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PATHOLOGICAL AND SEROPREVALENCE STUDIES ON INFECTIOUS BURSAL DISEASE IN CHICKENS IN AND AROUND BAHIR DAR, NORTH WEST, ETHIOPIA.

MSc Thesis

By

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June, 2015

Bishoftu, Ethiopia

PATHOLOGICAL AND SEROPREVALENCE STUDIES ON INFECTIOUS BURSAL DISEASE IN CHICKENS IN AND AROUND BAHIR DAR, NORTH WEST, ETHIOPIA

A Thesis submitted to the College of Veterinary Medicine and Agriculture of Addis Ababa University in partial fulfillment of the requirements for the degree of Master of Science in Tropical veterinary Pathology

By

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June, 2015

Bishoftu, Ethiopia

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As members of the Examining Board of the final MSc open defense, we certify that we have read and evaluated the thesis prepared by: **Natnael Teshager** entitled**: Pathological and Seroprevalence Studies on Infectious Bursal Disease in Chickens in and Around Bahir Dar, North West, Ethiopia** and recommend that it be accepted as fulfilling the thesis requirement for the degree of: Masters of Tropical veterinary pathology

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DEDICATION

This MSc thesis work is dedicated to memory of my absent father “Teshager Desta “whom I lost at grade 8. He nurtures me with lifelong hopes up to he was alived on: That help me to struggle even in uncomfortable circumstance of life to maintain self steem.

STATEMENT OF AUTHOR

I declare that the thesis hereby submitted for the MSc degree at the Addis Ababa University, College of Veterinary Medicine and Agriculture is my own work and has not been previously submitted to any other University or institution for the award of any degree. I concede copyright of the thesis in favor of the Addis Ababa University, Collage of Veterinary Medicine and Agriculture.

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LIST OF ABBREVIATIONS

BoAAO	Bureau of the Amhara Agriculture Office
CSA	Central Statistics Agency
IBD	Infectious bursal disease
IBDV	Infectious bursal virus
BB	Bovans brown
BW	Bovans white
LB	Local breed
HB	Hybrid breeds

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ABSTRACTS

A cross sectional study was conducted from January 2015 to May 2015 to characterize the pathological changes and to determine seroprevalence of IBD and its associated risk factors in chickens in and around Bahir Dar. The clinical signs observed in IBD infected chickens were whitish diarrhea mixed with blood, ruffled feather, and massive death within short period. The necropsy findings were petichial hemorrhage in bursa of fabricius, kidney, thymus, spleen, thigh and pectoral muscles. Bursa of fabricius became edematous in serosa and mucosal part, whitish-creamy and atrophied as the course of the disease progressed. Kidney became pale and ureter was turgid with urate in a numbers of cases. The histopathological changes in this study revealed that edema of bursa of fabricius, formation of cystic follicles, depletion of lymphocytes, fibrosis and follicular architecture lost. Severe, moderate and mild lesion score were observed in bursa of fabricius. Infiltrations of heterophils were noted both in lymphoid and non lymphoid tissues. Depletion of lymphoid cell in germinal center of spleen and total depletion of lymphoid cells were encountered in thymus gland. The kidney tubules were filled by exudates and necrotized cuboidal cells. Sera collected from a total of 320 chickens were subjected to IELISA test and disclosed an overall prevalence of 51.56% (CI: 45.95 – 57.14) in study area. The seroprevalence of IBD among chickens showed a statistically significant difference ($P<0.05$) among study sites namely Andassa (72.73%), Gombat (50%), Wonjeta (47.69%), Meshenty (44.44%) and Bahir Dar (42.42%). The susceptibility of chickens to IBDV revealed a statistically significant difference ($P<0.05$) among different breeds where Koekoek was found more prone to IBD infection with seroprevalence of 67.11% followed by Bovans brown (57.69%), local breeds (48.31%), and Bovans white (40.28%). The seroprevalence of IBD was also significantly associated ($P<0.05$) with the age of chickens and among farm systems. These studies clearly indicated that IBD infection is a common and wide spread problem affecting a number of chicken breeds under different management systems and hence an urgent control intervention should be in place.

Keywords: *Age, Bahir Dar, Breed, Chickens, Histopathology, Lesions, Seroprevalence, sex*

1. INTRODUCTION

Ethiopia has the largest livestock population in Africa, including more than 53.99 million cattle, 49.5 million small ruminants, approximately 0.92 million camels, 9 million equines and 50.38 million chickens. In Ethiopia about 96.9% of the chicken population consists of indigenous chickens, while the remaining 2.56% and 0 .54% consists of exotic and hybrid breeds (CSA, 2013). This indigenous poultry production in Ethiopia contributed to 98.5 and 99.2% of the national egg and poultry meat production, respectively (Alemu and Taddele, 1997). This flock Provide with yearly output of 72,300 metric tons of meat and 78,000 metric tons of eggs (Hailemariam *et al*., 2006). Chickens are wide spread in the Ethiopia and are important to subsistence, economic and social livelihoods of a large human population. 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.

There has been a gradual decline in the Ethiopian poultry population. According to Burley (1957) and the Central Statistical Authority (2004-2005), the Ethiopian poultry population was estimated at 85 and 31 million in 1954 and in 2005 respectively. The Sub-Sector Review (1984) estimated the average number of chickens per household at 6.5 in 1984 whereas the average number of chickens per household is estimated at 4.1 in 2003 (CSA, 2013). These figures show that the country's poultry population has turned down by 64% in the last 50 years, while the average number of chickens per farmer has reduced by 37% over the last 20 years. This problem attracts the attention of researchers in Ethiopia to improve health management and breeding aspect of chickens (Mazengia, 2012).

Despite, Ethiopia owned huge chicken flock; there are different constraints like poor nutrition, poor management and prevalent diseases that hinder the productivity of the chickens in most area of the country (Alemu, 1995; Dessie and Ogle, 1996). Among the above obstacles, the poultry diseases are the main constraints incriminated for reduction of total numbers and compromised productivity (Tadesse, 2000; Ashenafi, 2000).

According to the work done by (Tadesse, 2000; Ashenafi, 2000) some farmers have stopped rearing chickens due to disease problems. Infectious bursal disease, Newcastle, Coccidiosis, Salmonellosis and nutritional deficiency have been considered the major diseases inflicting heavy losses in Ethiopia. Beside the above problem, imported breeds and cross-breeds are multiplied in Ethiopia poultry hatchery center and day old chickens are imported from abroad. This day old chicken distributed to farmers by the Bureau of Agriculture to be maintained and produced under the backyard management system. This is aim to advance the livelihood and nutrition of poor farmers and in addition to increase the country economy (Tadelle and Ogle, 2001). Accordingly, the Bureau of the Amhara Agriculture Office schemed poultry production strategy that started in 2003.The goal of the strategy was to enable farmers to generate income through rearing day-old chickens of breeds of Bovans brown, Bovans white and Koekoek breeds which are hatched and distributed from poultry multiplication centers of Andassa, Kombolcha and Ethio - chickens. However, it is becoming a growing issue with introduction of diseases of various etiologies into chickens of backyard and intensive poultry farms. The distribution of these none indigenous breeds to farmers is creating a great treat to the indigenous backyard chickens (Alamargot, 1987; Zeleke *et al*., 2005). Among these distributed day old chickens to small holder farmers, about 31% of them died within 3-6 weeks and 2% of total died within 7 weeks of age (BOAAO, 2013).

Infectious bursal disease is among those diseases introduced with exotic breed that result in death in young chickens and reduction of egg in pullet that impose great economical loss in productivity of chickens in Ethiopia (Taddele and Ogle, 2001; Zeleke *et al*., 2005). Infectious Bursa Disease (IBD) is an acute and highly contagious viral disease of growing chickens. The virus infect primarily lymphoid cells, especially B-cells in which, Lymphoid tissues of the bursa fabricius are severely affected (Chou and Calnek, 1997). There are two serotypes of IBDV (serotypes 1 and 2). Strains of serotype 1 IBDV are pathogenic only in chickens, and are further classified as classical virulent IBDV (cvIBDV), vvIBDV, antigenic variant IBDV (avIBDV) and attenuated IBDV (atIBDV) (van den Berg *et al*., 2004).

In the world, the poultry industry has encountered heavy economic losses associated with very virulent (vv) IBDV strains in the last several years. These strains may cause high mortality in affected chicken flock and severe immunosuppression that involves both innate and adaptive immune responses (Negash, 2013).

Although prevalence and incidence of infectious bursal disease (IBD) have been reported in major field outbreaks of intensive farm and backyards in northwest and central Ethiopia in recent years (Zeleke *et al*., 2005; Mazengia *et al.,* 2009; Zeryehun and Fekadu, 2012), the pathological changes and susceptibility variation across breed, age, sex and location of IBD remain untouched and with its scanty current information on seroprevalence in backyard and intensive chickens farms in and around Bahir Dar. The present study was designed and proposed to undertake an investigation on the occurrence of IBD in and around Bahir Dar area and further characterize the involved pathological changes.

Thus, the objectives of this study include characterization of the major clinical signs, appreciation of the gross and histopathological changes in different tissues of naturally infected chickens as well as determination of the seroprevalence of IBD virus among chickens in different farm systems. Moreover, the association of IBD with different risk factors was assessed.

2. LITERATURE REVIEW

2.1. Infectious bursal disease

Infectious bursal disease (Gumboro disease) is infectious disease of global economic importance (Pitcovski, 2003). Infectious bursal disease virus (IBDV) is cause of IBD, currently responsible for huge economic impact on the worldwide poultry industry (Vandersluis, 1999). 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), whereas serotype 2 strains are non-pathogenic (Meferran *et al*., 1980). Serotype 1 IBDV isolates comprise the variant, classical virulent and vvIBDV strains, which greatly differ in their pathogenecity in chickens. Variant IBDVs do not cause mortality, whereas the classical strains cause up to 20% mortality (Muller *et al*., 2003). VvIBDV causes mortality exceeding 50% in susceptible chickens (Chettle *et al*. 1989; Berg *et al*., 1991; Muller *et al*., 2003).

Currently, this disease reported throughout the globe and an economical important disease causing 100% morbidity. Mortality reaches up to 90% in susceptible flock. The loss is attributing to high mortality, immunosuppression and condemnation of carcasses (Kibeng, 1988). The clinical disease of IBD, first described by Cosgrove in (1962), affects chicks between 3 and 6 weeks of age. It is characterized by ruffled feathers, whitish or watery diarrhea, anorexia, depression, trembling, severe prostration and finally death. The target organ of the virus is the lymphoid tissue, specially the bursa of Fabricius that has a gelatinous yellowish transudate covering the serosal surface. Longitudinal striations on the surface become prominent, and the normal white color turns to cream. The transudate disappears as the bursa returns to its normal size and becomes gray during the following period of atrophy. The infected bursa often shows necrotic foci and at times ecchymotic hemorrhages on the mucosal surface. Occasionally, extensive hemorrhage throughout the entire bursa has been observed (Sellers *et al*., 1999).

2.2.Etiology

Infectious bursal disease virus (IBDV) is classified as a member of the *Birnaviridae* family. 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 (Viruses in this family possess bi-segmented, double-stranded RNA (dsRNA) genomes, which are packaged into single shelled, non-enveloped virions. The capsid shell exhibits icosahedral symmetry composed of 32 capsomeres and a diameter ranging from 55 to 65 nm (Brown *et al*., 1994). 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), whereas serotype 2 strains are non-pathogenic (Meferran *et al*. 1980). 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). VvIBDV causes mortality exceeding 50% in susceptible chickens (Chettle *et al*. 1989; Berg *et al*., 1991; Muller *et al*., 2003).

Infectious bursal disease virus is highly resistant to adverse environmental conditions. It is more resistant to heat and ultraviolet light than reovirus (Petek *et al*., 1973) and is resistant to ether and chloroform. Once infected with IBDV, chickens are capable of shedding the virus in feces for as long as 16 days (Winterfield *et al*., 1972). Benton *et al*., (1967) reported that poultry houses which previously harbored infected flocks remained infective for at least 122 days and that fomites (water, feed, droppings) contaminated with IBDV contribute to viral dissemination (Benton *et al*., 1967). 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

Cosgrove (1962) reported a specific disease, (IBD) that affecting the bursa of Fabricius in chickens .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., 1997), and become devastating disease in Europe in the years of 1962 to 1971 (Faragher, 1972). With its pandemic movement from the year 1966 to 1974, the disease was reported in the southern and western Africa, Far East, Middle East, India and Australia (Faragher, 1972; Provost *et al*., 1972; Firth, 1974; Jones, 1986; Lasher and Shane, 1994; Van den Berg, 2000; Van der Sluis, 1999). Infectious bursal disease currently become an international issue, 95 % of the 65 countries that responded to a survey conducted by the (OIE, 1995) announced presence of infection (Eterradossi, 1995), including New Zealand which had been free of disease until 1993 (Jones, 1986).

