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**PATHOLOGICAL STUDY ON CLINICALLY SICK, DEAD AND SLAUGHTERED
CHICKENS IN AND AROUND BISHOFTU, ETHIOPIA**



BY

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MVSC IN VETERINARY PATHOLOGY**

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BISHOFTU, ETHIOPIA

**PATHOLOGICAL STUDY ON CLINICALLY SICK, DEAD AND SLAUGHTERED
CHICKENS IN AND AROUND BISHOFTU, ETHIOPIA**



**A Thesis Submitted to the College of Veterinary Medicine and Agriculture of Addis Ababa
University in the Partial Fulfillment of the Requirements for the Degree of Master of
Science in Veterinary Pathology**

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DEDICATION

I dedicate this thesis of manuscript to my beloved father, Tadessa Gurmessa, for nursing me with affection, love and for his dedicated partnership in the success of my life, who passed away on July 03, 2018 G.C without seeing my master's program achievement and to my beloved wife, Hawi Babu, who is special throughout my life.

STATEMENT OF THE AUTHOR

By my signature below, I declare and affirm that this Thesis is my own work and any scholarly material used in preparation of the Thesis has been given recognition through citation. I solemnly declare that it has not been submitted to any other institution anywhere for the award of any academic degree, diploma or certificate. This Thesis is submitted in partial fulfillment of the requirements for Master of Science degree at the Addis Ababa University.

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LIST OF ABBREVIATIONS

AAU	Addis Ababa University
APEC	Avian pathogenic <i>Escherichia coli</i>
CNS	Central nervous system
CSA	Central Statistics Authority
EARO	Ethiopian Agricultural Research Organization
ELISA	Enzyme-linked immunosorbent assay
ESAP	Ethiopian Society of Animal Production
FAO	Food and Agriculture Organization
FT	Fowl Typhoid
GDP	Gross domestic product
IBD	Infectious bursal disease
IBDV	Infectious bursal disease virus
IC	Infectious coryza
ILRI	International Livestock Research Institute
MD	Marek's disease
MDV	Marek's disease virus
NAHDIC	National Animal Health Diagnostic and Investigation Center
ND	Newcastle disease
NDV	Newcastle disease virus
OIE	Office International Des Epizooties
PCR	Polymerase chain reaction
PD	Pullorum Disease
PMV	Paramyxoviridae
PT	Paratyphoid
USAID	United States Agency for International Development

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ABSTRACT

In Ethiopia, the contribution of poultry production to small holder farmer and country economy is still restricted by various factors mainly due to infectious and non-infectious diseases. To contribute to this end a case study with a purposive sampling was conducted from October, 2018 to May, 2019 in and around Bishoftu on the 135 chickens (25 clinically sick, 10 dead and 100 slaughtered chickens) which aimed to investigate clinical signs, necropsy findings and histological lesions with isolation of bacterial agents and finally correlate the results with the problems. In the present study, all of the clinically sick chickens were found with at least two of the major clinical signs recorded and 24 (96%) of them were clinically depressed with diarrhea, respiration problems, discharges, decrease in egg production, retarded growth, paralysis and other signs frequently. By necropsy findings, the presence of gross lesions were examined in terms of consistency, texture, shape, size and color of organs in all cases and the main gross lesions recorded included mild to severe hemorrhage and congestion, severe and diffused necrosis, ulceration/necrosis, abnormal size and discoloration, nodules on the organs, ascites and fibrinous inflammation in different organs of the examined chickens. Among the examined organs grossly, the most affected organ was liver, 106 (78.5%) followed by caecal tonsil, 25 (71.4%), trachea, 22 (62.9%), duodenum, 20 (57.1%), jejunum and air sacs, 19 (54.3%) of both. Corresponded to these gross lesions, the major histopathologic lesions recorded were mild to severe infiltration with polymorpho nuclear and mono nuclear cells, mild to severe congestion, hemorrhages and necrosis, massive depletion of lymphoid tissues, development of cysts, sloughed mucosal epithelium, edema and degenerative changes. Moreover, *Escherichia coli*, *Salmonella spp.*, and *Staphylococcus aureus*, from most to least, were isolated and *Coccidia* oocytes were also identified by microscopic examination. The correlation of the overall results of the present study identifies the presence of infectious diseases as the main constraints hampering the productivity and reproductively of the chickens in the study area which needs long term solution based on the scientific investigation.

Key Words: *Chickens, clinical signs, Ethiopia, gross lesions, histopathologic lesions.*

1. INTRODUCTION

Poultry sector is a fastest growing among the animal production activities offers an opportunity to feed the fastest growing human population and provide income resources for poor farmers (Safari *et al.*, 2004; CSA, 2009). Moreover, poultry, in many parts of the modern world is considered as the chief source of not only cheaper protein of animal origin but also of high quality human food. Important factors in continued growth of the poultry industry in many countries are the efficiency of poultry in converting vegetable protein into animal protein, attractiveness and acceptability of poultry meat and egg to many people (Jordan, 1990; Jordan *et al.*, 2002).

Raising poultry has also a number of advantages over the other livestock sectors which include fast return on investment, spreading income throughout the year, high return compared to feed cost, low land requirement, adaptability to both small part time enterprise and large commercial enterprise and the operation can be highly mechanized with high output per hour or labor (Gillespie, 1998; Yenesew *et al.*, 2015).

Poultry breeding in Ethiopia has long traditional practice, which is mainly used as an immediate cash income for the rural communities in which especially, women are more involved in keeping backyard for egg collection and selling adult chickens. There are village or backyard, small scale and commercial poultry production systems. Backyard poultry production is the predominant system in Ethiopia and account for nearly 99% of the poultry population consisting mainly of local chicken breeds under individual farm household management and it is also common to find a few exotic breeds distributed through the extension programs. Small scale poultry production system comprises a flock size ranging from 50 to 500 exotic breed operation commercial bases and outdoor with a low bio-security level. Commercial poultry production system is highly intensive production system that involves greater than 10,000 birds kept under in door and heavily depends on imported breeds (Dawit *et al.*, 2008; Abraham and Yayneshet, 2010; Alem, 2014)

Despite these facts, the contribution of poultry production to small holder farmer and country economy is still restricted by various factors like low input of feeding, poor management, prevalence and wide distribution of infectious and non-infectious diseases augmented with poor veterinary services, lack of appropriate selection and breeding practices, even if the attempt are underway to enhance chicken productivity and their contribution through importing exotic chickens and also by cross breeding and distributing improved breeds to poor farmer living in rural part of Ethiopia (Giambron *et al.*, 1999; Taddelle *et al.*, 2003; Zelleke *et al.*, 2005).

In Ethiopia, the fast growing and young poultry industry is facing many of the above mentioned constraints from which the infectious diseases are the most predominant once with a significant impact on the poultry industry development. Most of the infectious diseases are endemic in the country and some of them are emerging and re-emerging diseases. Generally, poultry diseases are responsible for a number of adverse economic effects due to mortality and morbidity of chickens, cost of medication, miscarriage in production and international trade ban and public health significance (Dana *et al.*, 2008; Yenesew *et al.*, 2015).

Poultry mortalities due to disease are estimated to range from 20% to 50% but they can rise as high 80% during epidemics in Ethiopia. Diseases are also considered to be the most important factor responsible for reducing both the number and productivity of chickens. Poultry disease such as Newcastle disease (ND), Coccidiosis, Infectious Bursal disease (IBD), Avian Salmonellosis, Avian Colibacillosis and nutritional deficiency are considered to be the most endemic and the one to incur huge economic losses (Serkalem *et al.*, 2005; Zeleke *et al.*, 2005b; Aberra and Tegene, 2009).

Even though, there are many studies on diseases which are endemic to Ethiopia as a country, it is important to study and specify the diseases based on their clinical signs, necropsy findings, histopathological examination and as well as bacterial isolation in clinically sick, dead and slaughtered chickens which is poorly described and documented in Ethiopia till today. Unfortunately diagnosis of poultry diseases at field level in Ethiopia is limited within recording of clinical history and signs with post-mortem findings without these diagnostic methods where there is every chance of wrong diagnosis because the signs and post-mortem findings are more or

less similar in most of the poultry diseases. This is the reason why the report of correlation between clinical signs and necropsy finding as well as histological examination of the affected internal organs with isolation and identification of the bacterial agent in clinically sick, dead chickens and slaughtered chickens is yet available in the country.

Therefore, the objectives of the present study were conducted to:

- ✚ Identify the types and frequencies of macroscopic and microscopic lesions within the organs of the clinically sick, dead and slaughtered chickens.
- ✚ Correlate the clinical signs, gross and microscopic pathological lesions with the problem.
- ✚ To isolate bacterial agents as additional diagnosis in identifying and specifying the problem.

2. LITERATURE REVIEW

2.1. Poultry Production Systems in Ethiopia

Agriculture is by far the largest sector of the Ethiopian economy, encompassing diverse economic and physical environments. Crops, fisheries and livestock are the most important sub-sectors within the agricultural sector which contributing the highest percent of the agricultural Gross domestic products (GDP). Poultry production is an important and integral part of most Ethiopian households in rural, urban and peri-urban areas. In Ethiopia, chickens are the most widespread and almost every rural family owns chickens, which provide a valuable source of family protein and income (Tadelle *et al.*, 2003). The total chicken population in the country is estimated to be 56.5 million with native chicken representing 96.9%, hybrid chicken 0.54% and exotic breeds 2.56% according to the reports of Central Statistics Authority (CSA) (2014) which increased by about 3.5 millions within two years and estimated to be about 60 millions in 2016 (CSA, 2016). The most dominant chicken types reared in Ethiopia are local ecotypes, which show a large variation in body position, plumage color, comb type and productivity (Halima, 2007).

However, the economic contribution of the sector is not still proportional to the huge chicken numbers, attributed to the presence of many productions, reproduction and infrastructural constraints. The chicken production system in Ethiopia can be characterized by not market oriented, low input, scavenging and traditional management system consisting of local breeds using criteria such as breed, size of the flock, management conditions, purpose of production, degree of commercialization and location. Ethiopian chicken production systems can be classified as village (sometimes also called traditional or backyard), semi-intensive, and modern (or intensive) production systems. The indigenous birds are small in body size and low producers of meat and egg (Aberra, 2000; Halima, 2007).

2.2. Challenges and Constraints of Poultry Production in Ethiopia

Under farmer management poultry production, prevailing disease, predators, market problem, lack of water, lack of proper health care, poor feeding and extension together with veterinary services are reported as the major constraint by Moges *et al.* (2010), Getu and Birhan (2014), Negussie and Ogle. (1999) and Mengesha *et al.* (2011). Over the all, diseases are the first frequently occurred chicken production constraint. The other comprehensive study showed that Newcastle Disease (ND) is highly infectious and causes more losses than any other diseases in the tropics and it spreads rapidly through the flock and mortality could reach up to 100% (Nigussie *et al.*, 2003).

2.2.1. Genetic resources

Genetic resources are often cited as a constraint, due to the comparatively low productivity of indigenous chickens and unavailability of alternative breeds. The majority of Ethiopian chickens are low producing indigenous birds. The productivity of scavenging hens is low, not only because of their comparatively smaller size, slower growth rates and lower egg production, but also due to a long reproductive cycle due to the natural traits of incubation and brooding. (Tadelle and Ogle, 1996; Tadelle *et al.*, 2003).

2.2.2. Quality and availability of feed

Feed availability: Poultry feed and nutrition is one of the most critical constraints to poultry production under both the rural small holder and large-scale systems in Ethiopia. The problem is mainly associated with lack of processing facilities, inconsistent availability and distribution and sub-standard quality of processed feeds, when available. Regular availability of good quality ingredients and a fully balanced complete feed are essential for efficient poultry production. The most serious problems arise from the unavailability of suitable micro-nutrient sources: vitamins and minerals which are not obtained locally (Tadelle *et al.*, 2002; Demeke, 2004; Mazengia *et al.*, 2012; Dessie *et al.*, 2013).

