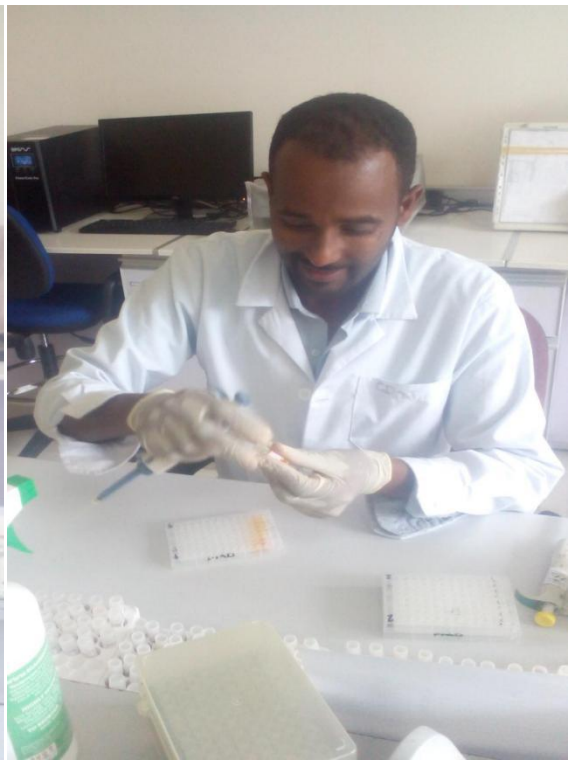


IBD
20/05/19
Serum
IBD
1:25
IBD
1/20

ELISA Reader