Only chickens develop IBD after infection by serotype 1 viruses. Turkeys may be asymptomatic carriers of serotype 2 (McFerran *et al*., 1980; Jackwood *et al*, 1982; Ismail *et al*., 1988), and at times, of serotype 1 viruses whose pathogenicity for turkeys is ill-defined (Reddy and Silim, 1991;Owoade and Durojaiye, 1995). Anti-IBDV antibodies have been detected in guinea-fowl, common pheasants and ostriches, which have also been found to carry, serotype 2 viruses (Guittet *et al*., 1992). Neutralizing or precipitating antibodies have been detected, *inter alia,* in various species of wild duck, goose, tern, puffin, crow and penguin, which may mean that wild birds act as reservoirs or vectors (Gardner *et al.,* 1997; Ogawa *et al*., 1998; Wilcox *et al.,* 1983). The age of maximum susceptibility to IBDV is between 3 and 6 weeks, which is the period of maximum bursa development, during which the acute clinical signs are observed. Infections occurring before the age of three weeks are generally subclinical and immunosuppressive. Clinical cases may be observed up to the age of fifteen to twenty weeks (Ley *et al*., 1979; Okoye and Uzoukwu, 1981). Light strains of laying stock are more susceptible to disease than the heavy broiler strains (Van den Berg and Meulemans, 1991; Bumstead *et al*., 1993; Hassan and Saif, 1996).

The IBD transmit with horizontal way only, with healthy subjects being infected by the oral or respiratory pathway. Infected subjects excrete the virus in faces as early as 48 hours after infection, and may transmit the disease by contact over a sixteen-day period (Vindevogel *et al*., 1976). The possibility of persistent infection in recovered animals has not been researched. The disease is transmitted by direct contact with excreting subjects, or by indirect contact with any inanimate or animate contaminated vectors. Some researchers have suggested that insects may also act as vectors (Howie and Thorsen, 1981). The extreme resistance of the virus to the outside environment enhances the potential for indirect transmission. The virus can survive for four months in contaminated bedding and premises (Benton *et al*., 1967) , and up to fifty-six days in lesser mealworms taken from a contaminated building (Lucio and Hitchner,1980). In the absence of effective cleaning, disinfection and insect control, the resistance of the virus leads to perennial contamination of infected farm buildings.

2.3.1. Status of IBD in Ethiopia

Table 1: Reported prevalence of IBD in Ethiopia

Study area	Prevalence	Authors
Central Ethiopia	82.2%	Zeryehun and Fekadu, 2012
Selected sites of Ethiopia	83.1%	Jebberie *et al*., 2012
Gondar and west Gojjam	73.5%	Kassa and Molla, 2012
Bahir Dar	29.4%	Mazengia *et al*., 2010
Farta	21.7%	Mazengia *et al*., 2010
Andassa poultry farm	100%	Woldemariam and Wossene, 2007
Debre Zeit	93.3%	Zeleke *et al.*, 2005

2.3.2. Morbidity and mortality

Infectious bursal disease is extremely contagious and in infected flocks, morbidity is high, with up to 100 % serological conversion, after infection, whilst mortality is variable. Until 1987, the field strains isolated was of low virulence and caused only 1% to 2 % of specific mortality. However, since 1987 an increase in specific mortality has been reported in different parts of the world. In the USA, new strains responsible for up to 5% of specific mortality were described (Rosenberger and Cloud, 1986). At the same time, 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. These hypervirulent field strains caused up to 100 % mortality in specific pathogen free (SPF) chickens (Van den Berg *et al*., 1991; Nunoya *et al.*, 1992).

2.4. Clinical signs

The incubation period is very short which range from 2 to 3 days. In acute cases, the chickens tired, prostrated, dehydrated, suffer from watery diarrhea, and feathers are ruffled. 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. Disease severity depends on the age and breed sensitivity of the infected birds, the virulence of the strain, and the degree of passive immunity. Initial infection on a given farm is generally very acute, with very high mortality rates if a very virulent strain is involved. If the virus persists on the farm and is transmitted to successive flocks, the clinical forms of the disease appear earlier and are gradually replaced by subclinical forms. Nonetheless, acute episodes may still occur. Moreover, a primary infection may also be in apparent when the viral strain is of low pathogenicity or if maternal antibodies are present (Faragher, 1972).

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; 1*)* the 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 or GLS strains, which partially resist neutralization by antibodies against the 'classical' viruses (Jackwood and Saif, 1987; Snyder, 1990). The acute form, first described in Europe, Africa and then in Asia, is caused by 'hypervirulent' strains of IBDV, and is characterized by an acute progressive clinical disease, leading to high mortality rates on affected farms (Chettle et al., 1989; Stuart, 1989; Van den Berg et al., 1991).

2.5.Pathology and lesions

Even although IBD affected different lymphoid organs (Sharma *et al*., 1993; Tanimura *et al*., 1995; Tanimura and Sharma., 1997), the principal target of the virus is the bursa of Fabricius (Kaufer and Weiss, 1980), which is the reservoir of B lymphocytes in birds. Indeed, the target cell is the B lymphocyte in active division, for which the infection is cytolytic (Burkhardt and Müller, 1987). Cell sorting studies have demonstrated that the B lymphocyte is susceptible in the immature stage, during which immunoglobulin M is carried on the surface of the lymphocyte (Hirai et al, 1981; Nakai and Hirai, 1981). This accounts for the paradoxical immune response to IBDV, in which immunosuppression co-exists with high anti-IBDV antibody titres. The mature and competent lymphocytes will expand as a result of stimulation by the virus whereas the immature lymphocytes will be destroyed.

A kinetic study using immunofluorescence (Mülle *et al*., 1979) has shown that, 4 hours after oral inoculation, the virus is found in the lymphoid tissues associated with the digestive tract, where the first cycle of viral replication occurs. The virus subsequently enters the general circulation via the hepatic portal vein. A phase of primary viraemia ensues, during which the virus reaches the bursa, 11 hours after infection, and a major

secondary replication cycle occurs. A phase of secondary viraemia then occurs, and the other lymphoid organs become massively infected.

Macroscopic lesions are observed principally in the bursa which presents all stages of inflammation following acute infection (Vindevogel *et al,* 1974; McFerran, 1993). Autopsies performed on birds that died during the acute phase (three to four days following infection) reveal hypertrophic, hyperemic and oedematous bursas. The most severe cases are characterized by a major infection of the mucous membrane and a serous transudate, giving the bursal surface a yellowish colour. This appearance is often accompanied by petechiae and haemorrhages. By the fifth day, the bursa reverts to normal size and by the eighth day becomes atrophied to less than a third of the normal size. The affected animals are severely dehydrated, and many birds have hypertrophic and whitish kidneys containing deposits of urate crystals and cell debris. Haemorrhages in the pectoral muscles and thighs are frequently observed, probably due to a coagulation disorder (Skeeles *et al*., 1980). Certain variants from the USA are reported that causes rapid atrophy of the bursa without a previous inflammatory phase (Lukert and Saif, 1997). Moreover, in the acute form of the disease caused by hypervirulent strains, macroscopic lesions may also be observed in other lymphoid organs (thymus, spleen, caecal tonsils, Harderian glands, Peyer's patches and bone marrow) (Hiraga *et al*., 1994; Inoue *et al*., 1994; Tsukamoto *et al*., 1995; Inoue *et al*., 1999) .

Pathological study in Bangladesh on natural infected chickens by (Islam and Samad, 2004) observed gross lesions in bursa which was swollen, oedematus and streaks of haemorrhagic on outer and inner surface of bursa. Cut surface of the bursa revealed slimy and gelatinous material. Thigh muscle also revealed petechial hemorrhage and in addition spleen was hemorrhagic and swollen. This investigation also indicated that hemorrhages at the junction of proventriculus and gizzard. Henry *et al*., (1980) have developed a system for evaluating microscopic lesions of the affected organs, with a score ranging from one to five according to lesion severity. The B lymphocytes are destroyed in the follicles of the bursa as well as in the germinal centres and the perivascular cuff of the spleen. The bursa is infiltrated by heterophils and undergoes hyperplasia of the reticulo-endothelial cells and of the intermolecular tissue. As the disease evolves, the surface

epithelium disappears and cystic cavities develop in the follicles. Severe panleukopenia is also observed and these microscopic lesions are exacerbated in the acute forms of the disease.

2.6. Immunosuppression

The destruction of immature B lymphocytes IBDV in the bursa creates an immunosuppression, which will be more severe in younger birds (Giambrone et al., 1976). In addition to the impact on production and role in the development of secondary infections, this will affect the immune response of the chicken to subsequent vaccinations which are essential in all types of intensive chicken production (Giambrone *et al*., 1976). The most severe and longest duration immunosuppression occurs when day-old chicks are infected by IBDV (Allan *et al*., 1972; Faragher *et al.,* 1974; Sharma *et al*., 1989; Sharma *et al*., 1994). In field conditions, this rarely occurs since chickens tend to become infected at approximately two to three weeks, when maternal antibodies decline. Evidence suggests that the virus has an immunosuppressive effect at least up to the age of six weeks (Wyeth, 1975; Giambrone, 1979; Lucio and Hitchner, 1980).

2.7. 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. The diseases like avian coccidiosis, Newcastle disease in some visceral forms, stunting syndrome, mycotoxicoses, chicken infectious anaemia and nephropathogenic forms of infectious bronchitis are the differential diagnosis for IBD. In all acute cases, the presence of bursal lesions allows for a diagnosis of IBD. In subclinical cases, an atrophy of the bursa may be confused with other diseases such as Marek's disease or infectious anaemia. A histological examination of the bursa will allow differentiation between these diseases (Lukert and Saif, 1997).

2.7.1. 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 (Inoue *et al*; 1994), spleen or bone marrow (Inoue et *al*., 1999) has been reported as a potential characteristic of hypervirulent IBDV strains. The histological diagnostic method has the advantage of allowing for diagnosis of both the acute and chronic or subclinical forms of the disease.

Detection of viral antigens: t*hin 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*, 1977) or by immunoperoxidase staining (Cho *et al*, 1987) 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 titre after four days (Winterfield *et al*, 1972; Vindevogel *et al*, 1976). The use of monoclonal antibodies in IHC techniques for detection of the virus enhances the specificity of the test (Cho *et al*, 1987).

2.7.2. 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. Isolation: A filtered homogenate of the bursa of Fabricius is inoculated in nine- to eleven-day-old embryonated eggs originating from hens free of anti- BDV 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 monospecific anti-IBDV serum. Isolation in embryonated eggs does not

require adaptation of the virus by serial passages, and is suitable for vvIBDVs. 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 (Hitchner, 1970; Rosenberger, 1989; Lukert P and Saif, 1997).

2.7.3. 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 titre of passive antibodies and determine the appropriate date for vaccination (Dewit, 1999; Kouwenhoven and van den Bos, 1994; Muskett *et al*, 1979) or in laying hens to verify success of vaccination (Lucio, 1987; 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).

2.7.4. Molecular identification

Most efforts at molecular identification have focused on the characterization of the larger segment of IBDV (segment A) and especially of the vVP2 encoding region. Several protocols have been published on characterization using restriction endonucleases of RT-PCR products. These approaches are known as RTPCR/RE or RT-PCR-RFLP (restriction fragment length polymorphism) (Jackwood, 1990; Lin *et al,* 1993; Zierenberg *et al*, 2001). The usefulness of the information they provide depends on the identification of enzymes that cut in restriction sites that are phenotypically relevant. Some sites involved in antigenicity have already been identified, however, restriction sites reliably related to virulence still need to be defined and validated. Nucleotide sequencing of RT-PCR

products, although more expensive than restriction analysis, provides an approach to assessing more precisely the genetic relatedness among IBDV strains. Markers have been demonstrated experimentally, using a reverse genetics approach, for cell culture-adapted strains, which exhibit amino acid pairs 279 N–284 T (Lim *et al.,* 1999) or 253 H–284 T (Mundt , 1999).

In most very virulent viruses, four typical amino acids are present (222 A, 256 I, 294 I and 299 S) (Brown *et al*, 1992; Lin *et al*, 1993; Eterradossi *et al*, 1999). 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 (4). 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).