Feed quality and Costs: Yami and Desie (1997) reported that the quality of mixed feed for commercial poultry production is generally poor in Ethiopia. Most formulations available do not have vitamin/mineral premixes. Ingredients and processed feeds vary in nutritive value and there is no regular quality control mechanism in the country. Unavailability of feed quality legislation and laboratory facilities for chemical analysis also contributes greatly to the poor quality of processed feeds. In relation to the feed cost it is believed that considerable scope exists to reduce the price of feed in some areas without reducing its nutritive value (Demeke, 2004; Dessie *et al.*, 2013).

2.2.3. Poor institutional linkage

In Ethiopia, institutions engaged in agricultural development do not have well-established links to each other or the developing poultry industry. As such, research outputs that could potentially contribute to the development of the sector are not readily available to the main industry stakeholders or to rural producers. This includes research relevant to increasing the production potential of indigenous chickens that have demonstrated promising results in trials, but are not implemented more widely (EARO, 2002; Avery, 2004; Abebe, 2006; FAO, 2008).

2.2.4. Limited production capacity

Institutions engaged in chicken production and development, such as research institutions, regional multiplication centers and private commercial poultry farmers, have limited capacity to meet the growing human population and the associated increased demand for meat. The extension system has been attempting to enhance production and productivity of the poultry sector, especially the village system which has been contributing to the largest share of egg and chicken meat supply of the country. However, the interventions are often piecemeal, failing to incorporate necessary inputs and account for local husbandry practices (Teklewold *et al.*, 2006; Augustine, 2010).

2.2.5. Diseases and predators

Diseases: In Ethiopia, the presence of endemic infectious diseases affects production in two main ways. Firstly, periodic disease outbreaks may result in the loss of almost the entire flock in the village chicken production systems. Secondly, the constant presence of infectious disease can lead not only to illness and death but also reduce productivity. Infectious diseases, such as Newcastle disease, salmonellosis, fowl cholera, coccidiosis and Gumboro are some of the major reported problems which are the causes of morbidity and mortality in small scale and large-scale poultry farms in addition to village poultry. The level of biosecurity implementation as a measure to prevent the entry of disease causing agents into intensive farms is low, due to limitations in knowledge and skill, as few producers have access to training. Moreover, vaccine packaging in large multi-dose vials that are not cost-effective for small producers, and a lack of veterinary service or an organized village level delivery system are major barriers to implementing vaccination for economically important poultry diseases in village chickens specially (Tamirat *et al.*, 2004; Sambo *et al.*, 2015; Habte *et al.*, 2017).

Predators: Predators are among the major constraints that cause chicken deaths, especially in the village production system. Snakes, rats, dogs, cats and foxes are the main predators that cause losses of younger birds. Wild birds (eagles, hawks, etc.) during the dry season and wild cats (locally known as shelemetmat) during the rainy season are the most dangerous predators that attack older chickens (Tadelle *et al.*, 2003; Sambo *et al.*, 2015).

3. MAJOR DISEASES OF POULTRY IN ETHIOPIA

There are different diseases which hampering the production and productivity of chickens in this country. These diseases are of bacterial, viral, fungal and parasitical origin. It is becoming a growing concern that there is introduction of diseases of various etiologies into several poultry farms concurrent with importation of exotic breeds to backyard chickens in Ethiopia from time to time which greatly affecting the economy of the country starting from the individual to national wide. Some of the major infectious disease of chickens reported by different authors in Ethiopia includes Newcastle disease (NCD), Infectious Bursal disease (IBD), Marek disease (MD), Avian Salmonellosis, Avian Colibacillosis, Coccidiosis and others (Eshetu,2001; Tadelles and Ogle, 2001; Zeleke *et al.*, 2005a; Zeleke *et al.*, 2005b; Abera and Tegene, 2009; Yenesew *et al.*, 2015; Habte *et al.*, 2017).

3.1. Chicken Viral Diseases

3.1.1. Newcastle disease

Newcastle disease (ND) is a highly contagious and the most dreaded disease of chickens, turkeys primarily and many other wild and domestic birds are susceptible (Alexander and Jones, 2001). It is a viral disease that can spread rapidly through a flock and the first disease which is suspected during any disease outbreak in Ethiopia. This disease is characterized by a high variability in morbidity, mortality, clinical signs and lesions. Several different strains of virus exist and are circulating in Ethiopia, ranging from mild strains to ones which can cause 100% mortality in an unprotected flock (Alexander, 2000; Mazengia *et al.*, 2010; Habte *et al.*, 2017).

Etiology: Newcastle disease virus (NDV) is an enveloped virus which is part of the recently described genus *Avulavirus* belonging to the family *Paramyxoviridae* (PMV). Different strains of NDV belong to serotype PMV-1 but antigenic variations can be identified within the group, mainly by using monoclonal antibodies (Aldous *et al.*, 2003; Meulemans, *et al.*, 2015).

Transmission: NDV transmission between poultry occurs through fecal-oral and aerosol route is probably the main mode of transmission since the infected birds shed virus in the feces. In this way the droplets and contaminated aerosol can be inhaled by healthy birds and affect the mucosa of their upper respiratory tract, while droppings can contaminate feed and drinking water and thus be eaten by other birds in the house. The dispersion of this virus may also be from farm to farm via the transportation of contaminated material (soil, feed, water, footwear, clothing, equipment and litter) and from wild birds (Alexander, 1988; Habte *et al.*, 2017).

Clinical Signs: The incubation period of ND after natural exposure has been reported to vary from 2-15 days (average 5-6 days). Newcastle disease virus isolates can be broadly grouped into pathotypes on the basis of clinical signs, which in turn are affected by the strain of virus. Other factors also important in establishing the severity of the disease are the host species, host age, host immune status, coinfection with other organisms, environmental stress, social stress, route of exposure, and the virus dose (Lancaster and Alexander, 1975; Collins *et al.*, 1994; Hailu, 2012).

In general the different strains of PMV-1 are classified into five pathotypes based on the clinical signs observed in susceptible chickens: **a) Velogenic viscerotropic strains** which cause high mortality (up to 100%) associated with characteristic intestinal damage; **b) Velogenic neurotropic strains** that cause very high mortality (up to 100%) associated with respiratory and nervous disorders; **c) Mesogenic strains** which are responsible for respiratory and nervous disorders associated with a low mortality rate in adults but a high mortality rate among young birds (up to 50%); **d) Lentogenic strains** those cause only respiratory disorders without mortality neither in young nor adult birds and **e) Lentogenic asymptomatic strains** cause no clinical signs but affect the economy greatly which are identified only by isolation from droppings (Beard and Hanson, 1984; Alexander and Senne, 2008; Mazengia *et al.*, 2010; Meulemans, *et al.*, 2015).

Moreover, the mild form of the virus generally causes respiratory symptoms (nasal discharge, dyspnea, coughing), and low mortality; but the severe one generally causes high mortality, with respiratory and nervous system problems including twisting of the head (torticollis), green

diarrhea, swollen heads, conjunctivitis, cyanosis of the comb and wattles, periocular swelling and paralysis followed by death in two to three days are often noticed. In layers, almost complete stop in egg-laying is indicative of this disease (Alexander and Senne, 2008; Habte *et al.*, 2017).

Pathological changes: The pathologic changes associated with ND vary greatly from bird to bird, flock to flock and from one geographic region to another (Chuhhan and Roy, 1998).

a) Gross lesions: In addition to the host and all the other factors that may affect the severity of the disease, the gross lesions and the organs affected in birds infected with NDV are mainly dependent on the strain and pathotype of the infecting virus. No pathognomonic lesions are associated with any form of the disease and also gross lesions may be absent. But, the presence of prominent hemorrhagic lesions in the intestines, proventriculus and caeca of infected chickens is the evidence of very virulent NDV (Hanson, 1980; Alexander and Jones, 2001).

Cadavers of birds that dead because of virulent NDV, usually have a dehydrated appearance which is particularly prominent in the proventriculus, small intestine and caeca. These organs are markedly hemorrhagic which apparently results from necrosis of the intestinal wall or lymphoid tissues, such as caecal tonsils. The presence of predominant hemorrhagic lesion and congestion of the trachea as well as air sacculitis may be the gross lesion evident in respiratory tract in birds infected by this disease. Egg peritonitis, flaccid and degenerative ovarian follicles and hemorrhage and discoloration of the other reproductive organs may also seen in laying hens affected with virulent NDV. Gross pathology seen in other organs includes hemorrhage in the lower conjunctiva, focal necrosis of the spleen, and tracheal edema, most generally observed near the thoracic inlet (Alexander and Allan, 1974; Beard and Hanson, 1984; Chuhhan and Roy, 1998; Murphy *et al.*, 1999; Alexander and Jones, 2001; Meulemans, *et al.*, 2015).

b) Microscopic Lesions: The histopathology of NDV infections is as varied as the clinical signs and gross lesions and can be affected greatly by the same parameters (Beard and Easterday, 1976). In general, in most tissues and organs involved, the lesions include hyperemia, necrosis, cellular infiltration, hemorrhage and edema. Lesions seen in the central nervous system (CNS) are those of a non-purulent encephalomyelitis with neuronal degeneration, foci of glial cells,

perivascular infiltration of lymphocytes and hypertrophy of endothelial cells. Congestion, edema, and hemorrhage are found associated with the blood vessels of many organs in vascular system. Hyperplasia of the mononuclear phagocytic cells in various organs, especially the liver, may take place in subacute infections and necrotic lesions presence in the spleen. Focal vacuolation and destruction of lymphocytes, in the cortical areas and germinal centers of the spleen and thymus as well as marked degeneration of lymphocytes in the medullary region are seen in the bursa of Fabricius (Beared and Hanson, 1984; Murphy *et al.*, 1999; Al-Garib, 2003).

Hemorrhage and necrosis of mucosal lymphoid tissue are seen in the intestinal tract with infections of some virulent forms of ND. In the mucosa of the upper respiratory tract, congestion, edema, and dense cellular infiltration of lymphocytes and macrophages are seen. Changes in female reproductive organs included atresia of follicles with infiltration of inflammatory cells and the formation of lymphoid aggregates (Stevens *et al.*, 1976; Alexander and Senne, 2008; Hailu, 2012).

Diagnosis: Even if the clinical signs, lesions and the general epidemic context can often lead one to suspect ND, it should be confirmed by isolation and identification of the virus. Direct detection of viral antigens by immunohistologic techniques offer a rapid method for the specific demonstration of the presence of virus or viral antigens in organs or tissues. And also molecular techniques, especially polymerase chain reaction (PCR) is useful for the diagnosis of this virus with other tests (Hamid *et al.*, 1988; Lockaby *et al.*, 1993; Gohm *et al.*, 2000).

Prevention: Effective control of ND requires good sanitation, management, quarantine, an appropriate vaccination program, and monitoring system, including serotyping and pathogenicity testing of isolated virus. Biosecurity, including sanitation, is considered the first line of protection against the introduction of any pathogenic agent and in particular against ND. A good biosecurity program must also prevent the direct and indirect contact of poultry with wild birds such as pigeons and waterfowl (Meulemans, 1988; Nasser, 1998).

3.1.2. Gumboro/Infectious bursal disease

Infectious bursal disease (IBD) is an acute, highly contagious viral infection of young chickens that has lymphoid tissue as its primary target with a special predilection for the bursa of Fabricius (cloacal bursa). At the beginning it was recognized by Cosgrove (1962) in 1962 and it was referred to as “Avian Nephrosis” because of the extreme kidney damage found in birds that succumbed to infection, as a specific disease entity. The disease is considered endemic in most part of Ethiopia (Cosgrove, 1962; Woldemariam and Wossene, 2007; Eterradossi and Saif, 2008).