3. MATERIALS AND METHODS

3.1.Study area

The study was conducted in and around Bahir Dar, which include Bahir Dar city, Gombat, Andassa, Meshenty and Wonjeta in Northwest of Ethiopia. The day old chickens were highly distributed by government to the small holder farmers, semi-intensive and intensive farms. Bahir Dar district is located in the Northwest part of Ethiopia at about 578 kms from the capital city, Addis Ababa. Bahir Dar town has an altitude range of 1730 - 2300 meter above sea level. The district has mid-altitude agro-climatic zone, with an annual rainfall of 800 to 1250 mm. (BoAAO, 2003). Except Andassa, Gombat, Wonjeta and Meshenty have the same climatically condition as Bahir Dar. Andassa is located 17 km away from Bahir Dar town in southern direction**.** It located 11^0 29' N latitude and 37^0 29' E longitudes with an elevation of 1730 m.a.s.l. It receives average annual rainfall of 1150mm with the mean annual temperature varying from a maximum of 29.5^{o}C in March to a minimum of 8.8^{o}C in January.

3.2.Study animals

Exotic and indigenous breeds of chickens within 3- 9 weeks of age, which were owned by farmers, semi-intensive and intensive farm owners considered and then stratified on the basis of age, sex, and breed. Following, the study samples were drawn by systemic random method from each stratified group. The age of the chickens was categorized in weeks, which ranges between 3- 9week old (Abdu, 1986; Abdu *et al*., 1987).

3.3. Study design

Semi-structured questionnaire was prepared (Annex A) and administered to small chicken holder farmers, semi-intensive and intensive farm owners which were selected every fifth by systematic random sampling method. The selected respondents were

interviewed about their chickens, vaccination history, diseases occurrence and mortality proportion in early age of chickens. A cross sectional study was implemented to assess pathological changes and seroprevalence of IBD with its associated risk factors in study flocks in and around Bahir Dar. The sample size was according to the formula described by Thrusfield, 2004.

$$n = \frac{1.96^2\, p\,(1-p)}{d^2}$$

Where,

n = sample size of the study population

D = desired precision

p = previous/expected prevalence in the study area 29.4%

CI = confidence interval (95%)

Accordingly, a total of 320 chickens (from backyard, semi-intensive and intensive production systems) were considered.

3.4. Study methodology and data collection

3.4.1. Data collected

History of vaccination, clinical signs, pathological changes from affected tissues, serological results and questionnaire survey data from backyard and intensive chickens were recorded.

3.4.2. Blood sample collection, serum separation and IELISA test

Approximately 1.5 to 2 ml of blood was collected from the humeral region of the wing vein with a 3-ml syringe. The syringe was laid nearly horizontally until the blood clotted. The sample was kept at 37 °C for 24 hours or left before the serum was separated. After clotting at laboratory, the syringe was returned to a vertical inverted position to facilitate the serum to ooze out. The separated serum was transferred to cryovials, labeled, and

stored at –20 °C until the indirect enzyme-linked immunosorbent assay (IELISA) was performed to detect antibodies against the IBD virus (Onunkwo and, Okoye, 1991; Smith, 1992) at Amhara Regional Animal Health and Diagnostic Laboratory Center. IELISA test was valid if the mean of optical density (OD) of the positive control is greater than 0.25(ODpc > 0.025). The ratio of the mean of the OD of positive and negative controls (OD_{PC} and OD_{NC}) is greater than 3, so if the calculated S/P ratio is > 0.3, the serum taken as positive to IBD and ≤ 0.3 considered as free of IBD.

3.4.3. Clinical examination and Postmortem findings

The clinical signs observed and histories were recorded, clinically suspected chickens from vaccinated and none vaccinated flocks were opened with standard post mortem procedure. The observed the gross pathological changes in the bursa of fabricius, spleen, kidney, thymus, pectoral and thigh muscle and the junction of proventriculus and gizzard muscle were recorded (annex II). Representative sample (0.5cm-1cm) from each tissue was collected and fixed with the10% neutral buffered formalin for histopathology processing (Philip *et al*, 2014).

3.4.4. Histopathology procedures

For histopathological examination, gross lesions in affected tissues (bursa, thymus, the junction of proventriculus and gizzard, spleen and kidney) were trimmed to the thickness of 5 mm up to 1cm in size and fixed with the 10% buffered formalin. Following, tissues samples were subjected to different steps of fixative, it was dehydrated in a series of alcohol concentrations (70%, 80%, 100%, 100%, 100%), cleared by zylene I and II. Then, it was impregnated in paraffin wax at temperature of 60-62 ^{0}C and embedded by molten wax. The embedded tissues were sectioned by rotary microtome with thickness of 5 micrometer (Luna, 1968) at the Pathology unit of the National Animal Health diagnostic and investigation Center (NAHDC). The sectioned ribbon were added to floating bath and picked it up by slide and labeled. The labeled slide was stained with Meyer's hematoxylin and eosin dye and after stained, it was mounted with Canada

balsam. The microscopic changes were examined by 10x 40x objective and the major histopathological were recorded. Tissues were histopathologically evaluated on the basis of the extent of necrosis and degeneration of follicular lymphocytes, the presence of follicle and muscle wall edema, hyperemia, and heterophilic infiltration (Bennoune, 2011). Cloacal bursa and other tissue microscopically lesions was scored based on the following system: 1 = normal, no IBDV related lesions; 2 = mild, scattered IBDV related cell depletion in a few follicles; 3 = moderate, 1/2 of the follicles with IBDV related atrophy or depletion of follicular lymphocytes; and 4 = acute / sever, totally depletion of lymphoid cells and disappearance of bursa follicle architecture (Hair-Bejo *et al*., 2004). Spleen was assessed for increased germinal center formation, as well as, lymphoid depletion in the germinal centers and around the periellipsoid and periarteriolar lymphoid sheath (Elankumaran *et al.,* 2002).

3.5.Data management and analysis

Data entry and management was done by Microsoft excel window 2007, while statistical analysis was done by SAS university edition version 9.4. Descriptive statistics like frequency, 2 x 2 tables, chi – square (χ^2) and fishery exact test were used to compare the distribution and score of lesion in affected tissues, seroprevalence of IBD, across age and breeds. Logistic regression was used to test association of prevalence of IBD with age, breed and management factor in backyard and intensive farm chickens.

4. RESULTS

4.1.Questionnaire survey

There were five chicken breeds in the study area. These are local; Koekoek, Bovans white, Bovans brown and hybrid (Table 2). The Koekoek, Bovans brown, and Bovans white breeds were introduced to backyard semi-intensive and intensive from Andassa, Gerado and Ethio-chickens multiplication center without immunization against IBD. Among, the interviewed 100 farmers, semi-intensive and intensive owners, 100% of them responded that there was high chicken mortality in their flocks. Most of the respondent (77%) didn't have the information as to the presence of vaccine for infectious bursal disease and they did not use it at all for their flock. However, only 23 % the flock owners use IBD vaccine for their flock (Table 3). High death rate was observed in Bovans brown, the lowest death occurrence in local breeds (Figure 1). The respondents emphasize that high death of growing chickens occurred in month of September to November and June to August (Figure 2).

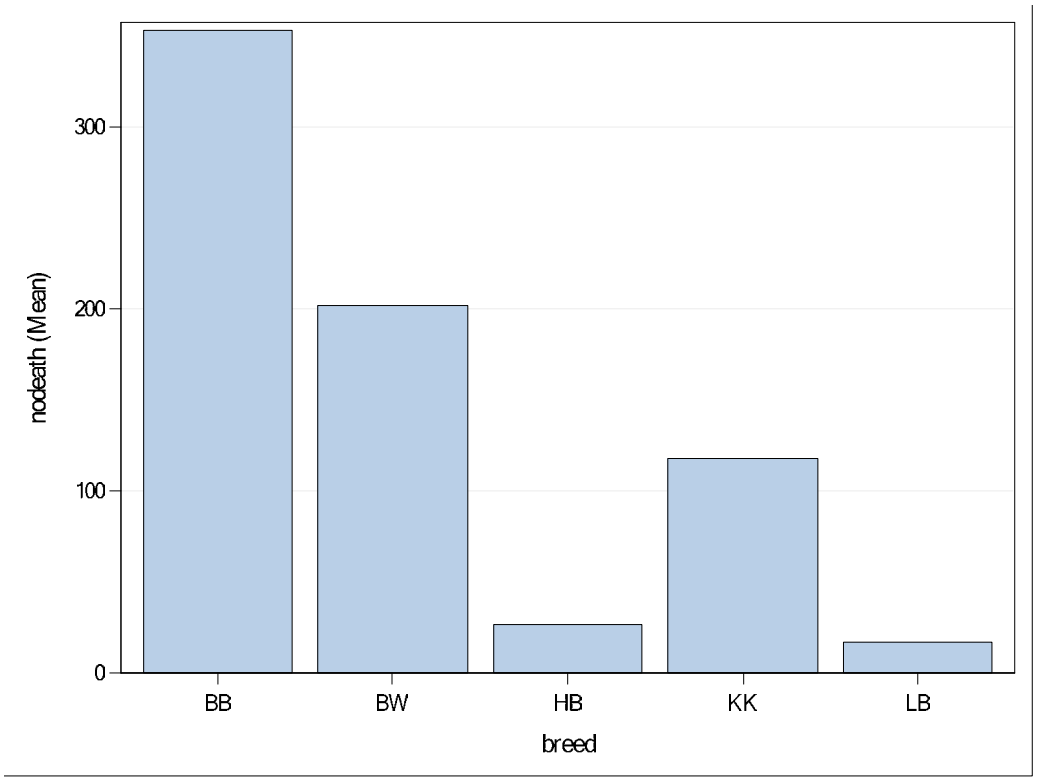
Table 2: Distribution of chicken breeds used for the study in and around Bahir Dar

Breeds	Percent (%)
Bovans brown	16
Bovans white	15
Koekoek	22
Local	28
Hybrid	19
Total	100%

Table 3: IBD vaccine utilization per farming system in & around Bahir Dar.

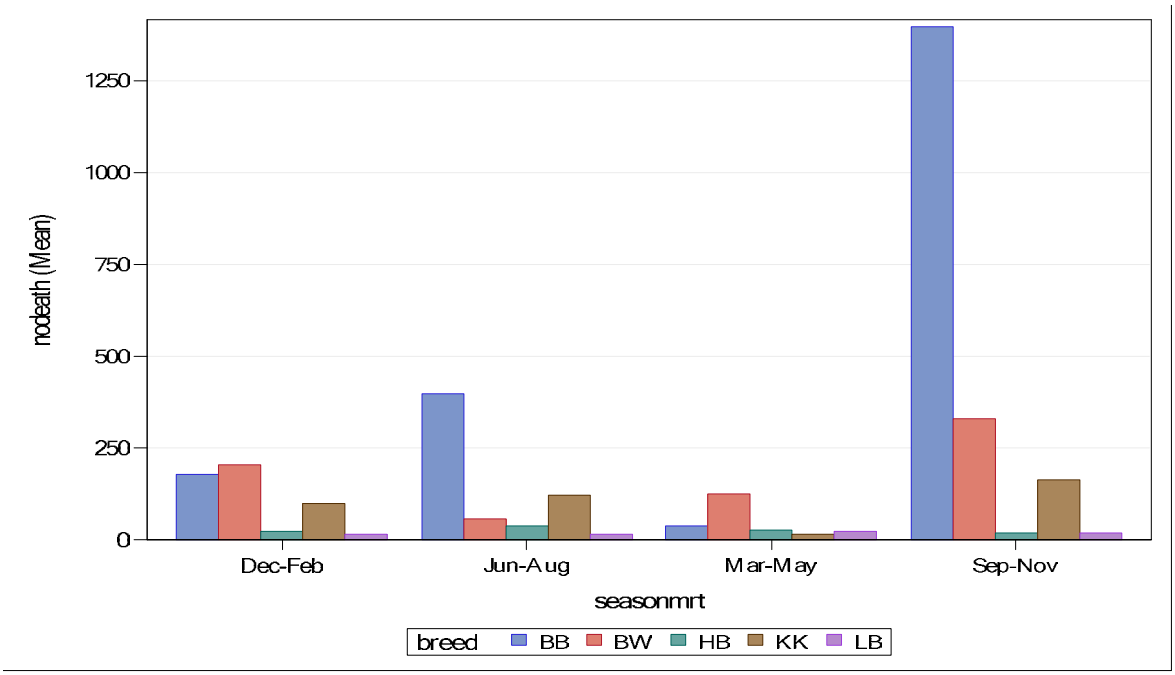
Farm types	Use of IBD Vaccine		Total
	No	Yes	
Intensive	28.57% (4/14)	71.43% (10/14)	14
Semi- intensive	46.67% (7/15)	53.33% (8/15)	15
Backyard	92.96%(66/71)	7.04 % (5/71)	71
Total	77.00 (74/100)	23.00 (26/100)	100

X^2 =51.61; P = 0.000



nodeath = number of death.

Figure 1: The death rate of the different breeds ranked by the owners in & around Bahir Dar.



Seasonmrt = season of mortality; nodeath = number of death

Figure 2: The frequency of death in different breeds at different seasons of the year as responded by the owners in and around Bahir Dar.

4.2.Clinical manifestation

In the study flock, prominent clinical sign in IBD infected chicken were recorded. Among which, tired, clumping together, recumbent on the break, whitish diarrhea mixed with blood, emaciated, ruffled feather, dropping of the wing, anorexia, depression and massive death within short period of time (Figure 1: A, B, C and D).

4.3.Characterization of gross pathological lesions

Postmortem finding revealed that the affected chicken become dehydrated and emaciated. Congestion of subcutaneous blood vessels was observed in all part of the affected chickens (Annex 3: figure E). Petichial hemorrhage and congestion were observed on the pectoral and thigh muscles (Annex 3: figure D). Among clinically active cases, 76.67% of Bursa of fabricius was found as swollen, edematous and petichial hemorrhage in

serosa and mucosal part (Figure E and F). It also became atrophied, whitish-creamy as the course of the disease progressed (6.66%) and 16.67% of it filled with gelatinous exudates on its serosa (Figure 2: E and F). The spleen becomes swollen, dark red in color and petichial hemorrhagic (Figure 2: E). Petichial hemorrhage and swollen was observed on thymus gland (Figure 2: D). A caudal part of kidney became swollen; petichial hemorrhagic and pale, ureter was turgid in a numbers of cases (Figure 2: A and B). Ecchymotic hemorrhage was also observed on the junction of proventriculus and gizzard (Figure 2: C).

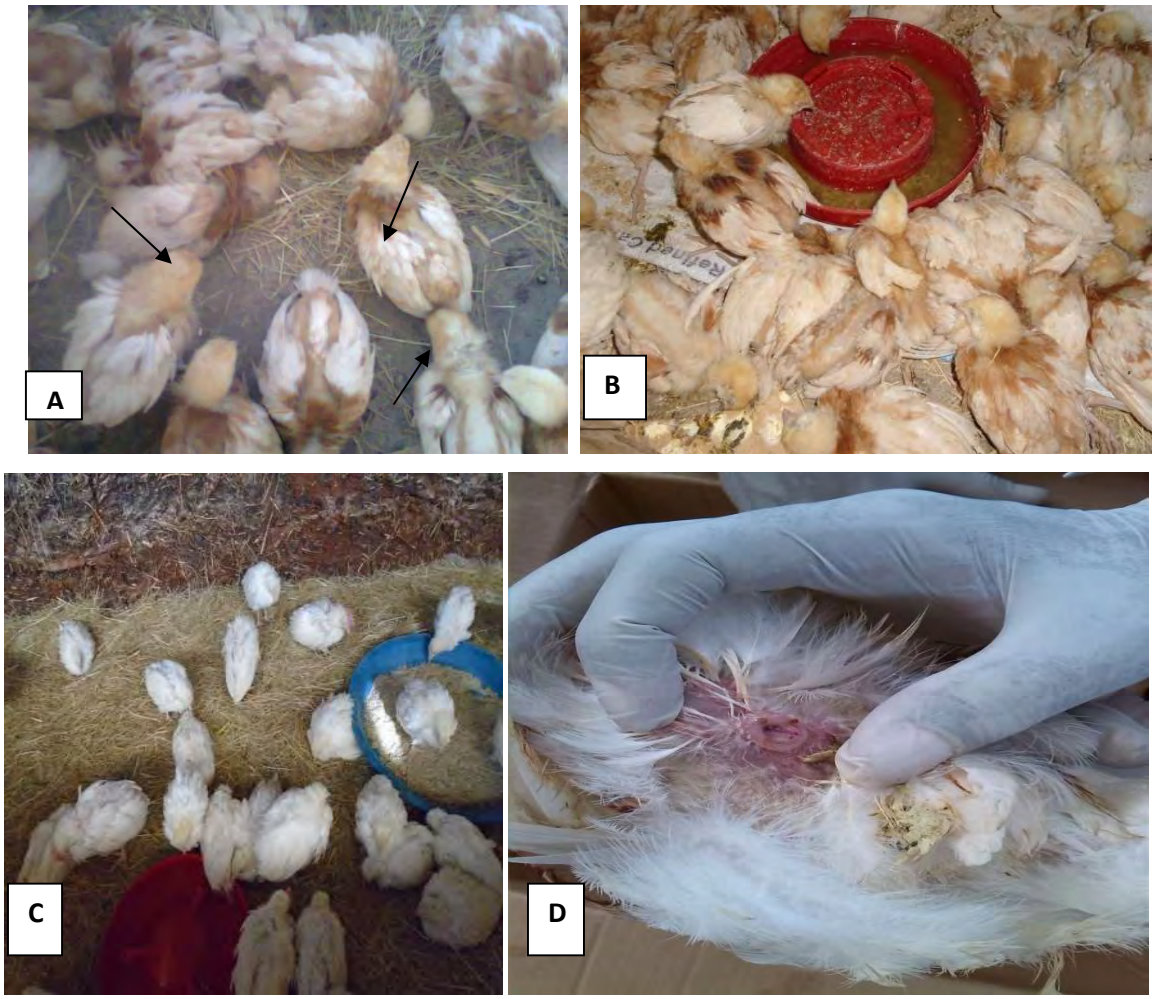


Figure 3: Clinical signs observed during active case of IBD in 28 – 52 day old chickens in and around Bahir Dar.

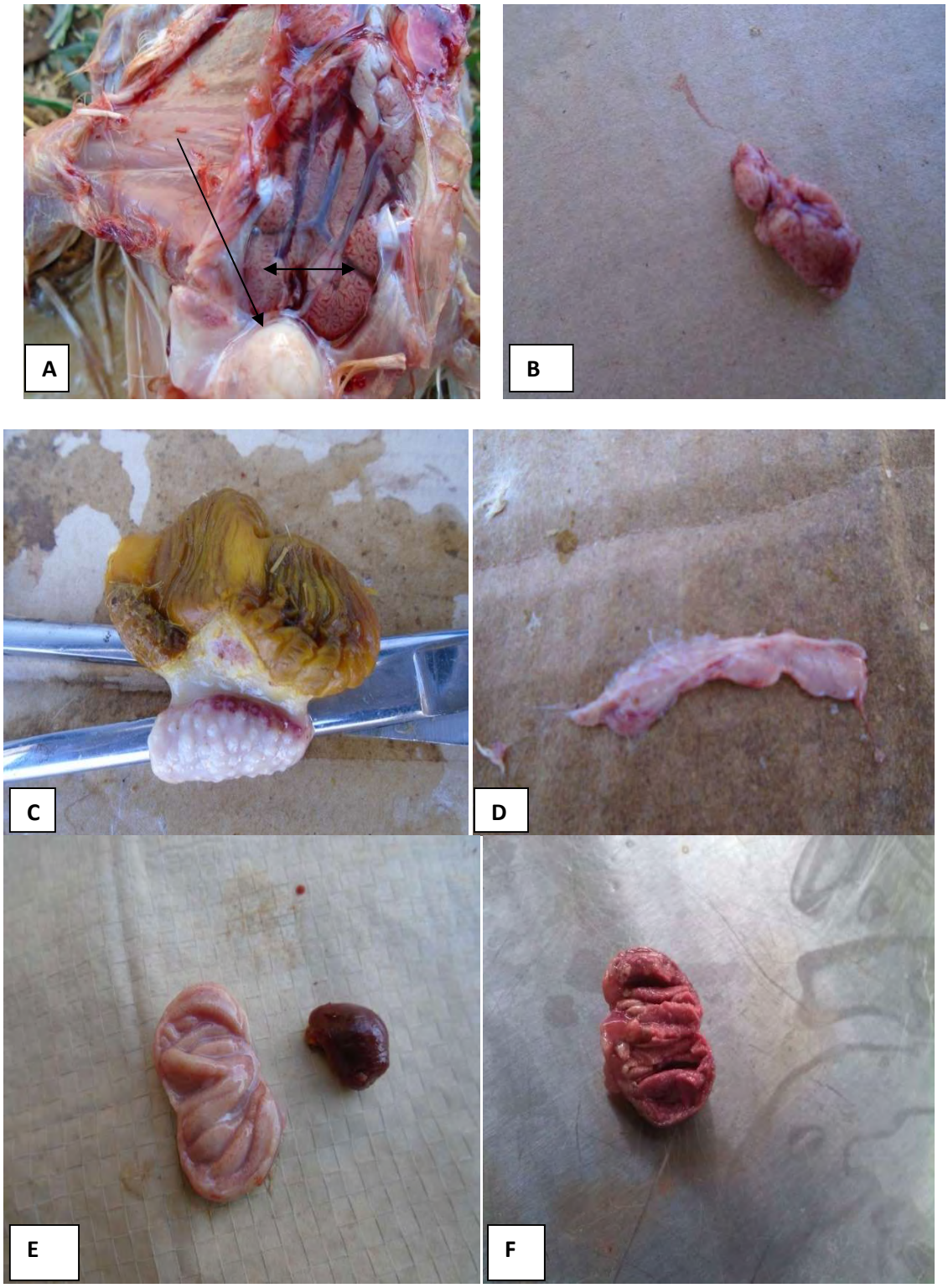


Figure 4: Gross pathological changes in different organ of 21-54 day old chicken infected by IBDV in and around Bahir Dar.

4.4. Histopathological changes

The major microscopic change in the bursa of fabricius was depletion of lymphocye in follicles, accompanied by interfollicular hemorrhage, congestion, infiltration of heterophils, edema, karyorrhetic nucli of lymphocyte, formation of cortical rim, and follicular cyst (Figure 6, 7 and 8). The lesion score in bursa of IBD infected from vaccinated and unvaccinated ranges from mild to severe depletion of lymphoid cells (Table 4). The tubules of kidney became congested with fibrinous exudates and necrotized cuboidal epithelial cells; hemorrhage within and between tubules. Hemorrhages, congestion, depletion of lymphoid cells and infiltration of heterophils have been notice as the prominent changes in the spleen (Figure 8). Hemorrhage, congestion and infiltration of heterophils observed as the main microscopic changes in the proventriculus (Figure 11). A histopathological change in thymus was total depletion of lymphocytes cells in follicles and formation of tissue cords (Annex 3).

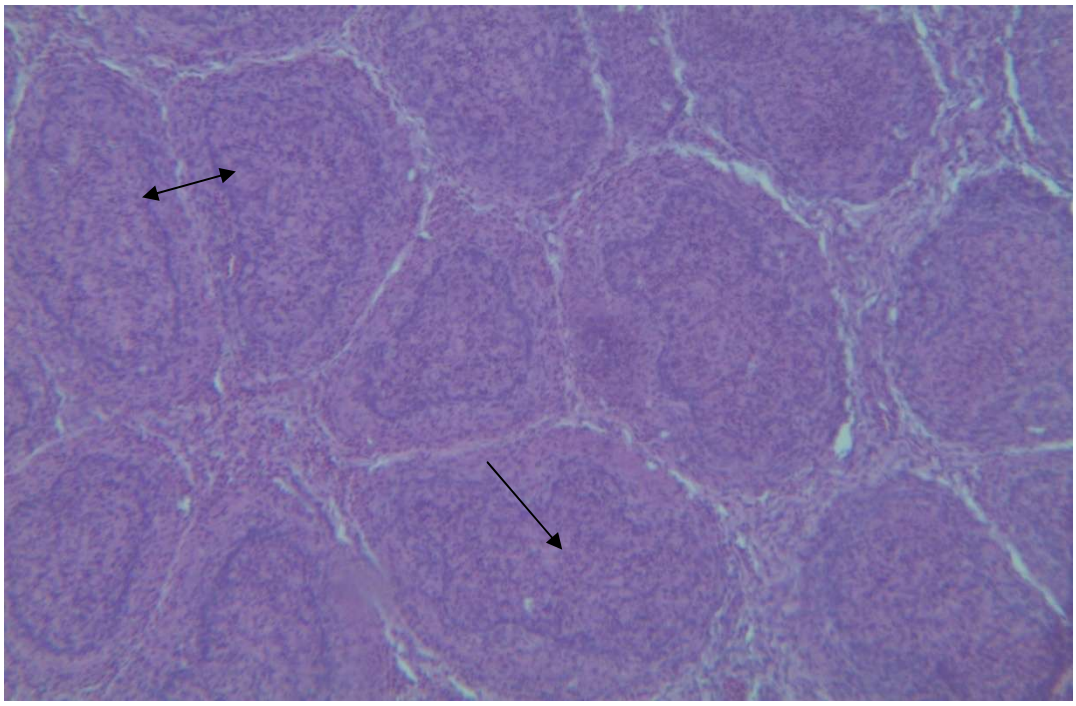


Figure 5: Moderate lymphocyte depletion in follicles (arrow), formation of cortical rim (double arrow) in IBD infected bursa of chicken. HE x10.