Etiology: Infectious bursal disease virus (IBDV) is a member of the *Birnaviridae* family. The family has 3 genera designated as *Aqua birnavirus* which infects fish, molluscs, and crustaceans; *Avibirnavirus* which infects birds; and *Entomobirnavirus* which infects insects. This IBDV causes an immunosuppressive disease in young chickens. The virus replicates in the bursa of fabricius and destroys B lymphocytes and also causes a significant reduction in the functions of T-cells. (Müller *et al.*, 1979; Brown, 1986; Müller *et al.*, 2003; Delmas *et al.*, 2004).

Transmission: IBDV is persistent in the environment of a poultry house and is very infectious and spreads easily from bird to bird via droppings through which the infected birds excrete virus in their dropping at least for 14 days. Infected clothing and equipment may also act as a mean of transmission within and between farms. Chickens are the only known avian species to develop clinical disease and distinct lesions when exposed to IBDV. The most common mode of infection is through the oral route and conjunctival and respiratory routes may also be involved in general (Lukert and Saif, 1997; Sharma *et al.*, 2000; Baxendale, 2002; Habte *et al.*, 2017).

Clinical Signs: The clinical signs of the disease are seen within 2-3 days after exposure. But IBD infection may result in a chronic or acute form of disease in which both forms targets the bursa of fabricius. Acute IBD occurs in birds around 3-6 weeks of age and causes both immunosuppressive and disseminated infection with damage throughout the body. The virus causes a clinical sings characterized by the tendency of some birds to pick at their own vents at the first, anorexia, high morbidity and low mortality, depression with ruffled feathers, often

huddle together, trembling, mild whitish or watery diarrhea, soiled vent feathers and severe prostration and finally, death (Lukert and Saif, 1997; Le Nouen *et al.*, 2006; Jackwood, 2015).

Pathological changes: a) Gross lesions: Frequently, hemorrhages in the skeletal muscles (thigh, breast and pectoral muscles) and large edematous bursa (often yellowish in color) are observed in the infected chicken. Infected bursa increase in size and weight because of edema, hyperemia and a gelatinous yellowish exudate covering the serosal surface. Necrotic foci and petechial or ecchymotic hemorrhages on the mucosal surface are also the character of the infected bursa. The slightly enlarged size of spleen with very often small gray foci uniformly dispersed on the surface, the presence of hemorrhages in the mucosa at the juncture of the proventriculus and gizzard occasionally and more severe lesions in the caecal tonsils, thymus, spleen, and bone marrow are observed in the IBD grossly. There is also increased mucus in the intestine, and renal changes may be prominent in birds that die or are in advanced stages of the disease (McFerran, 1993; Tanimura *et al.*, 1995; Eterradosi and Saif, 2008).

b) Microscopic Lesions: Microscopic lesions of IBD occur primarily in the lymphoid tissues (i.e., cloacal bursa, spleen, thymus and caecal tonsil), but it is severe in the cloacal bursa than others. Histological lesions in the bursa include severe lymphocyte depletion accompanied by inflammation. At the early infection time, there is degeneration and necrosis of lymphocytes in the medullary area of bursal follicles and lymphocytes (B-cells) are soon replaced by heterophils, pyknotic debris, and hyperplastic reticuloendothelial cells. The increase in bursal weight seen at this time is mainly caused by severe edema, hyperemia, and marked accumulation of heterophils. Hemorrhages often appeared but are not a consistent lesion. All lymphoid follicles are affected by 3 or 4 days post-infection. As the inflammatory reaction declined, cystic cavities develop in medullary areas of follicles (depletion of B-cells), necrosis and phagocytosis of heterophils and plasma cells occurred; and there is a fibroplasia in interfollicular connective tissue (Hanson, 1978; Chuahan and Roy, 1998; Baxendale, 2002; Negash, 2004; Withers *et al.*, 2005; Jackwood, 2015).

The spleen has hyperplasia of reticuloendothelial cells around the adenoid sheath and thymus and caecal tonsils exhibited some cellular reaction in the lymphoid tissues in early stages of

infection, but, in the spleen, the damage is less extensive than in the bursa. In addition to these, histologic lesions of the kidney are non-specific and probably occur because of severe dehydration of affected chickens. The lesions observed are large casts of homogeneous material infiltrated with heterophils. The liver may have slight perivascular infiltration of monocytes (Withers *et al.*, 2005; Eterradossi and Saif, 2008; Jackwood, 2015).

Diagnosis: Confirmation of the diagnosis can be made at necropsy by examination for characteristic grossly visible changes in the cloacal bursa. Infections of chickens of any age with variant strains of IBDV will be detected only by histopathology of the cloacal bursa or by virus isolation. Both serology enzyme-linked immunosorbent assay (ELISA) and molecular methods can be used to diagnose infection in a flock (McFerran *et al.*, 1980; Jackwood and Saif, 1987; Mekkes and de Wit, 2002).

Prevention: The sanitary precautions that are applied to prevent the spread of most poultry infections must be rigorously used in the case of IBD. Additionally, control and prevention of this immunosuppressive disease can be achieved by vaccination with live-attenuated and/or inactivated viruses. Because the immunosuppressive effects of an IBDV infection are most pronounced in birds infected at a young age, maternal antibodies transferred via the yolk are used to protect chicks during the first several weeks of life (Whitfill *et al.*, 1995; Gagic *et al.*, 1999; Negash *et al.*, 2004).

3.1.3. Marek's disease

Marek's disease (MD) is a common lymphoproliferative disease of chickens, usually characterized by mononuclear cellular infiltrates in peripheral nerves and various other organs and tissues including iris and skin. Classical Marek's disease affects the nervous system leading to paralysis typically birds with a 'hurdle jumper' appearance and lead to eye infection that may cause blindness. Infection leads to transformation of lymphocytes in the blood causing them to become cancerous. This leads to lymphoma or cancer of the blood system which is usually fatal (Nair and Kung, 2004; Karel *et al.*, 2008; Habte *et al.*, 2017).

Etiology: Marek's disease virus (MDV) is a *herpesvirus* (*Gallid herpesvirus 2* or *GaHV*) which are cell-associated viruses, belonging to the family *Herpesviridae*, the subfamily *Alphaherpesviridae* subgroup *Mardivirus*. These viruses are closely related antigenically, and are further classified into three serotypes that include: serotype 1 (MDV1) viruses which are oncogenic and the etiological agents of MD; serotype 2 isolates which are common in chickens and are non-oncogenic and the serotype 3 isolates which are thought to be ubiquitous in turkeys and are non-oncogenic. All three serotypes are antigenically cross-reactive (Chuahan and Roy, 1998; Hailu, 2012).

Transmission: MDV is transmitted readily by direct or indirect contact between chickens, apparently by the airborne route. Epithelial cells in the keratinizing layer of the feather follicle replicate fully infectious virus, and these cells serve as a source of contamination to the environment and to other chickens. Virus associated with feathers and dander is infectious and contaminated poultry house dust remains infectious for at least several months at 20-25°C and for years at 4°C. In intensive commercial production, the virus is easily spread by feather dander and dust as birds are kept in close proximity to each other in enclosed housing (Bigg, 1985; Schat, 1985; Calnek, 1986; Miles, 2015).

Clinical Signs: Clinical signs of MD usually appear at about 3 weeks of age and peak between 2 and 7 months. Depression, gray eyes and blindness due lymphoid infiltration in to the iris, lameness/ incoordination or stilted gait, paralysis due to infected brain, skin problem due to infected the feather follicles and unthriftiness are the main clinical manifestation of the disease. A particularly characteristic clinical presentation is a bird with one leg stretched forward and the other back as a result of unilateral paresis or paralysis of the leg (jumper leg) (Ficken *et al.*, 1991; Gimeno *et al.*, 1999; Gimeno *et al.*, 2001; Witter and Schat, 2003).

Pathological changes: a) **Gross lesions:** these pathologic changes mainly of nerve lesions and visceral lymphomas. The most commonly affected nerves that are easily seen on post mortem examination are the brachial and sciatic plexus nerves, celiac plexus, abdominal vagus and intercostal nerves. Severely affected peripheral nerves may show loss of cross striations, gray or yellowish appearance and edematous and often are 3-4 times than their normal thickness due to

the cellular infiltration of peripheral nerves (Chuahan and Roy, 1998; Murphy *et al.*, 1999; Payne, 2004).

Lymphomatous lesions can be found in the gonad (especially the ovary), lung, heart, mesentery, kidney, liver, spleen, bursa, thymus, adrenal gland, pancreas, proventriculus, intestine, iris, skeletal muscle, and skin. Visceral lymphomas/tumors are common in more virulent forms of the disease. Lymphomas may occur as focal, nodular growths of varying size. Nodules are white or gray in color and are firm, and the cut surface is smooth. In integumentary system, skin lesions with infected feather follicles are evidence of the disease. The nodular lesions may involve few scattered follicles, or they may be numerous and coalesce. The distinct whitish nodules, especially evident in dressed carcasses, may become scab like with brownish crust formation in extreme cases. In addition to this, gross ocular changes also include loss of pigmentation in the iris (“gray eye”) and irregularity of the pupil in which both are the result of mononuclear infiltration of the iris (Payne, 1985; Witter, 1997; Ficken *et al.*, 1991; Murphy *et al.*, 1999; Venugopal *et al.*, 2001; Payne, 2004).

b) Microscopic Lesions: Numerous workers tried to describe the histopathologic changes associated with MD. The neoplastic, consisting of masses of pleomorphic lymphocytes, in some cases, demyelination and Schwann cell proliferation and the inflammatory cells which are characterized by diffuse, light-to-moderate infiltration by small lymphocytes and plasma cells and /or sometimes with a few macrophages, and usually with edema are associated with the microscopic lesions in the peripheral nerves. Mild perivascular cuffing usually accompanied by gliosis but without primary demyelination as the principal central nervous system lesion is identified in the brain. Lymphomatous lesions in visceral organs are more uniformly proliferative in nature than those in nerves and the cellular composition is similar to that of the proliferative lesions in the nerves (Payne, 1972; Payne, 2004; Karel *et al.*, 2008).

Lesions in the skin appear largely inflammatory but may also be lymphomatous which are usually are localized around infected feather follicles with the compact aggregates of proliferating cells, often perivascular, and a few plasma cells and histiocytes those may be seen in the dermis. Mononuclear infiltration of the iris that may also be found in eye muscles,

especially in rectus lateralis and ciliaris is the most constant change in the eye. Acute cytolytic changes which occur in both bursa of Fabricius and thymus due to the *herpesvirus* replication in them are accompanied by atrophy. The critical lesion in transient paralysis is vasculitis which resulted in vasogenic brain edema in central nervous system (CNS). Induced atherosclerosis include proliferative and fatty-proliferative changes in aortic, coronary, celiac, gastric, and mesenteric arteries are reported as the main microscopic lesions associated with MDV in the blood vascular system. Furthermore, blood leukocyte counts may be elevated, largely because of increased numbers of large lymphocytes and lymphoblast (Kornegay *et al.*, 1983; Calnek, 1986; Swayne *et al.*, 1989; Ficken *et al.*, 1991; Miles, 2015).

Diagnosis: Even if the clinical signs, gross and histologic examination can enhance the accuracy of diagnosis, the diagnosis of the clinical disease remains difficult in practice for several reasons. Since the gross lesions of MD can resemble those of other neoplasms and unrelated conditions characterized by tumors in visceral organs or grossly enlarged nerves, there is no truly pathognomonic gross lesion exists for MD. However, the serology (ELISA) and molecular methods, such as PCR, can be used to detect carrier birds. Moreover, the principal methods to identify the presence of infection are isolation of the virus, demonstration of viral Deoxyribonucleic Acid (DNA) or antigens in tissues, and detection of antibody (Sharma, 1998; Davidson and Borenstein, 1999; OIE, 2004; Zelnik, 2004).