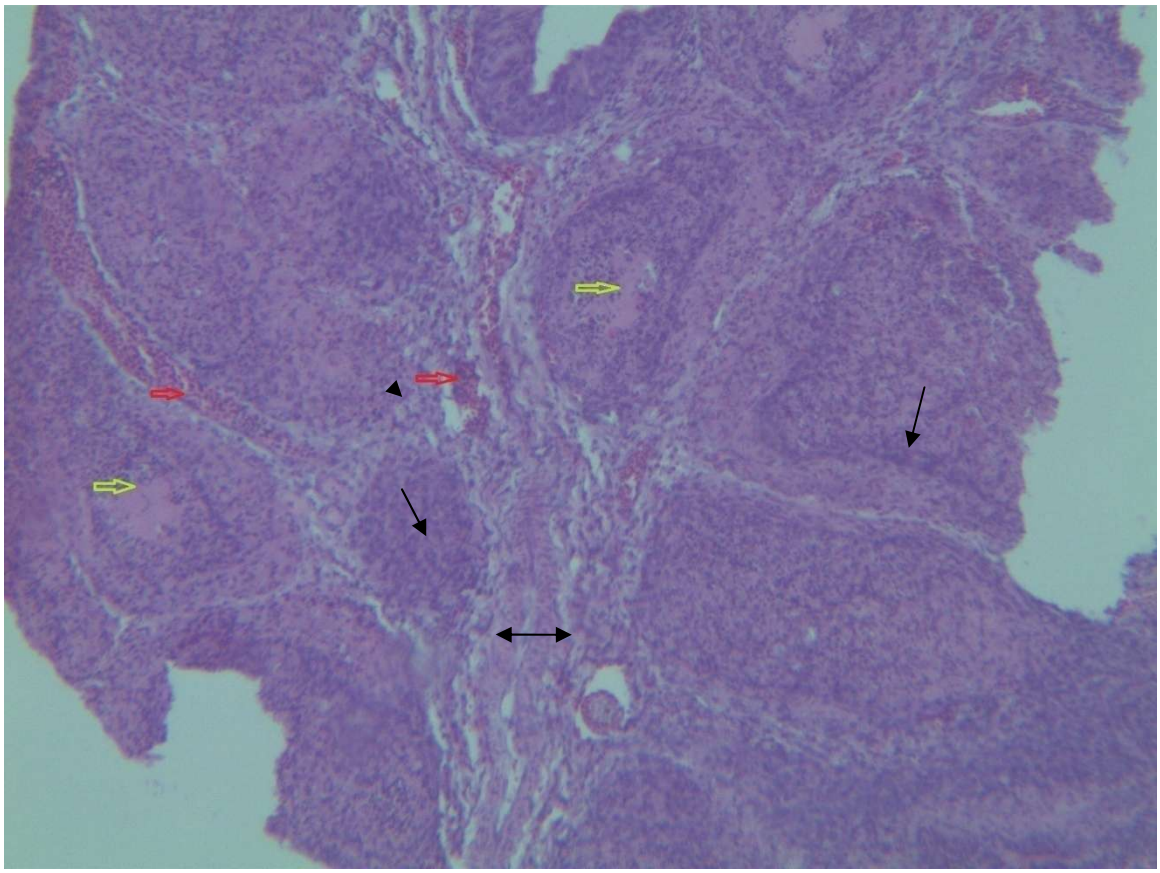


Figure 6: Bursa with intrafollicular edema (yellow arrow), interfollicular hemorrhage (red arrow), congestion, depleted lymphoid cell with pyknotic nuclei (black arrow) and interfollicular fibrosis (double arrow). HE X10.

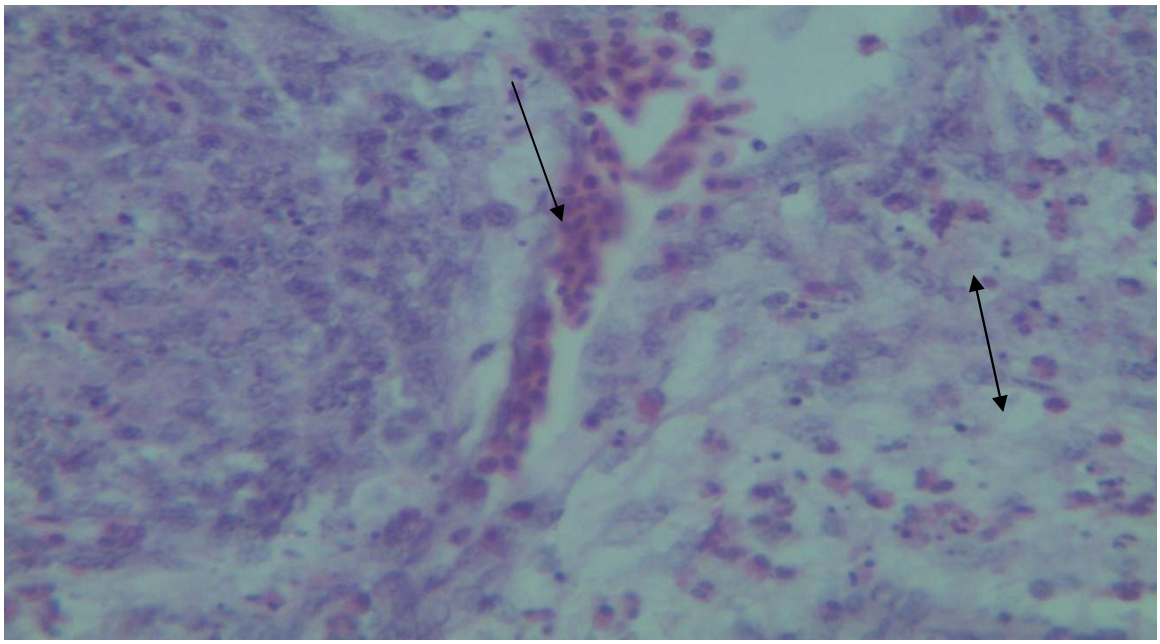


Figure 7: Bursa with marked lymphoid depletion and necrosis, interfollicular hemorrhage (single arrow) and follicular cyst (double arrow). HE X40.

Table 4: Lesion score in IBD infected Bursa in vaccinated and unvaccinated chickens

Vaccine history	Lesion score			
	Sever	Moderate	Mild	Total
Unvaccinated	10 (62.50%)	2 (12.50%)	4 (25.00%)	16 (100%)
Vaccinated	4 (28.57%)	5 (35.71%)	5 (35.71%)	14 (100%)
Total	14 (46.67%)	7 (23.33%)	9 (30.00)	30 (100%)

Fisher exact test = 0.01820 (p= 0.1856) there was no lesion score difference between the lesion score in vaccinated and unvaccinated IBD infected chicken's bursa.

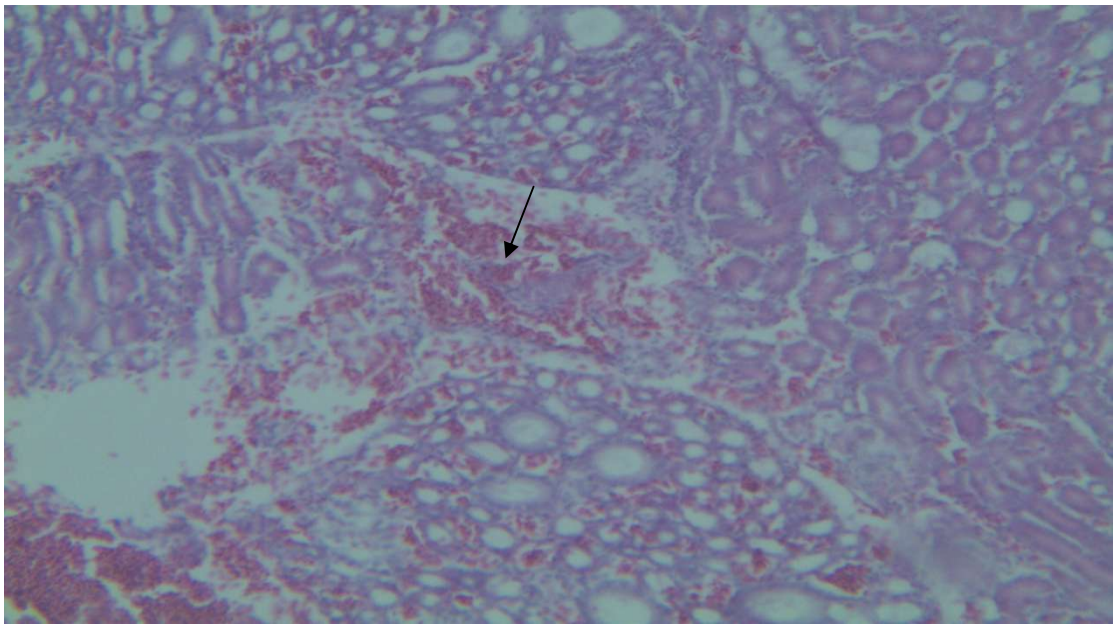


Figure 8: The tubules of kidney became congested with purulent exudates; hemorrhage within and between tubules (arrows). HE x10.

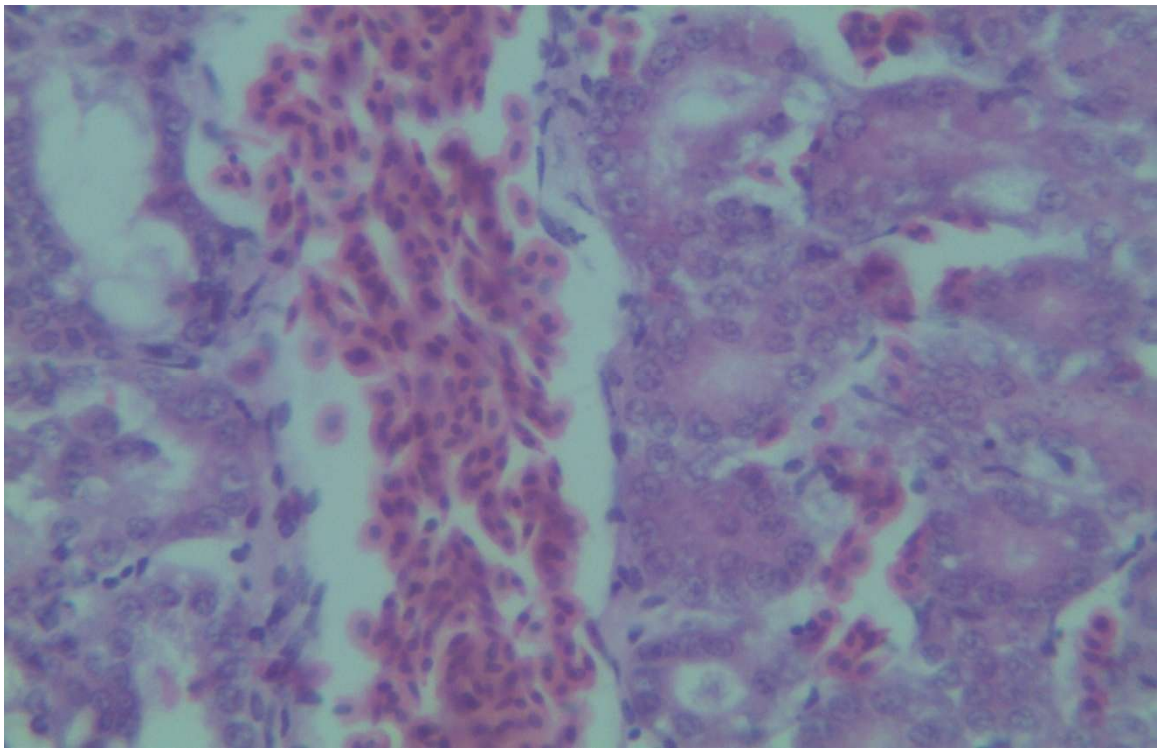


Figure 9: The tubules of kidney became congested with fibrinous exudates that contain necrotized cuboidal cell; hemorrhage within and between tubules. HE x40.