Prevention: Proper application of an appropriate vaccine and strict biosecurity practices to limit the extent of early MDV exposure is the best way of prevention and control of this disease (Pattison, 1985; Kreager, 1996; Venugopal *et al.*, 2001; Hailu, 2012).

Table 1: Summary of occurrences of Newcastle and infectious bursal diseases in certain areas of Ethiopia.

Disease	Location	Period/year	Mortality rates (%)	Prevalence/ Incidence (%)	References
Newcastle disease	Central highlands of Ethiopia (Dembi, Shola and Lemlem poultry farms)	1983-1995	14.90	58.50	Nasser, 1998
	State poultry farms of Ethiopia	1983-1995	2.3	-	Nasser, 1998
	Central high lands of Ethiopia	1999-2000	43.7	-	Ashenafi, 2000
	Rift valley	2004-2005	22.51	-	Zelege <i>et al.</i> , 2005a
	Wet highland districts	2004-2005	14.13	-	“ “
	Bahir Dar District	October 2007-April 2008	-	29.60	Mazengia <i>et al.</i> , 2010
	Farta District	October 2007-April 2008	-	21.70	“ “
	Debre Zeit overall (mortality)	2004-2005	49.89	-	Zelege <i>et al.</i> , 2005b
Infectious bursal disease	Layer (morality)	2004-2005	25.08	-	“ “
	Broiler (mortality)	2004-2005	56.09	-	“ “
	Debre Zeit (seroprevalence)	2004-2005	-	93.30	“ “
	Andasa poultry farm (young mortality)	Oct-Nov 2006	72	-	Woldemariam and Wossene, 2007
	Andasa poultry farm (adult mortality)	Oct-Nov 2006	7	-	“ “
	Andasa poultry farm (seroprevalence)	Oct-Nov 2006	-	100	“ “
	Bahir Dar	October 2007-April 2008	38.9	29.40	Mazengia <i>et al.</i> , 2009, 2010
	Farta	October 2007-April 2008	17.40	21.70	“ “

Source: (Hailu, 2012).

3.2. Chicken Bacterial Diseases

3.2.1. Avian Salmonellosis

Infections bacteria with the genus *Salmonella* are responsible for a variety of acute and chronic diseases in poultry. Infections of poultry with *salmonellae* can be grouped into three categories: *S. pullorum* and *S. gallinarum* which are non-motile, host-specific for avian species, motile and non-host-adapted *Salmonella* collectively called as *paratyphoid salmonellae* and the third one is *S. enterica subsp. enterica arizonae*, a motile serotype (Shivaprasad, 2000; Gast, 2008; Shivaprasad, 2015).

I) Pullorum Disease (PD) and Fowl Typhoid (FT): Even if these two diseases are caused by different bacteria (i.e, *S. pullorum* and *S. gallinarum*, respectively), they are similar in terms of their history, clinical signs, epizootiology, lesions, and control and eradication procedures. Both are septicemic diseases affecting primarily chickens and turkeys, but other birds such as quail, pheasants, ducks, peacocks, and guinea fowl are also susceptible. (Pomeroy and Nagaraja, 1997; Snoyenbos, 1997; Shivaprasad and Barrow, 2008).

Etiology: PD and FT diseases are caused by *S. pullorum* and *S. gallinarum*, which have been placed in a single species, *S. enterica subsp. enterica serovar Pullorum-Gallinarum* of the family *Enterobacteriaceae*, which is highly host adapted, respectively (Shivaprasad, 2000).

Transmission: PD and FT can be transmitted in several ways. The infected bird (reactor and carrier) is by far the most important means of perpetuation and spread of the organism. Birds may infect not only their own generation by horizontal transmission, but also succeeding ones through egg transmission by trans-ovarian infection. Horizontally they transmitted via infected droppings (Feces), dead bird carcasses, contaminated feed, water, litter, utensils (any other fomites) and infected clothes and shoes of attendants, feed dealers, chicken buyers, visitors and who move from house to house and from farm to farm used on the farm. And also Contact transmission is reported (Barrow *et al.*, 1987; Shivaprasad *et al.*, 1990; Pomeroy and Nagaraja, 1997; Snoyenbos, 1997).

Clinical Signs: FT is a more significant disease in growing and adult chickens and turkeys than PD. **a) In chicks:** Dead chicks may be observed in the incubator, weakness, depressed appetite, poor growth, and adherence of chalky white material to the vent, an inclination to huddle together under heaters, having droopy wings, and distorted body appearance are appreciated. In PD Blindness, swelling of the tibiotarsal (hock joint) and the humero-radial and ulnar articulations due to synovitis and lameness in chicks has been described (Johnson *et al.*, 1992; Salem *et al.*, 1992; Mayahi *et al.*, 1995; Habte *et al.*, 2017).

b) In growing and mature chickens: A sudden drop in feed consumption, showing ruffled feathers and pale and shrunken combs, drop in egg production, decreased fertility, and diminished hatchability, observed in both PD and FT, depending upon the severity of infection (Bouzoubaa and Nagaraja, 1984; Erbeck *et al.*, 1993; Shivaprasad and Barrow, 2008).

Pathological changes: a) Gross lesions: i) In chicks: Gross lesions such as, enlarged and congested liver, spleen, and kidneys, the yolk sac contents may be of creamy or caseous consistency, white nodules in the lungs (occasionally), heart and pancreas are observed in acute cases of PD and FT. In chronic cases congestion of the liver and ascites, thickened pericardium with the pericardial sac contain yellow serous or fibrinous exudate and similar nodules may be present in the muscle of the gizzard and occasionally in the wall of the caeca and rectum and swollen joints containing yellow viscous fluid are evident (Chishti *et al.*, 1985; Wong *et al.*, 1996; Kokosharov *et al.*, 1997; Shivaprasad, 2015).

ii) In adult chickens: Gross lesions such as a small nodular or regressing ovarian follicle, misshapen, discolored cystic or nodular ovules, the oviduct which contain caseous exudate in the lumen, extensive peritonitis and adhesions of the abdominal viscera, fibrinous peritonitis, perihepatitis, frequent pericarditis, the thickened pericardial sac are observed. In addition to this, the advanced cases have chronic thickening of the pericardium and epicardium and partial obliteration of the pericardial cavity by adhesions and pancreas with white foci or nodules frequently observed (Erbeck *et al.*, 1993; Mayahi *et al.*, 1995; Shivaprasad, 2000).

b) Microscopic Lesions: Severe vascular congestion mainly in liver, spleen, and kidney are presented in per acute cases of PD and FT. In acute to subacute cases, there is multifocal necrosis of hepatocytes with accumulation of fibrin and infiltration of heterophils mixed with a few lymphocytes and plasma in the liver. With the presence of large nodules in the heart and liver, they will have chronic passive congestion with interstitial fibrosis; spleen may have severe congestion or fibrin exudation of vascular sinuses and the caeca may have extensive necrosis of the mucosa and submucosa, with an accumulation of necrotic debris mixed with fibrin and heterophils in the lumen and interstitial inflammation of the lungs and kidneys in the chronic cases of PD and FT. The most characteristic histopathologic lesion in the heart is necrosis of myofibers with infiltration of heterophils mixed with lymphocytes and plasma cells which is replaced by massive numbers of uniform histiocytes in later stages. Microscopic lesions in the ovary range from acute fibrinosuppurative inflammation to severe pyogranulomatous inflammation of the ovules (Kokosharov *et al.*, 1984; Wong *et al.*, 1996; Shivaprasad and Barrow, 2008; Shivaprasad, 2015).

Diagnosis: A tentative diagnosis of both PD and FT can be made based on flock history, clinical signs, mortality, and lesions and a definitive diagnosis requires the isolation and identification of etiology of the both i.e *S. pullorum* and *S. gallinarum*, respectively. In addition to this, various serological tests such as microscopic tube agglutination test, rapid serum test, stained antigen whole blood test, and microagglutination test using tetrazolium stained antigens can be performed. ELISA is also available and can be used for screening large numbers of blood samples (Gast and Beard, 1990; Shivaprasad *et al.*, 1990; Waltman and Horne, 1993; Gast and Holt, 1998).

Control and Prevention: Reasonably effective prophylactic and therapeutic drugs have been developed against PD and FT. The antibiotics frequently used to treatment these diseases are Sulfonamides that include sulfadiazine, sulfamerazine, sulfathiazole, sulfamethazine, and sulfaquinoxaline. Since egg transmission plays an important role in the spread of these two diseases, only eggs from flocks known to be free of PD and FT should be introduced into the hatcheries and others management practices such as obtaining chicks from sources free from FT and PD, regular serological testing, elimination of carriers, placing chicks in an environment that

can be cleaned and sanitized and feeding ingredients free of *salmonella* and a sound biosecurity program will go a long way in preventing PD and FT. Moreover, various vaccines use of mutant strains of *Salmonellae* also available (Pomeroy and Nagaraja, 1997; Snoyenbos, 1997; Shivaprasad, 2000).

II) Paratyphoid Salmonella: The numerous motile members of the bacterial genus *Salmonellae* are often referred collectively as *Paratyphoid Salmonellae* (PT). More recently this PT salmonellae have been identified as important agents of foodborne human disease. Young chicks, poults or ducklings are commonly affected and rarely in birds over 4 weeks of age (Gast, 2008; Owen, 2015).

Etiology: Family *Enterobacteriaceae*, gram negative, motile *Salmonella* serotypes, other than those in the *S. arizonae* sub genus is often referred to as *Paratyphoid Salmonellae*. These organisms can infect a wide variety of hosts including humans. In some cases, they result in asymptomatic intestinal carriers and in some cases they produce clinical diseases. The most commonly isolated serotypes from chickens (i.e. *PT salmonellae*) are: *S. Typhimurium*, *S. enteritidis*, *S. thomson*, *S. montevideo*, *S. Hadar*, *S. kentucky* and, *S. Heidelberg* (Krieg and Holt, 1984; Gast, 1997; Grimont *et al.*, 2000).

Transmission: Both vertical transmission to the progeny from infected breeder flocks via contaminated eggs internally or externally and horizontal transmission within and between flocks through mediated by mechanisms such as direct bird-to-bird contact, ingestion of contaminated feces or litter, contaminated water, or personnel and equipment are reported in *PT salmonellae* as the mode of transmission. And also insects, other invertebrates and mice participate in the transmission of this disease by being as source and vectors (Cox *et al.*, 1983; Rose *et al.*, 1999; Davies and Breslin, 2003; Jone and Richardson, 2004).

Clinical Signs: Even if the severe signs of PT infection in young chicks are similar to those observed PD and FD and acute septicemic bacteria, there is embryo mortality and the rapid death of newly hatched birds before clinical signs are observed. The main and typical signs recorded in the PT infection of chicks include depression, anorexia, emaciation, progressive somnolence

with closed eyes, drooping wings, ruffled feathers, shiver, huddle near heat sources, profuse watery diarrhea which is frequently observed followed by dehydration and pasting of the vent. In addition to this, blindness and lameness have occasionally been reported (Gast and Beard, 1990; Shivaprasad *et al.*, 1990; Dhillon *et al.*, 2001).

Pathological changes: a) **Gross lesions:** Although they are not pathognomonic for *PT salmonella* infection, the main macroscopic lesions observed include coagulated yolk sac contents, necrotic foci in the liver and spleen with enlargement and congestion, and, in more advanced cases, focal necrotic lesions in the mucosa of the small intestine, fibrinopurulent perihepatitis and pericarditis. On the other hand less frequently gross lesions such as purulent arthritis, air sacculitis, typhilitis, and omphalitis and Cheesy caecal cores have been reported on numerous occasions. In addition to this, peritonitis and oophoritis may be observed in laying hens with *S. Enteritidis* infection (Gast, 1997; Roy *et al.*, 2001).

b) **Microscopic Lesions:** There are no exact distinctive histopathologic lesions indicative of *PT salmonella* infection. The lesions seen are typical for a non-specific inflammatory disease and include heterophil infiltration ranging from focal to diffuse in distribution (especially in the ovaries and oviducts) and diffuse cellular necrosis (Hoop and Pospischil, 1993; Roy *et al.*, 2001).