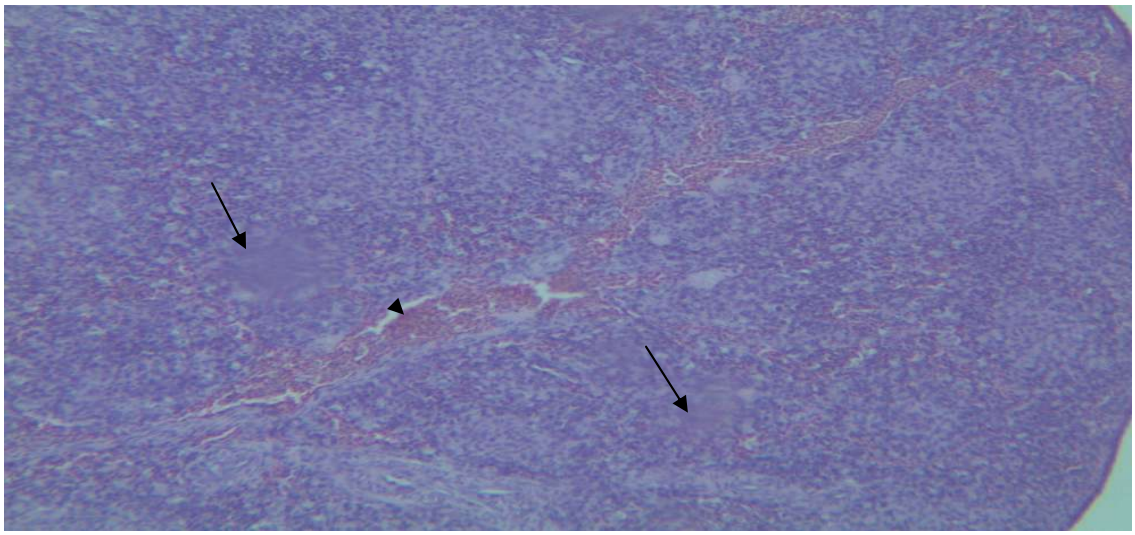


Figure 10: The depletion of lymphocyte in the germinal center of the spleen (arrow), infiltration of heterophils and hemorrhage in red pulp area (head arrow). HE x10.

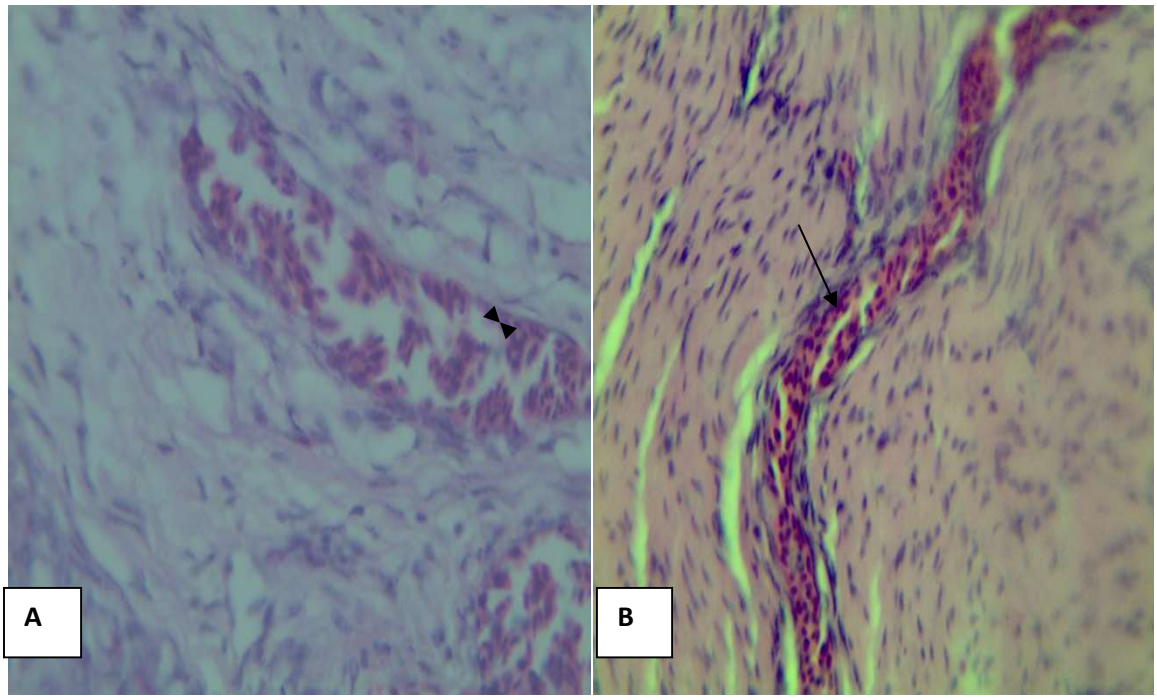


Figure 11: congestion (double head arrow: A) and hemorrhage (B), infiltration of heterophils on the junction of proventriculus and gizzard (B). HE x10.

4.5. Seroprevalence of IBD Virus

A total of 320 sera were collected from backyard, semi- intensive and intensive farm and tested by indirect ELISA and results in over all prevalence of 51.56 % (165/320) IBD in the investigation area (Table 5). Andassa has the highest prevalence of 72.73% (48/66) IBD among sub-district while Bahir Dar experienced comparatively with low prevalence of 42.42% (Table 6). The Koekoek breeds have more susceptible to IBD with prevalence 67.11%, (51/76) than the Bovans white, Bovans brown, local breed and Hybrid breeds (Table 6).

The prevalence of IBD in sex wise was proportional with 51.63% (127/246) and 51.35 % (38/74) in female and male chickens respectively (Table 6). High IBD was observed in the age range of 3-6 week old chickens, but it was lower prevalence in age group of 7 – 9 weeks old chickens (Table 6). This disease has different prevalence magnitude among farm systems. It was 57.14 % in backyard farm system, 34.69% in semi-intensive chickens farm and 21.05% in intensive farm system (Table 6).

The seroprevalence of the IBDV in study area was influenced by a number of risk factors which include age of chicken, farm type, study site and breeds of chickens (Table 7). The odds of IBDV infection was 15.4% lower in intensive farm system than that of backyard production system and odds of it infection was significantly associated with farming type (OR =0.1543; P =0. 0032). The odds of IDBV infection in Bahir Dar was 16.96% lower than that of Andassa and odds of it infection was significantly associated with study site (OR = 0.1696; P = 0.0001).

Table 5: The overall seroprevalence of IBDV by IELISA in and around Bahir Dar.

	Serological test / IELISA Test (N=320)		
	Number positive	Prevalence (%)	Confidence interval (95 %)
Result	165	51.56	45.95 – 57.14

Table 6: Seroprevalence of IBD in different study sites, management systems, sex and breed in around Bahir Dar.

Variable	Seroprevalence (%)	X^2 (p-value)
Farm type		
Backyard	57.14 (144/252)	
Intensive	21.05 (4/19)	15.8061 (0.0004)
Semi-intensive	34.69 (17/49)	
Sex		
Female	51.63 (127/246)	0.0017 (.967)
Male	51.35 (38/74)	
Breed type		
Koekoek	67.11 (51/76)	
Bovans brown	57.69 (30/52)	
Bovans white	40.28 (29/72)	14.23 (0.0066)
Hybrid	38.71 (12 /31)	
Local breed	48.31 (43/89)	
Study sites		
Bahir Dar city	42.42 (28/66)	
Andassa	72.73 (48/66)	20.0589 (.0033)
Meshenty	44.44 (28/63)	
Wonjeta	50.00 (34/68)	
Gombat	45.00 (27/60)	
Age (in weeks)		
3	66.67 (50/75)	
4	58.21 (39/67)	
5	51.67 (31/60)	20.3578 (.0024)
6	41.67 (30/72)	
7	38.89(14/36)	
8	16.67 (1/6)	
9	0.00 (0/4)	

Table 7 : Association of risk factor with IBD by using multivariate logistic regression in and around Bahir Dar.

Variables	OR	95% CI for OR	P Value
Age (in weeks)			
6 *vs.* 3	0.422	0.199 - 0.897	0.0497
7 *vs.* 3	0.333	0.124 - 0.893	0.0290
8 *vs.* 3	0.041	0.004 - 0.424	0.0504
Breeds			
HB/BB	0.347	0.120 - 1.001	0.0479
LB/ BB	0.431	0.187 - 0.992	0.0479
Study sites			
Bahir Dar *vs.* Andassa	0.169	0.071 - 0.399	0.0001
Gombat *vs.* Andassa	0.278	0.115 - 0.672	0.0045
Meshenty *vs.* Andassa	0.300	0.125 - 0.720	0.0070
Wonjeta *vs.*.Andassa	0.274	0.117 - 0.645	0.0030
Farm types	0.28		
Intensive *vs.* backyard	0.154	0.044 - 0.535	0.0032
Semi-intensive *vs.* backyard	0.409	0.17 3– 0.965	0.0413

5. DISCUSSION

The present study clearly showed that IBD is one of the major diseases of chickens in and around Bahir Dar. This study was conducted to characterize the pathological changes, determine the seroprevalence of IBD and associated risk factors in and around Bahir Dar in exotic and local breeds of chickens maintained under different productions systems in areas like Bahir Dar city, Andassa, Wonjeta, Gombat, and Meshenty. Clinical signs, gross and histopathological changes as well as seroprevalence of IBD was assessed.

Among, the interviewed 100 farmers, semi-intensive and intensive owners, 100% of them responded that there was high chicken mortality in their flocks. Most of the respondent (77%) didn't have the information as to the presence of vaccine for IBD and they did not use it at all for their flock. However, only 23 % the flock owners use IBD vaccine for their flock. This high mortality of growing chickens might be related with the absence of scheduled vaccination in the study area (Nunoya *et al.*, 1992). The respondents emphasize that high death of growing chickens occurred in month of September to November and June to august. This might be related to time of high number of day old chickens distributed by government without immunization against major chicken diseases. In addition to that, the season of market in investigation area in which chicken transported with life threaten infectious disease like IBD might increase death of young chicken (Rosenberger and Cloud, 1986).

The clinical signs like depression, ruffled feather, anorexia, white diarrhea tinged with blood, massive death, emaciation, dropping of the wing were among the major manifestations. These clinical manifestations agreed with findings of (Zeleke *et al*, 2005; Shekaro and Josiah, 2015) but clumping of the infected chicken was not noticed by these investigators even if this was the prominent manifestation in this study. This may be due to the difference in study breeds of the chickens and season of the investigation period (Kegne and Chanie, 2014).

The gross pathological findings observed in this study include hemorrhagic, edematous, enlarged and gelatinous lesions in bursa of fabricius, congestion of subcutaneous blood

vessels in all parts of the affected chickens, petichial hemorrhage and congestion on the pectoral and thigh muscles. These changes were similarly reported by previous researchers (Zeleke *et al*, 2005; Mazengia *et al,* 2009 and Rozina *et al*, 2014). However, none of the investigators did not reveal the gross pathological changes in spleen and thymus gland. In our case, swollen and petichial hemorrhagic lesions were common in spleen and thymus. A caudal part of kidney became swollen; petichial hemorrhagic, pale and ureter was turgid with urate in a numbers of cases. Petichial and ecchymotic hemorrhage was also observed on the junction of proventriculus and gizzard which were agreed with the previous finding of (Islam and Samad, 2004; Babiker *et al*, 2008; Kegne and Chanie, 2014).

Depletion of lymphoid cells, formation of cortical rim, intrafollicular and interfollicular hemorrhage, edema, and formation of cystic follicles were the major microscopic findings in IBD infected bursa of chickens (Figures 6, 7 and 8). These histopathological findings in bursa were related with the studies of (Henry *et al*., 1980; Skeeles *et al*., 1980; Chowdhury *et al*.**,** 2015). The microscopic evidence of IBD infected chicken's bursa revealed that different lesion score and have been grouped according to study of (Hair-Bejo *et al*., 2004) which were taken from outbreaks of vaccinated flock as well as from none vaccinated flock. These lesion scores were classified as sever, moderate and mild. Sever lesion score consists of 62.50% (10/16) from none vaccinated flock and 28.57% (4/14) from vaccinated flock. This lesion score was characterized by total depletion of lymphoid cell in the follicle, formation of cystic follicles and disruption of follicular architecture with fibrosis. The moderate lesion score in microscopic lesion of bursa comprised 25.00% (4/16) from none vaccinated and 35.71% (5/14) have observed in vaccinated flock's bursa. The moderate lesion score designated by 3 and characterized by depletion of 1/3-1/2 lymphocyte cell in the bursa follicles, interfollicular and intrafollicular hemorrhage, infiltration of heterophils between follicles, prominent edema and formation of cortical rim. The mild lesion score in IBD infected bursa comprised 12.50% (2/16) and 35.71% (5/14) from none vaccinated and vaccinated flock respectively. The microscopic feature in the mild lesion scored bursa was edema and scattered lymphocyte depletion has been observed in the follicles. There was no

significant difference among lesion score (P = 0.1856) in bursa of none vaccinated and vaccinated flock which were collected from outbreaks of IBD in both flocks. This magnificent lesion score in IBD infected bursa of chicken both in vaccinated and none vaccinated flock indicated that either the vaccine was not properly given or it may be transported and stored in poor conditions. The other reason is that, virus strain that causes outbreak in the flock may super pass the protective level of the given vaccine (Onunkwo and Okoye, 1991; Gary and Butcher, 2013).

The spleen in IBD infected chicken was infiltrated with heterophils, hemorrhage was observed in arteriole, the periarteriolar lymphoid sheath (PALS), and depletion of lymphocytes in germinal centere (Figure 8). This histological finding in IBD infected chickens spleen was related with study of (Hair-Bejo *et al*., 2004; Gary and Butcher, 2013). While, the histopathological change in thymus was total depletion of lymphocytes cells in follicles and formation of tissue cords. The study conducted by (Mohammad *et al,* 2012) also revealed that thymus was found devoid of lymphocyte cell in its follicles following naturally IBD infected broiler chickens which support the present study.

The tubules of kidney were filled with fibrinous exudates; hemorrhage between and within tubules. The microscopic changes include nephritis, detached and necrotized cuboidal epithelial cells in the tubules and eosinophilic cast in renal ductus, and infiltration of heterophils adjacent to kidney tubules. These microscopic changes in kidney were not specific to IBD infection, but it is a responsive reaction to acute inflammation and dehydration due to diarrhea and hemorrhage. Study conducted in Nigeria by (Oliwayelu *et al*, 2002) encountered similar histological changes in kidney with the present findings. Microscopic changes in the junction of proventriculus and the gizzard, includes congestion, hemorrhage and infiltration of hetrophils at the junction of two organs. These microscopic changes only observed in early clinical stages of IBD as the disease progressed, it subsides quickly and could not notified in later stage of the disease (Chowdhury *et al.,* 2015).

The current seroprevalence findings of IBD in unvaccinated backyard, semi-intensive and intensive chicken flocks result in the overall prevalence of 51.56% in and around Bahir Dar. This result indicated that IBD is epidemic chicken threatening disease in and around

Bahir Dar. This prevalence was higher that the study of (Mazengia *et al*, 2009) in Bahir Dar and Farta district with the prevalence of 29.04% and 21.70%, respectively.

The seroprevalence of IBD from this study (51.56%) was higher than the findings of Reta (2008), who reported a prevalence of 39.2% in unvaccinated backyard chickens in East Shoa zone using AGID (Agar gel immuno-diffusion) test. Kassa and Molla (2012) reported the overall prevalence of 73.5% of backyard chickens in northern Gonder and west Gojjam which was higher than the current prevalence report. Study conducted at Andassa poultry multiplication center by Woldemariam and Wossene (2007) reported a seroprevalence of 100% which was much higher than current prevalence in Bahir Dar and its surrounding areas. Zeryehun and Fekadu (2012) investigated the seroprevalence of IBD in chickens managed under backyard production system in central Oromia and reported that the overall prevalence to be 82.2%, which was higher than the present seroprevalence in and around Bahir Dar. This may be related with intensification of poultry farm in central Oromia which may facilitate more contamination to backyard chicken than less intensification in and around Bahir Dar. Retrospective study of IDB in Nigeria at Zuria by (Mbuk *et al*, 2010) reported the overall prevalence of 7.26% in broiler chickens. This prevalence was much lower than the current study in and around Bahir Dar and the previous IBD seroprevalence reports from Ethiopian.

There was no statistically significant difference (x^2= 0.0017; P= 0.967) between the seroprevalence of IBD in female 51.63% (127/246) and male 51.35 % (38/74). In Early age, female and male chickens received the same maternal immunity level and thus female and male chickens might have equal resistance level to IBDV (Hassan and Saif, 1996). The other reason could that female and male chickens in the study area share common house, and feed trough both in intensive, semi-intensive and backyard production systems. This facilitates similar exposure to IBDV to male and female chickens, which agree with the prevalence reported by (Zeryehun and Fekadu, 2012) from central Oromia.

The seroprevalence of IBD was significantly associated with study area (x^2 = 15.77, P= 0.0033). Andassa was highly exposed area with IBDV than Wonjeta, Gombat, Meshenty and Bahir Dar with the prevalence of 72.73% (Table 6). Chicken multiplication and distributor center have been established around Andassa town. This multiplication center, imported different exotic chicken breeds (Koekoek, White leg horn, Bovans white etc) from abroad for multiplication purpose. After multiplication these imported chickens, the center redistribute the day old chickens to farmers and grouped youths without immunizing against IBD. The absence of programmed vaccination against IBDV in exotic breeds of chickens in the study area resulted in high seroprevalence of IBD compared to others.

The odds of IBD infection in Andassa was 0.1696% times higher than that of Bahir Dar and the odds of IBD infection was significantly associated with location (OR= 0.1696; P= 0001). The odds of IBD infection was 0.2783% times lower in Gombat than that of Andassa chickens and the odds of IBD infection was significantly associated with location (OR=0.2783; P= 0.0045). Seroprevalence between the sub-districts, 0.2783% times prevalent than Gombat, 0.3005% times prevalent than Meshenty, and 0.2749% times more prevalent than Wonjeta (Table 6). Bahir Dar has the least seroprevalence of 42.42 % among these study areas; since more than half of the intensive farms owners in Bahir Dar vaccinated their flocks against IBDV, which lowers the seroprevalence of IBD compared to Andassa, Meshenty, Gombat, and Wonjeta. This result agrees with study of Zeryehun and Fekadu (2012) from central Oromia with different seroprevalence in their study sub-districts. For instance, Swaia, *et al.,* (2011) reported statistically significant IBD prevalence which varies from 37.5 % to 91 % between districts and from 75 % to 90 % between regions in northern Tanzania free ranging chickens.

The seroprevalence of IBD was significantly associated with the age of chickens (x^2 = 20.3578; P= 0.0024). As the chickens got older and older, the seroprevalence decreases significantly (Table 6). Higher seroprevalence of IBD in the current study chickens was observed from age ranging 3-6 weeks old. At this age range, bursa of fabricius attains its maximum growth and development with sufficient B lymphocyte for IBDV

multiplication which agrees with the findings of Zeryehun and Fekadu (2012) where age was considered as risk factor for IBDV infection in chickens. Bursa of fabricius begins to atrophy starting from seven weeks old and the number of B lymphocyte decrease gradually. As result of this, IBDV infectivity decrease as B lymphocyte decrease with age of the chickens (Rashid *et al*, 2013). The odds of IBD infection was 33.38% lower in 7 weeks aged chickens than that of 3 weeks aged chickens. The odd of IBD infection was significantly associated with age (7/3) of chicken (OR =0.3338; P = 0.0290). The odds of IBD infection is 4.14% lower in 8 weeks aged chicken than that of 3 weeks aged chickens. The odds of IBD infection was significantly associated with age (8/3) of chicken (OR =0.0414; P =0.0074). This odd implies that as the age of chicken increase beyond 6 weeks old it become a protective factor for INDV.

The susceptibility of chickens to IBDV was significantly associated with breed (χ^2 =14.23; P = 0066). The highest seroprevalence (67.11%) of IBD among the five breeds of chickens from the present study observed in Koekoek chicken might originated from the contaminated poultry multiplication center of Andassa. Bovans brown is the second most susceptible to IBDV with the prevalence of 57.69% as compared to the rest of the breeds. Local breed of chickens was also the third susceptible with the seroprevalence of 48.31% among the five breeds. This indicated that the indigenous breed of chickens was also exposed to IBDV and this agrees with the study of Mazengia (2009) in Bahir Dar and Farta and Zeryehun and Fekadu (2012) in central Oromia. Bovans white and hybrid breeds have almost similar seroprevalence, 40.28% and 38.71%, respectively.

Management, bio-security and vaccination play the key role in chickens farming system to control infectious diseases. This study observed high IBD seroprevalence (57.14%) in backyard chickens. In backyard, there is poor bio-security, poor management and no vaccination (Table 3). There is also chance mobility of chickens from villages to market and vice-versa, all these reasons favors IBDV to be endemic problem in backyard production system (Kassa and Molla, 2012)..

In semi-intensive chicken production system, there was a relatively higher seroprevalence (34.69%) than that of backyard chicken farm (21.05%) but lower than intensive production system (Table 6). The odds of IBDV infection was 15.43% lower in intensive chicken's farm system than that of backyard production system and odds of its infection was significantly associated with production system/ type of farming (OR = 0.1543; P = 0.0032: Table 7). The odds of IBD infection in semi-intensive production system was 0.4092 times lower than that of backyard production, so this study indicated that intensification of production system can act as a means of protective factor against IBDV (Negash, 2013).

6. CONCLUSION AND RECOMMENDATIONS

This study concludes that IBD is epidemic chicken's disease in and around Bahir Dar as confirmed by pathological and serological findings. Prominent Gross and histopathological changes were observed in bursa of fabricius, spleen, kidney, thymus, thigh and pectoral muscles and at junction of proventriculus and gizzard. High seroprevalence of IBD virus was found to circulate in various study sites in and around Bahir Dar. The IBDV infection in study area was influenced by age of the chicken, breed, and study site and farming system. It was more prevalent in 3 - 6 week old chickens, backyard production system, unimmunized flock, and in the breeds of Koekoek and Bovans brown. This high prevalence of IBD reduces the income of producer from chicken in and around Bahir Dar.

In line with the above concluding remarks, the following recommendations are forwarded:

- Management factors like, scheduled vaccine program in backyard, proper bio-security in semi-intensive and intensive farm should be implemented to reduce the magnitude of IBDV infection in investigation area.
- The regional government should set immunization schedules in chicken before and after distribute day old chickens to backyard, semi- intensive and intensive producers.
- Molecular diagnostic study should be conducted to identify the current circulating strain of IBDV in the chicken population.
- The current vaccine efficacy and the circulated IBDV strain should be investigated.

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8. ANNEXES

Annex II: Questionnaire survey

Addis Ababa University College of veterinary medicine and agriculture

A questionnaire survey prepared aim to collect information about the presence of infectious bursal diseases (IBD) in chicken flock at purposively selected small holder farmers or semi-intensive farm.

Date ...../...../.....

Questionnaire No. ..... Breed..... Small holder farmer..... semi-intensive farm.....

Answer the questionnaire by marking 'X' on correct answer corresponding to your chicken flock.

1. Is there chicken mortality in your flock? ☐Yes no ☐
2. If your answer in question 1 is yes, at which age range highly mortality of chicken observed? ☐1-2 weeks ☐ 3-6 weeks ☐ 7-10 weeks ☐11- 15 weeks ☐ 16-21 weeks.
3. How many of your chickens died per year from flock?
4. At which season high mortality of chicken occur?
   a) At summer b) winter c) spring d) autumn
5. How many days it takes to die from the observation of the first clinical signs
   a) 1-2 days b) 3-5 days c) 6- 8 days
6. Which clinical sign observed before the chicken die?
   a)……
   b)…….
   c)…….
7. Did your chicken get vaccine in this year? ☐Yes ☐ no
8. If your answer yes for question 7, for which disease your chicken have vaccinated?
   a) …….. c) ……
   b) …….. d) ……
9. Did you sell the diseased / recovered chicken at market? ☐ Yes ☐ no

10. Is there retardation of growth in your chicken flock? ☐ Yes ☐ no

11. Did your chicken get treatment at time of morbidity? ☐ Yes ☐ no

Annex II**:** Serological and pathological sample data collection sheet

Date ...../...../.....

1. Case no. ..... Age ..... breed..... semi-intensive farm ..... backyard.....

1.1. Physical abnormality observed/ antemortem examination and history

a)..... e).....

b)..... f).....

c)..... g).....

1.2. Serum..... Serology test result.....

1.3.Postmortem findings in different tissues and its gross interpretation

(1) Bursal fabricious	(2) spleen	(3) thymus
a)	a).....	a).....
b)	b).....	b).....
c)	c).....	c).....

(4) Proventriculus and gizzard	(5) pectoral muscle
a).....	a).....
b).....	b).....
c).....	c).....

1.4. Pathological sample taken for histopathological procedure and microscopic interpretations

a. Bursal fabricious result.....
b. Spleen result .....
c. Thymus result .....
d. Proventriculus & gizzard result .....

Annex III: Gross and histopathological changes in different tissue of IBD infected chickens.