Diagnosis: History of high mortality in young birds, clinical observations and gross lesions observed by necropsy finding may use as suggest of a PT infection. But the final diagnosis depends on the isolation and identification of causative organisms. Numerous faster alternative strategies such as serologic testing for detecting and identifying *salmonellae* have also been proposed in recent year (Hassan *et al.*, 1991; Waltman *et al.*, 1991; Waltman *et al.*, 1998; Gast and Holt, 2003).

Control and Prevention: Preventive treatments with a variety of different antibiotics like tetracycline, neomycin, bacitracin and sulfonamides are employed effectively and used both as therapeutic and prophylactic agents as part of control efforts for PT infection in chickens. To control *PT salmonella* biosecurity, using of available tools to control shedding of *salmonella* and

vertical transmission and vaccination with either killed or live preparations are available in this time (Goodnough and Johnson, 1991; Miles and Butcher, 1993; Chadfield and Hinton, 2003).

3.2.2. Avian Colibacillosis

Avian colibacillosis is one of the important bacterial diseases of poultry caused by *Escherichia coli* (*E.coli*), producing considerable morbidity and mortality and associated with heavy economic losses to the poultry industry. It is an association with various disease conditions as a primary pathogen or secondary pathogen. This infectious disease of chickens encompasses a number of different localized and systemic infections caused by an *avian pathogenic Escherichia coli* (APEC). Certain serotypes can cause disease in poultry such as Colisepticaemia, Egg peritonitis, Yolk sac infection, and Coligranuloma (Hjarre's disease) which are the well-recognized results of *E.coli* infection (Barnes *et al.*, 2008; Kabir, 2010; Nolan *et al.*, 2015).

Etiology: The etiological agent of Colibacillosis is *Escherichia coli* (*E. coli*), in the family *Enterobacteriaceae* which is a gram-negative, rod-shaped bacterium and a normal inhabitant of the digestive tract of mammals and birds, of which, the most strains are non-pathogenic (Lan and Reeves, 2002; Bopp *et al.*, 2003; Wirth *et al.*, 2006).

Transmission: In addition to direct or indirect contact with other animals or feces which can introduce new strains into the poultry flock, *E.coli* is obviously normal inhabitant of the intestinal tracts of most animals and shed in the feces, often in high numbers which get in to the body/system via oral or intra-air sac inoculation. And also larval and adult darkling beetles likely contribute to *E. coli* transmission and its spread among poultry houses and farms following consumption of infected larvae or beetles or contact with their feces by the birds (Goodwin and Waltman, 1996; Barnes *et al.*, 2008).

Clinical Signs: The incubation period is short which is 1-3 days in experimental studies and 5-7 days in the field/natural infection which varies with the specific type of disease produced by *E.coli*. Clinical signs may be absent when the lesion is mild or localized and when birds die per acutely. With bacterial septicemia in broiler chickens, an increase in overnight mortality and salpingitis/peritonitis in coliform which cause of sporadic mortality in caged layers and broiler

breeder hens are evidence of this problem. Birds with colisepticemia may become lethargic, stop eating and drinking, become moribund and unresponsive (in severely case), dehydrated in which young birds raised folds of skin and occasionally black toe nails. Chronically affected birds are often stunted and unthrifty. When joints, tendons, and/or bones are affected, birds will show lameness, walk with a characteristic hopping motion, and can become arched back and sit on their hocks if either both legs and the spine are affected. Feces are green with white to yellow urates as a result of anorexia and dehydration. The degree of reduced water consumption indicates the severity of the disease (Gross, 1991; Lee and Maurer, 2005; Nolan *et al.*, 2015).

Pathological changes: The several localized and systemic forms of Colibacillosis affect poultry are often named based on the tissue(s) affected or disease process. The major ones in chickens are described below.

i) Localized Forms of Colibacillosis: a) Coliform Omphalitis/Yolk Sac Infection: In this form, fecal contamination of eggs is considered to be the most important source of infection. In this case, infected yolk sac is not absorbed; hence, it is distended, usually smelly, and abnormal in color and consistency (liquid, flocculent, coagulated). Affected birds are often dehydrated, stunted, and may have vent pasting and an enlarged gall bladder. Swelling, edema, redness, and possibly small abscesses characterize acute inflammation of the navel and the abdomen is distended and blood vessels are hyperemic. Tissue around the navel is often wet and red (inflamed); which is why the disease is often called “mushy chick or poult disease” (Sandhu and Layton, 1985; Montgomery *et al.*, 1999).

Microscopically, the wall of the infected yolk sac is edematous with mild inflammation. There is an outer connective tissue zone adjacent to a layer of inflammatory cells containing heterophils and macrophages, a layer of giant cells, a zone of necrotic heterophils and masses of bacteria, and then the inner, abnormal yolk contents. A few plasma cells may be found in some yolk sacs (Sandhu and Layton, 1985; Barnes *et al.*, 2008).

b) Coliform cellulitis: Although this inflammation of the subcutis, which extends beneath normal skin, has many causes, *E. coli* infection is the most common cause in chickens. For this

reason, it has been termed as coliform cellulitis. **c) Swollen head syndrome:** it is acute to subacute form of cellulitis affecting the subcutaneous tissues of the periorbital area, giving a swollen appearance to the face/head of chickens, turkeys, and guinea fowl caused by inflammatory exudate following upper respiratory viral infections. Fibrinoheterophilic inflammation and heterophilic granulomas are evidenced in the air spaces of the cranial bones, middle ear, and facial skin by microscopic. In addition to this, conjunctivitis and tracheitis with formation of germinal centers have been observed by microscopic (Droual and Woolcock, 1994; Ishii *et al.*, 1997; Olkowski *et al.*, 1999; Gomis *et al.*, 2002).

d) Coliform salpingoperitonitis: Inflammation of the oviduct (salpingitis) caused by *E. coli* results in decreased egg production and sporadic mortality. Salpingoperitonitis occurs when *E. coli* spreads from the oviduct into the abdomen. Accumulations of caseating exudate in the body cavity resemble coagulated yolk, which is the reason for the common name “egg peritonitis”. Microscopically the tissue reaction in the oviduct is surprisingly mild in view of the marked gross lesions which consist of multifocal to diffuse heterophil accumulations subjacent to the epithelium and caseous exudate in the lumen that often contains bacterial colonies (Bisgaard and Dam, 1981; Jordan *et al.*, 2005).

ii) Systemic forms of Colibacillosis: a) Colisepticemia: This may be acute, subacute, or chronic with granulomatous inflammation. The characteristic features of this at necropsy finding are green discoloration tissues and a characteristic odor, atrophic or inflamed of the bursa of Fabricius, pericarditis which is usually associated with myocarditis, vessels in the pericardium become increasingly prominent because of hyperemia and the pericardium becomes cloudy and edematous that followed by fibrinous exudate and as the disease progresses exudate increases, becomes more cellular (fibrinoheterophilic), and undergoes caseation. Microscopically the same progression of lesion development is seen. Serous and serofibrinous exudate followed by increasing numbers of heterophils, macrophages, accumulations of lymphoid cells within the myocardium, close to the epicardium, presence of plasma cells, constrictive pericarditis, reduce arterial blood pressure and liver fibrosis due to chronic passive congestion are some of the major microscopic lesions (Nakamura *et al.*, 1985; Nakamura *et al.*, 1986; Barnes *et al.*, 2008; Nolan *et al.*, 2015).

b) Respiratory-origin Colisepticemia: This affects both chickens and turkeys and is the most common type of colisepticemia in which *E. coli* gains access to the circulation following damage to the respiratory mucosa by infectious or noninfectious agents. Severity of the resulting disease, which is commonly referred to as air sac disease, chronic respiratory disease (CRD), or multicausal respiratory disease, is directly related to the number of agents that are involved. Lesions are prominent in respiratory tissues (trachea, lungs, and air sacs), pericardial sac, and peritoneal cavities and are typical of the subacute polyserositis stage of Colibacillosis and the affected air sacs are thickened and often have caseous exudate on the respiratory surface. Edema, heterophil infiltrations followed by mononuclear phagocytes and later by macrophages become common, with giant cells along margins of necrotic areas, Pneumonia, pleuropneumonia, pleuritic, air sacculitis and pericarditis are some of the major microscopic lesions visible (Pourbakhsh *et al.*, 1997; Ginns *et al.*, 1998; Ask *et al.*, 2006).

c) Enteric-Origin Colisepticemia: In this case, *E. coli* gain access to the circulation and tissues following damage to the intestinal mucosa by infectious agents such as hemorrhagic enteritis virus. The most characteristic lesions are congestion or green discoloration of the liver, marked enlargement and congestion of the spleen, and congested muscles. In some cases, multiple, pale foci in the liver are seen. Microscopically the spleen is congested with multifocal necrosis, fibrin thrombi and acute necrosis in liver sinusoids, renal glomeruli occasionally and granulomatous hepatitis in survivors (Newberry *et al.*, 1993; Van den *et al.*, 1994; Pierson *et al.*, 1996; Kabir, 2010).

Diagnosis: Diagnosis is based on isolation and identification of *E. coli* from lesions as well as a multiplex PCR has been developed to distinguish between commensal and pathogenic isolates (Lee and Arp, 1998; Nolan *et al.*, 2015).

Control and Prevention: Treatments by antimicrobial drugs have been used extensively to reduce losses from Colibacillosis. Recently, fluoroquinolones became available in different countries for treatment of this disease in poultry, which generally proved to be highly efficacious in addition to tetracyclines, sulfonamides, ampicillin, streptomycin and others following antibiotic resistance developed. Determining and correcting risk factors are essential to

controlling Colibacillosis. In case of prevention methods, it should be starts with obtaining newly hatched birds from disease-free breeder flocks to flock management (e.g., good air and litter quality with adequate ambient temperature, access to quality feed and water). Furthermore, different vaccines (inactivated and live or recombinant) are available commercially in different countries even if few have proven very efficacious in the field (Sharada *et al.*, 2001; Glisson *et al.*, 2004; Hart *et al.*, 2006).

3.2.3. Infectious coryza

Infectious coryza (IC) is an acute respiratory disease of chickens caused by the bacterium *Avibacterium paragallinarum* (once known as *Haemophilus paragallinarum*). Chickens of all ages are susceptible and the susceptibility increases with age. The organism is widely regarded as one that is a secondary agent which is associated with other primary pathogens such as viruses or mycoplasmas (Blackall *et al.*, 2005; Blackall and Soriano, 2008; Akter, 2012; Ali *et al.*, 2013).

Transmission: The transmission is typically by direct contact, airborne droplets and contamination of drinking water. Chronic or healthy carrier birds have long been recognized as the main reservoir of Infectious coryza. Egg transmission does not occur (Blackall *et al.*, 1990).

Clinical Signs: IC has a short incubation period that develops clinical signs within 1-2 days after inoculation in to the chickens. The most prominent signs of IC are an acute inflammation of the upper respiratory tract including involvement of nasal discharge and sinuses with a serous to mucoid nasal discharge, sneezing, facial edema and swelling of the face under eye and conjunctivitis. In addition to this, there may be diarrhea, decrease in feed and water consumption and a reduction in egg production in layer chickens (Sandoval *et al.*, 1994; Mohan *et al.*, 2000).

Pathological changes: a) **Gross lesions:** Grossly acute catarrhal inflammation of mucous membranes of the nasal passages and sinuses, frequently a catarrhal conjunctivitis, subcutaneous edema of the face and wattles are evident of the IC. And also lesions such as conjunctivitis, abscesses in the head and wattles, sinusitis, tracheitis, air sacculitis, hepatitis, endocarditis,

salpingitis, oophoritis, peritonitis and synovitis have been reported. In acute cases, lesions may be limited to the infraorbital sinuses and there is a copious semifluid exudate from the nostril synovitis (Hoerr *et al.*, 1994; Talha *et al.*, 2001; Blackall and Soriano, 2008; Akter, 2012).

b) Microscopic Lesions: The main histologic changes occur in the nasal cavity, infraorbital sinuses, and trachea consisted of sloughing, disintegration, and hyperplasia of mucosal and glandular epithelia, and edema and hyperemia with heterophil infiltration in the tunica propria of the mucous membranes. In birds with involvement of the lower respiratory tract, acute catarrhal bronchopneumonia with heterophils infiltration and cell debris filling the lumen of secondary and tertiary bronchi are evident. Catarrhal inflammation of air sacs is characterized by swelling and hyperplasia of the cells, with abundant heterophils infiltration. Moreover, the products of mast cells, heterophils, and macrophages may be responsible for the severe vascular changes and cell damage leading to coryza. Additionally, pyogranulomatous pneumonia, air sacculitis, pericarditis, hepatitis, synovitis, and myositis and severe lymphoid depletion of the bursa of fabricius are reported by Shivaprasad and Droual. (2002) (Sawata *et al.*, 1985; Blackall and Soriano, 2008; Ali *et al.*, 2013).

Diagnosis: Isolation and identification of causative agent from chickens in a flock is one of the diagnostic techniques for infectious coryza. But the definitive diagnostic test for this disease is a PCR test that specifically detects *Av. paragallinarum*. Although a hemagglutination-inhibition test is the best of the available tests, no suitable serological test exists for the diagnosis of infectious coryza (Terzolo *et al.*, 1993; Chen *et al.*, 1998; Ali *et al.*, 2013).

Control and Prevention: To control infectious coryza in infected chicken by early treatment, antibiotics such as erythromycin and oxytetracycline are usually beneficial. However, “All-in/all-out” farm programs with sound management and good biosecurity are the best way to avoid the disease. Additionally replacements should be raised on the same farm or obtained from flocks known to be free of this disease. Moreover, vaccines are widely available for infectious coryza in chickens (Blackall, 1995; Blackall *et al.*, 1997; Huberman *et al.*, 2005).

3.3. Chicken Parasitic Diseases

3.3.1. Coccidiosis

Poultry coccidiosis is an economically important disease in chicken caused by the intracellular protozoa parasite of *Eimeria* species in the genus *Eimeria*, family *Eimeridae* (Taylor *et al.*, 2007). The protozoan parasites of the genus *Eimeria* multiply in the intestinal tract and cause tissue damage, with resulting interruption of feeding and digestive processes or nutrient absorption, dehydration, blood loss, loss of skin pigmentation and increased susceptibility to other disease agents. The species of *Eimeria* identified in Ethiopia are *E. tenella*, *E. necatrix*, *E. maxima*, *E. brunetti*, *E. acervulina* and *E. mivati* was also reported (Methusela *et al.*, 2002; McDougald, and Fitz-Coy, 2008; Tamiru *et al.*, 2015; Dinka *et al.*, 2017).

Transmission: Oocysts (unsporulated) are released by the rupture of intestinal cells and are excreted with the feces and the infected chickens may shed oocysts in the feces for several days or weeks. The oocysts in feces become infective through the process of sporulation in about 2 days. Ingestion of viable sporulated oocysts (eggs) is the only natural method of transmission by which this sporulated oocyst can potentially lead to the production of about 2-3 millions of new oocysts in 5 to 7 days. Although no natural intermediate hosts exist for the *Eimeria spp.*, oocysts can be spread mechanically by many different animals, insects, contaminated equipment, wild birds, and dust (Reyna *et al.*, 1882; McDougald, 2003; Guyonnet, 2015).

Clinical Signs: The severity of the clinical signs is vary depending on the species of *Eimeria* involved (with often more than one species involved) and extends of the damages produced on the intestinal wall. A reduced weight gain or even a loss of weight is one of the most common and early observation which result reduction in the absorption and conversion of nutrients as well as a decrease in feed consumption. Reduced water consumption and a mucoid or hemorrhagic diarrhea (bloody diarrhea) along with the unthriftiness of the chickens is also the main clinical signs (McDougald, 2003; McDougald, and Fitz-Coy, 2008; Tamiru *et al.*, 2015).

Pathological changes: The severity of the pathological lesions is also vary depending on the species of *Eimeria* involved and extends of the damages produced on the intestinal wall as in the clinical sings.

a) Gross lesions: Some of the gross lesions in a few various *species* of *Eimeria* are described as follows: a) *E. tenella*: hemorrhages are apparent and the caecal pouch may become greatly enlarged and distended with clotted blood and pieces of caecal mucosa in the lumen; b) *E. acervulina*: in this *Eimeria spp* white streaks, oriented transversally along the intestine (often described as ladder-like) represent a thickening of the intestinal mucosa due to the aggregation of gametocytes and oocysts; c) *E. maxima*: the thickening of the mucosa (due to the development of large gametocytes in the epithelial cells) and petechiae which gives characteristic orange tinge to the mucus are evidence of this problem; d) *E. necatrix*: the typical “Salt and Pepper” aspect with the juxtapositions of petechiae and white zones containing large schizonts in the intestine is evidence of this disease in this type (McDougald, 2003; McDougald, and Fitz-Coy, 2008; Guyonnet, 2015).

b) Microscopic Lesions: The most significant microscopic lesions are linked to cellular infiltration, epithelial cell losses, edema, epithelial hyperplasia and vascular damages which vary from species to species. Small focal areas of hemorrhage and necrosis may appear near blood vessels of the inner circular muscles of the muscularis layer (McDougald, 2003, Dinka *et al.*, 2017).

Diagnosis: A solid diagnosis will be the result of the post-mortem observation of specific lesions along with the scrapping of intestinal surface and the observation of unsporulated oocysts. The size of oocysts (length and width) will further assist in the differentiation of *Eimeria spp.* involved. Diagnosis is dependent upon finding caecal lesions with prominent blood and often firm bloody cores and accompanying clusters of large schizonts and oocysts in some species of *Eimeria* (especially *E. tenella*)(Methusela *et al.*, 2002; McDougald, and Fitz-Coy, 2008; Guyonnet, 2015).

Control and Prevention: Even if few products are available for the treatment of coccidiosis, any treatment will be effective only if given very early on. For instance treatment with Amprolium, Amprolium+ Clopidol, sulfonamides or other compounds, good and effective as soon as signs of infection are observed at early occurrence of this disease. Sanitary measures like the removal of dirty litter (wet and caking spots), the cleaning and disinfection of equipment and buildings at the end of each grow-out period will greatly contribute to the reduction in the contamination of the environment in addition to treatment, as the attempt of prevention of outbreaks as much as possible. Moreover, considerable research on coccidiosis vaccines in recent years has produced new live products (Conway and McKenzie, 1991; Dasgupta and Lee, 2000; Lillehoj and Okumura, 2003; Guyonnet, 2015).

3.4. General Chicken Diseases Control and Prevention Methods

Prevention of disease in chickens should be the priority in order to minimize unnecessary costs related with both loss and treatment. The two most important aspects in the control of disease in the flock are maintaining high levels of the biosecurity and management, and, where possible and appropriate, administration of suitable vaccines for populations at risk. However, even where these measures (and others) are used, it is not possible to guarantee the health of chickens and prevent disease related losses (Abebe, 2006; Yenesew *et al.*, 2015; Habte *et al.*, 2017).

For example, Newcastle disease virus is endemic to Ethiopia and prevention of this disease using only biosecurity and management measures often is not effective because the disease can occur in any farm if there is even a slight biosecurity breach, including via wild birds. Similarly, vaccination may not always successfully prevent Newcastle disease due to the risk of vaccination failure, which can happen due to several different reasons. For this reason it is important to always apply vaccines as part of a broader control strategy. Successful control and prevention of disease in poultry needs to bring together vaccination with improved biosecurity, hygiene, management and animal welfare, rather than control through a single measure (Fisseha *et al.*, 2010; Yenesew *et al.*, 2015). Moges *et al.* (2010) and Mengesha *et al.* (2011) suggested that improvement in veterinary and advisory service could also help to achieve control and prevention of diseases at village level.

4. MATERIALS AND METHODS

4.1. Study Area

The study was conducted from October, 2018 to May, 2019 in the Oromia regional state in and around Bishoftu town. Bishoftu is located 45 Km southeast of the capital city Addis Ababa and located at 9° N latitude and 40° E longitude with the altitude about 1880 meter above sea level. The average annual rainfall is 866mm with a bimodal distribution which includes a long rainy season extends from June to September in which 84% of the rain is expected followed by a dry season from October to February and a short rainy season lasts from March to May with average rain fall of 800mm. The mean annual minimum and maximum temperatures are 14°C and 26°C respectively and the mean relative humidity is 61.3%. These agro-climatic zones are inhabited with different plant and animal species and the farmers in the vicinity of Bishoftu town use a mixed crop and livestock farming system. Moreover, Bishoftu is a focus of intensive and semi-intensive chicken production in Ethiopia (CSA, 2004; FAO, 2008; CSA, 2009; USAID, 2010).

4.2. Study Populations

The study was conducted on the clinically sick chickens brought to Veterinary teaching hospital (VTH), dead chickens at clinic and different farms and slaughtered chickens at Alema chicken abattoir as the study populations. In this investigation, a total of 135 chickens were involved. Among these, 35 (25 clinically sick and 10 dead) chickens of different farms, sex, age, types (layers and broilers), history and clinical status were involved. The remaining 100 chickens among the slaughtered chickens of the same type (broilers), almost the same age and management were included in this study. Except only one layer chicken from local (backyard) and extensive management system, all of the chickens used in this investigation were from exotic breeds and intensive production system.

4.3. Study Design and Sampling Methods

A case study design with purposive sampling method was conducted to collect the necessary important data from all respective study populations for this research purpose. In this study any clinically sick chickens came to clinic and immediate dead chicken at clinic and farms (informed by an assigned contact person) was purposively sampled for further study process in addition to history and clinical signs recording. Additionally, among the slaughtered chickens in the Alema chicken abattoir, any positive case (chicken observed with any gross lesion by routine inspection) examined and identified during every visit was also grossly characterized and purposively sampled in this investigation for the further examination of the pathological disorders and associated bacterial isolation.

4.4. Sample Collections

At the time of sample collection, any clinically sick chicken brought to the clinic was examined carefully to detect all possible clinical signs as per appropriate procedure according to Chauhan and Chandra. (2007) and Corrie *et al.* (2008). In this way, sick chickens were closely examined for any visible clinical sign and every clinical sign observed was recorded properly for each case. Accordingly, the physical examination included were: assessment of general appearance, feathering, body weight, pigmentation of skin, physical injuries, facial tissues, eyes, feces (Droppings), nasal or respiratory discharges, gait, leg (Joint deformities) and any external swelling of the body part.

In addition to recording these clinical signs for clinically sick chickens, detailed necropsy/postmortem examination by euthanizing (Cervical dislocation) of the chickens was performed and any visible gross pathological changes/lesions in terms of organ color, consistency, shape, size and texture was recorded carefully in both clinically sick and dead chickens (based on the permission of the owners) according to the standard operational protocol in chicken necropsy (**Annex I**). The presences of gross lesions such as necrosis, inflammations, hemorrhages, edema, thickened wall, air sacculitis, ulceration, ascites, discoloration, enlargement,

abnormal consistency/rough surface and any other abnormality on internal organ was carefully considered and recorded accordingly in each case.