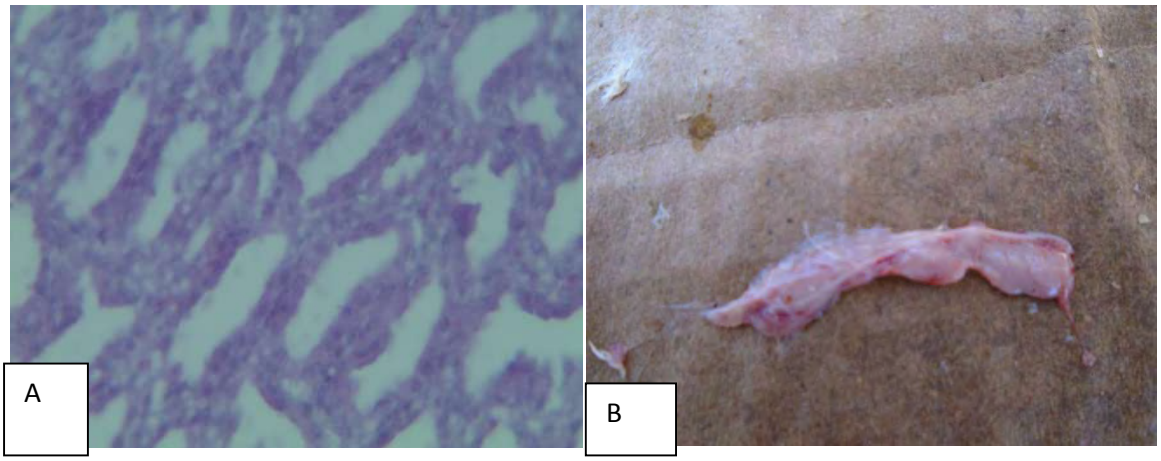

Figure A: M microscopical change (A) and gross changes (B) in chicken thymus infected with IBD was total depletion of lymphocyte cells in follicles and formation of tissue cords with necrotic cells. HEx40.

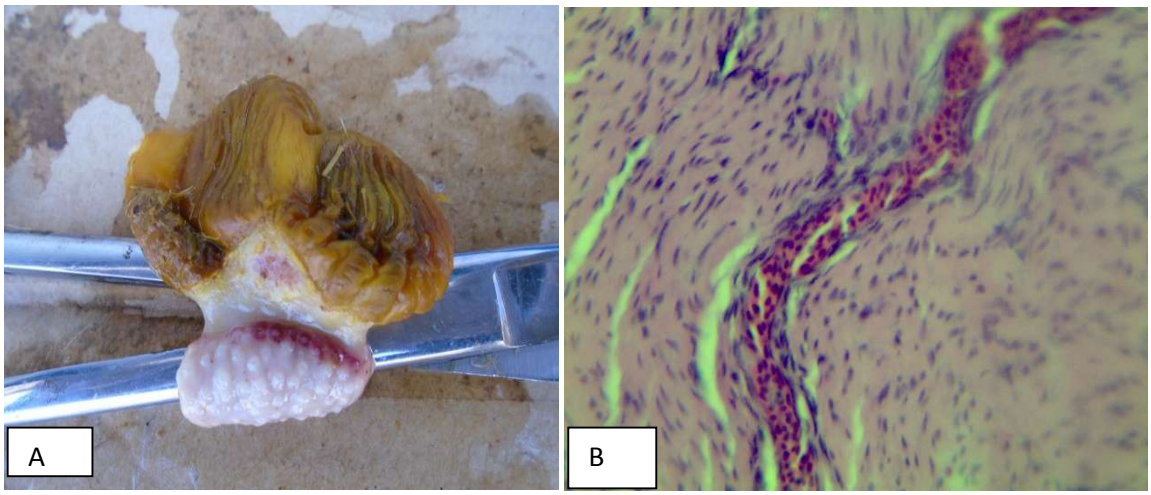

Figure B .Gross pathology (A) and microscopic view (B) of proventriculus and gizzard junction in IBD infected chickens. Hemorrhage was the major pathological changes.

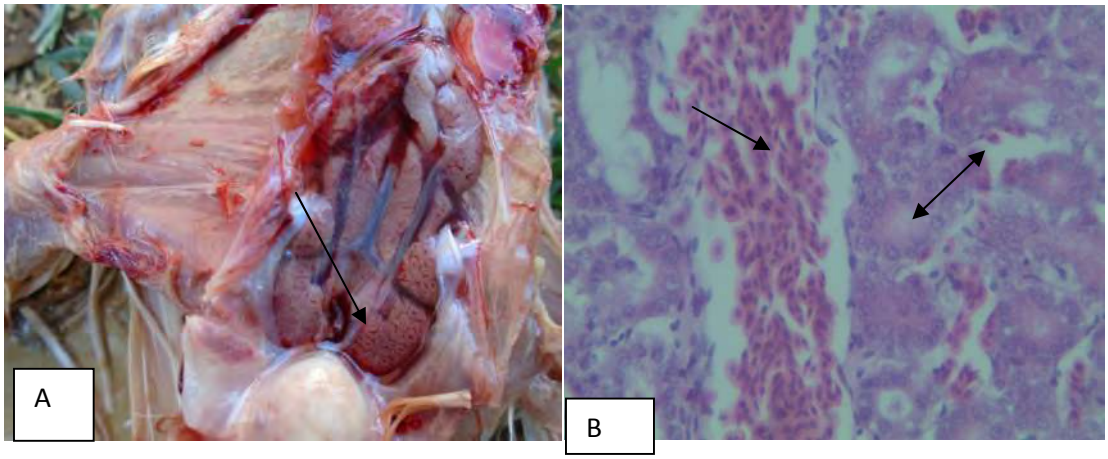

Figure C: Gross (A) and microscopic changes (B) in chicken kidney due to IBD infection. Swollen caudal lobe kidney (arrow), hemorrhage, proteinous edema in tubules and infiltration of heterophils ((B: arrow and double arrow)

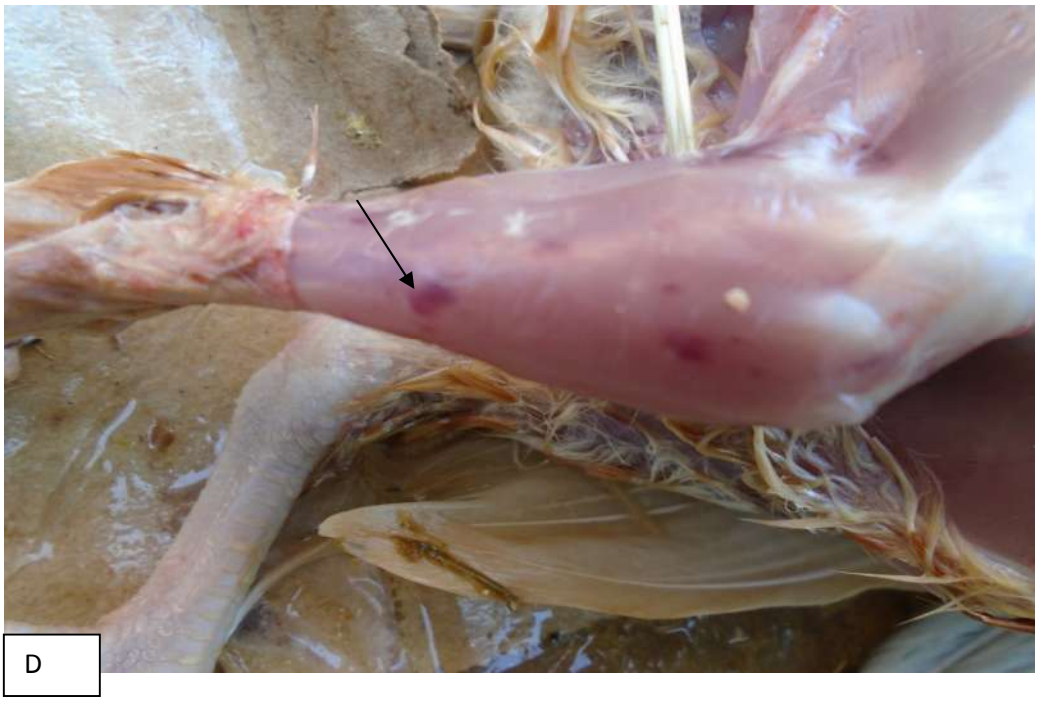

Figure D: Petichial hemorrhage (arrow) on thigh muscle of IBD infected 52 day old chicken.

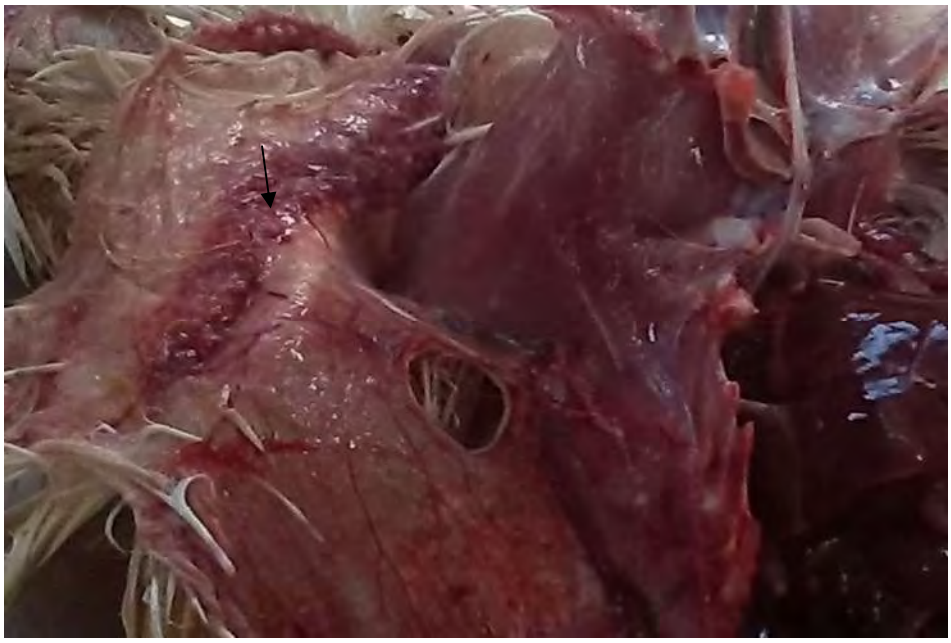

Figure E: Congested cutaneous blood vessel (arrow) in IBD infected chickens

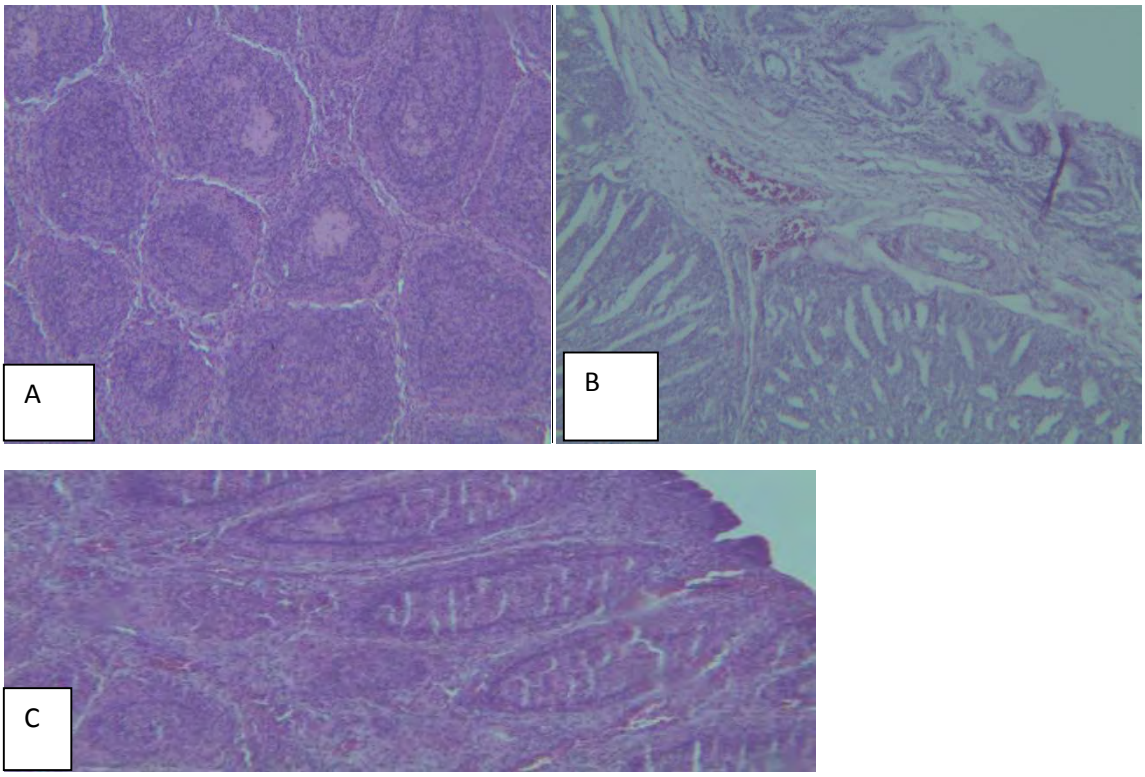

Figure F: Lesion score in bursa of IBD infected chickens (mild A), sever (B) ad moderate (C).