On the other hand, among the examined slaughtered chickens in the Alema chicken abattoir; any organ from positive chicken (chicken with any gross lesion visible on the organ) was collected for gross and histopathological characterization as well as for the isolation of associated bacterial agents by detailed and careful examination in the slaughtering house at every visit.

The representative pieces of tissue samples from the respective organs of the all involved cases were collected by using bottle containing 10% formalin immediately for histopathology examination and lesions swabs from the affected organ surface and/or by incising and intestinal tract (caeca and cloacal) swabs were collected for bacterial isolation by using a sterile wooden cotton swab and inserted in to the 10ml test tube that contains sterile buffered peptone water (BPW) which used as both transporting media and a pre-enrichment media for 24hrs at 37°C aseptically.

4.5. Sample Transportation

All samples were labeled legibly with permanent marker in which the clinical signs and gross lesions were recorded carefully with important information. Samples for bacteria isolation were transported to Addis Ababa University College of Veterinary Medicine (AAU CVMA) Microbiology laboratory by using 10ml test tube that contains sterile buffered peptone water (BPW) used as a pre-enrichment media as well as transporting media with icebox containing ice bag and analyzed. On the other hand, the pieces of representative tissues collected by containers/tubes with 10% formalin for histological examination were transported to National Animal Health Diagnostic and Investigation Center (NAHDIC), Sebeta, for histopathology processing by using ice box.

4.6. Sample Processing

4.6.1. Histopathology procedures

Pieces of the representative tissue samples from the respective organs such as trachea, brain, liver, lung, heart, intestines, spleen, kidney, bursa and caeca tonsil (about 5cm) were collected into a bottle with 10% formalin for histopathology examination and tissue processing was done according to the procedures described by Talukder (2007). Briefly, after adequate fixation, the tissues was trimmed to about 1-2 mm thickness and dehydrated in ascending grades of ethyl alcohols (70, 95 and 100%), then cleared in xylene and embedded with melted paraffin wax (Melting point 58°C). These all processes were done within the automatic tissue processing machine. After this the paraffin block was prepared and then the tissue was sectioned at about 5 micrometer thickness by using semi-automatic microtome machine. The paraffin embedded sections (tissue sectioned), (which were adhered to microscopic slide after floated on the warm water bath), were then passed through sequential steps of deparaffinization in xylene, rehydrated by passing through descending grades of ethyl alcohol (100, 95 and 70%) to running tap water and stained by routine haematoxylin and eosin stain (H and E) by dehydrated in ascending grades of ethyl alcohols (70, 95 and 100%) and cleared by xylene. After this the slides (stained sections) were mounted by canada balsam and allowed to dry well, then examined under the light microscope starting from the lower (10x) to higher magnification (100x) power by which the histopathological alterations were assessed and every microscopic lesion identified was carefully described, analyzed and recorded properly (**Annex II a and b**). Finally the photographs of the slides were taken.

4.6.2. Bacteriological isolation procedures

Isolation and identification of some bacteria was done according to the procedures described by Quinn *et al.* (2004). For this purpose lesions and intestinal tract, including ceca and cloacal swabs were aseptically collected. The collected samples were inserted in the 10ml test tube that contains sterile buffered peptone water (BPW) used as a pre-enrichment media for 24hrs at 37°C. After transferring the pre-enriched culture to 10 ml of Selenite F Broth (SFB) and Rappaport

Vassiliadis Soy broth (RVSB) and incubated at 37°C for 24hrs and 48hrs respectively, loop full of the this sample was inoculated and incubated on to Xylose Lysine Deoxycholate (XLD) at 37°C for 48hrs for *Salmonellae* isolation. Then the suspected sample was sub cultured on the nutrient agar media and finally confirmed by using secondary biochemical tests (**Annex III a**).

For *Escherichia coli* isolation, the selective enrichment sample was streaked onto MacConkey agar and incubated aerobically at 37 °C for 24hrs and followed by sub-culturing on Eosin Methylene Blue (EMB) agar, respectively for primary isolation. Then the suspected samples for *E. coli* were inoculated on nutrient agar and finally secondary biochemical tests were performed to confirm the presence of *E. coli* (**Annex III b**).

For Staphylococcus isolation collected and enriched sample was initially streaked onto mannitol salt agar (MSA) and incubated at 37°C for 24 hours. Then the presence of *S. aureus* colonies was evaluated using morphological aspects (round, smooth and yellow colonies). To get a pure culture, presumed *S. aureus* colonies was further sub-cultured on nutrient agar plates and incubated at 37°C for 24 hours and followed by coagulase test by which a full of the pure colony was mixed with a drop of saline water and then with a drop of rabbit plasma on the clean glass slide turn by turn, respectively as the secondary biochemical assays. By this the presence or absence of the coagulation on the mixture was tested by which *S. aureus* were identified as coagulase test positive.

4.7. Data Management and Analysis

All the generated data were arranged, checked, coded and entered to Excel spread sheet (Microsoft® office excel 2007). STATA version 12 was used for descriptive analysis to describe the proportion of the result analyzed as frequency and percentages of the lesions in relation to the organs affected in all cases. In this way the types of the gross lesions recorded with the frequency and percentage within each case in every organ of the body in all included chickens were known and characterized accordingly.

5. RESULTS

5.1. Clinical Signs

Careful clinical examination was performed on 25 clinically sick chickens for any abnormal clinical sign manifested following the history gathered from the owners properly. Accordingly various clinical signs with different frequency were recorded (**Table 2**). Some of the major clinical signs recorded includes: mild to severe depression with drooping their wings, anorexia (complete cease of feeding in some cases), different types of diarrhea (white, green, bloody and yellowish diarrhea), thick and sticky dropping/pasty vent, unilateral paralysis of leg and wings and paralysis of both legs and wings as well as torticollis (twisting of the neck), huddling together/reluctant to move, swelling of eye lids, respiratory problem such as gasping, sneezing, grunting and gurgling with open mouth breathing, discharge such as bloody nasal discharges, fluid discharges via mouth, drop in egg production (mild to severe almost become cease in some cases), retarded growth and reduced body condition (mild to severe), swelling of infraorbital sinus (facial edema), closure of the affected eye and swollen joint (hock joints). Few major clinical signs recorded were supported by figures (**Figure 1A-F**). Almost all of these clinical examinations processes were done at AAU CVMA veterinary teaching hospital followed by detailed post mortem examination.

Table 2: Major clinical signs recorded on clinically sick chickens, N=25

Type of Clinical signs	Frequency	Percentage (%)
Mild to severe depression with/without drooping of feathers/wings	24	96
Anorexia (complete cease of feeding in some cases, received form history)	17	64
Different types of diarrhea (white, green, bloody and yellowish diarrhea) with pasting of vent	16	64
Torticollis, paralysis of legs and wings either unilateral or bilateral	5	20
Huddling together/reluctant to move	9	36
Swelling of eye lids	2	8
Respiratory problem (gasping, sneezing, grunting and gurgling with open mouth breathing)	12	48
Nasal and mouth discharges	4	16
Mild to severe drop in egg production	3	12
Retarded growth and reduced body condition (mild to severe)	8	32
Swelling of infraorbital sinus (facial edema)	3	12
Closure/blinded of the affected eyes	3	12
Swollen joint (hock joints)	4	16



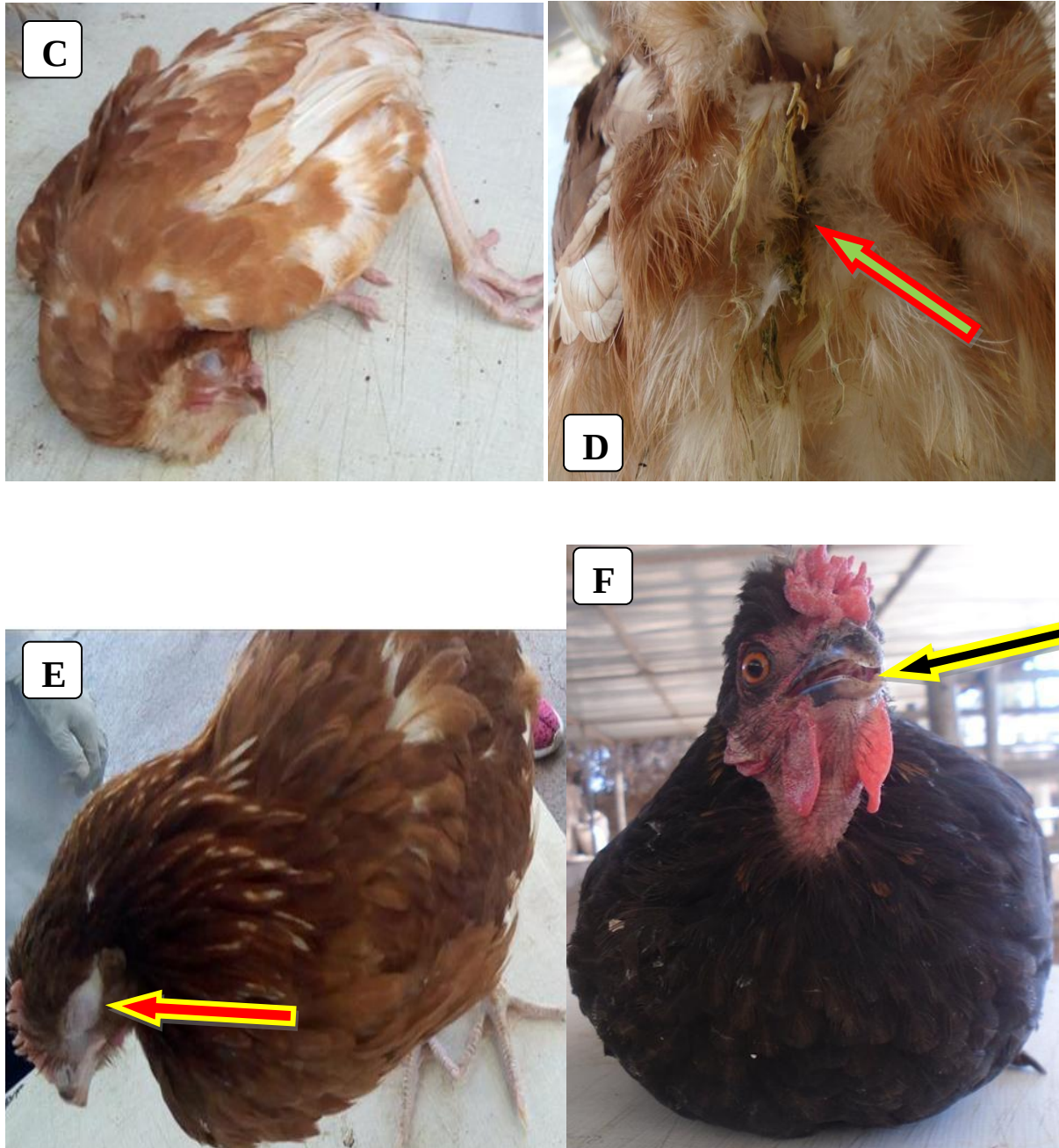


Figure 1: Different clinical signs of sick chickens recorded at the clinic.

Keys: (A) Severe depression with drooping of feathers; (B) Swelling of infraorbital sinus (facial edema); (C) Torticollis with paralysis of legs and wings; (D) Green diarrhea with pasting of vent (E) Swelling of eye lids with closure affected eye; (F) Open mouth breathing (dyspnea).

5.2. Necropsy Findings

5.2.1. Gross lesion findings in the clinically sick and dead chickens

The main gross lesions recorded in the 25 clinically sick euthanized and 10 dead chickens were almost similar to each other in the individual organ affected with few differences in the lesions frequencies and severity (**Table 3**). In both cases, in each chicken, every system of the body with their respective number of organs was examined carefully and detailed by necropsy findings and the gross lesions found were recorded. The general frequently affected and identified organs with gross lesions in the present study were presented with their frequencies and percentages by the **Figure 8**.

Necropsy findings of digestive system: Among the 35 proventriculus examined, 12 (34.3%) were found to have gross pathological lesions, of which, all of them were identified by having mild to severe hemorrhages (**Figure 2A**). Out of the 35 examined intestines by considering each segment, 20 (57.1%) of the duodenum were observed with gross lesions, of which, 18 (51.4%) were moderate to severely hemorrhagic and 2 (5.7%) were both hemorrhagic and ulcerated/necrotized. And also among the examined jejunum, 19 (54.3%) were presented with gross lesions in which 17 (48.6%) revealed mild to moderate hemorrhages and 2 (5.7%) of them showed fibrinous inflammations. In similar way, out of the examined ileum, 17 (48.6%) were evidenced with gross hemorrhages. In addition to this, 5 (14.3%) of colon and 3(8.6%) of the cloaca were observed with hemorrhagic lesions, respectively.

Among the intestinal segments, 16 (45.7%) ceacum were also observed with gross lesions, out of which, 1 (2.9%) was harbored with the adult parasite (*Ascaridia galli*) (**Figure 2C**) and the remaining 15 (42.9%) were observed with moderate to severe hemorrhages. Moreover, among ceacum examined, 7 (20%) were confirmed as positive for coccidial oocyte by microscopic examination based on the suspicion due to the observed severe hemorrhages and bloodied feces contents of the caecum at necropsy finding (**Figure 2D**). On the other hand, out of the 35 livers examined grossly, 14(40%) were found to have gross pathological lesions, of which, 11(31.4%) were highly enlarged with congestion and discoloration, 2(5.7%) with hemorrhagic and bulged cut

surface, 1(2.9%) was found with white foci of necrosis. Some of the discolorations observed within livers were dark brown, pale and greenish (**Figure 2B**). Some cases with such gross lesions and other indicative signs were being positive for the presence of bacterial infection as either primary pathogens or secondary complication of the other problem based on the bacterial isolation.

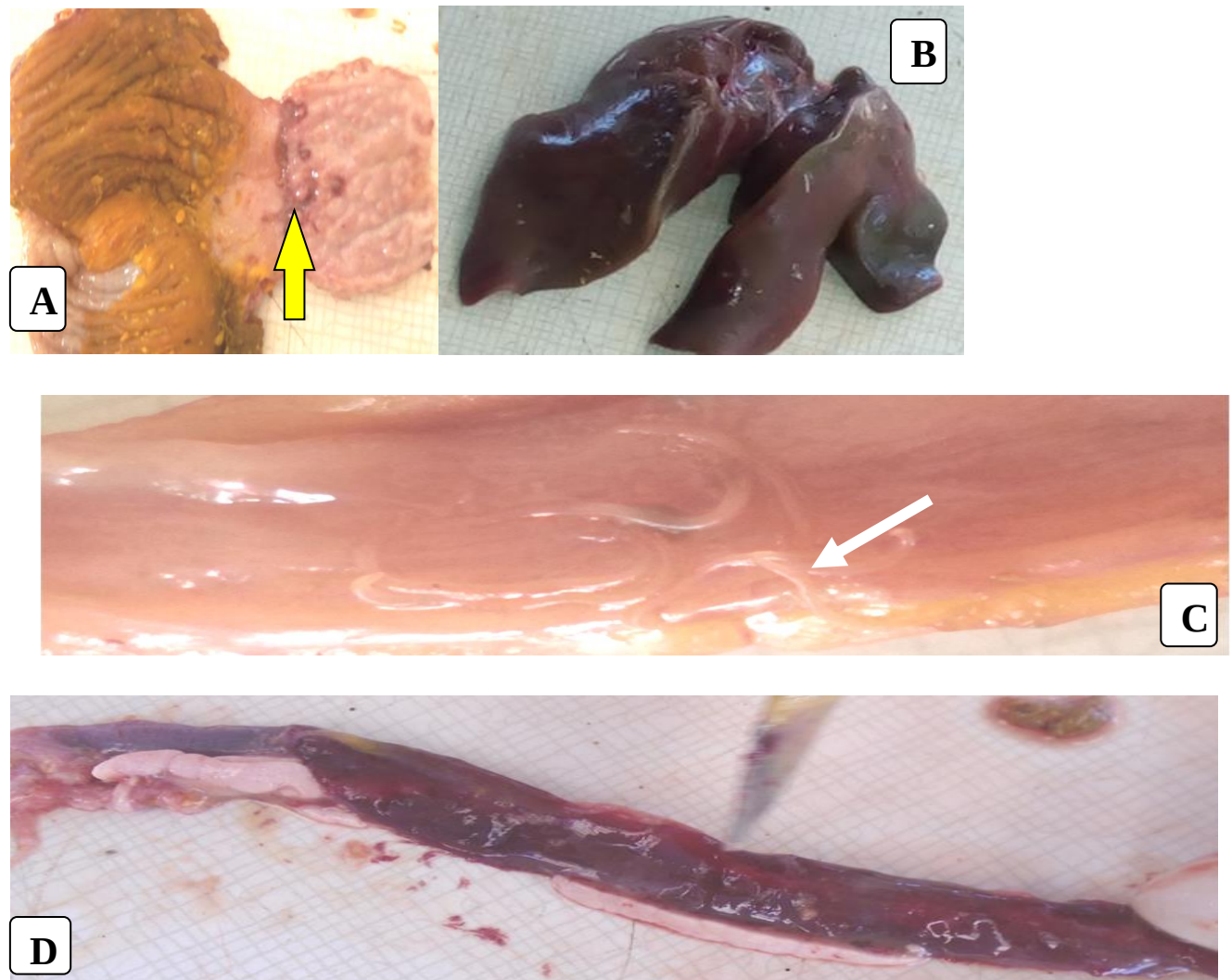


Figure 2: Various gross pathological lesions found in digestive system of clinically sick and dead chickens.

Keys: (A) Hemorrhagic proventriculus; (B) Discolored liver to brownish (C) Caecum harboring large number of adult parasites (*Ascaridia galli*) and (D) Severely hemorrhagic cecum with bloodied fecal contents.

Necropsy findings of respiratory system: Out of the 35 examined tracheas, 22 (62.9%) were found with gross lesions, of which, 17 (48.6%) were observed with mild to severe hemorrhages and 5 (14.3%) of the trachea with hemorrhages and mucoid exudates (**Figure 3A**). Among the examined 35 lungs, 10 (28.6%) were observed with gross pathological lesions. Out of these, 2(5.7%) were found to have mild hemorrhagic, 8(22.8%) were evidenced by consolidation with black spot pigmentation in some cases (**Figure 3B**). Additionally, out of the 35 examined air sacs, 19 (54.3) were observed with gross lesions, of which, 17 (48.6%) were found to have cloudy (thicken), 2 (5.7%) were evidenced by the presence of the cloudiness with hemorrhages. Some of these air sacs had also whitish spot and yellowish surfaces (**Figure 3C**).

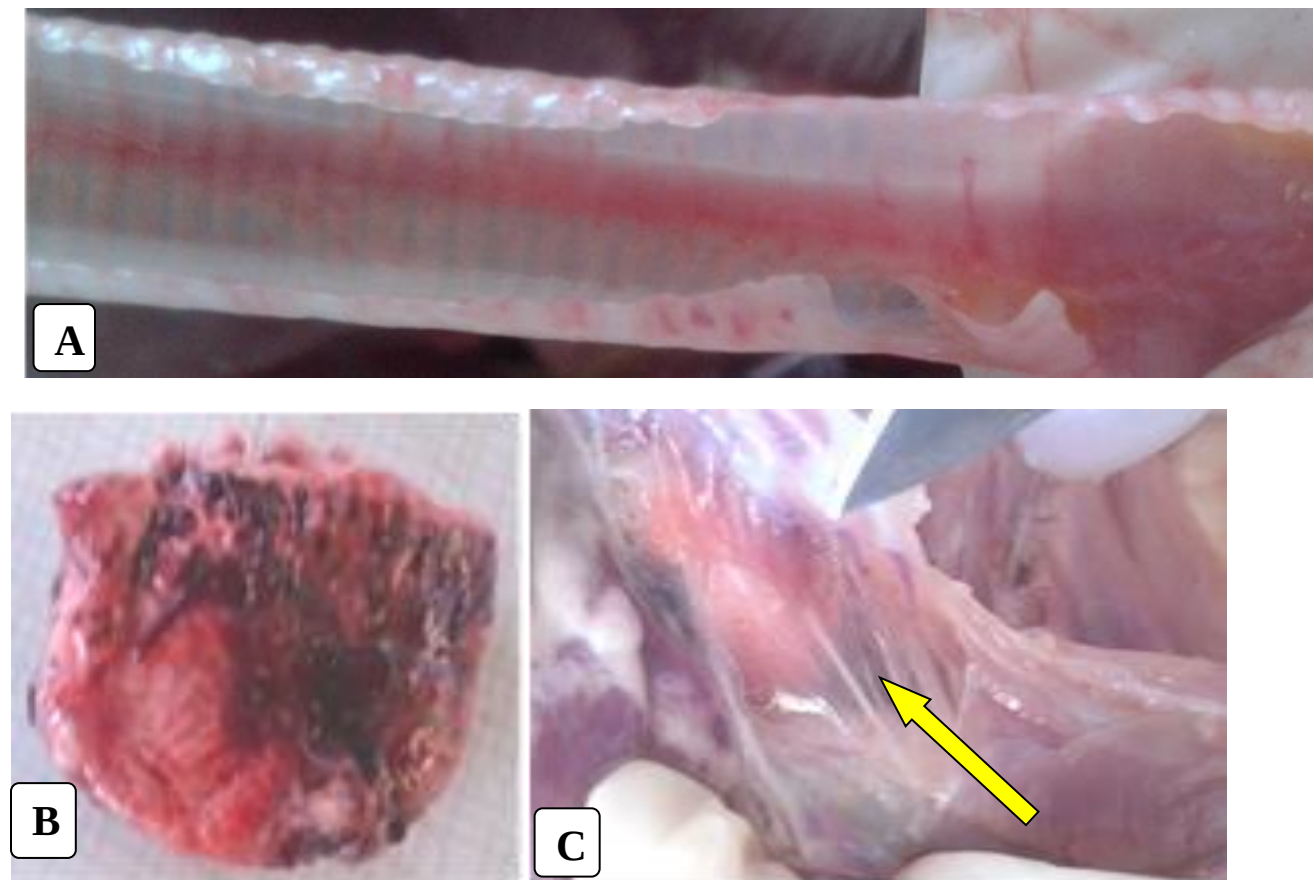


Figure 3: Various gross pathological lesions found in respiratory system of the chickens.

Keys: (A) Tracheal hemorrhage; (B) Consolidation of lung with blackish pigmentation and (C) Cloudy and thick air sacs.

Necropsy findings of the Hearts: Out of 35 examined hearts, 10 (28.6%) were observed with gross lesions which were presented by gray to white small nodules which were many around and over apex of the heart (**Figure 4A and B**). Some of the cases demonstrated with such gross lesions within the heart with additional evidences were suspected for the bacterial infection (mostly *Salmonellae*) and confirmed by bacterial isolation in few cases.



Figure 4: Gross nodules found on the surface of hearts (**A and B**).

Necropsy findings of lymphoid organs: Out of the examined 35 caecal tonsils, 25 (71.4%) were identified with different gross lesions, of which, 21 (60%) were found with moderate to severe hemorrhages and enlarged/swollen in size, 4 (11.4%) were found with hemorrhages and necrotic foci (**Figure 5A and B**). And also out of the 35 examined bursa of Fabricius, 4(11.4%) were found with gross pathological lesions, of which, 2 (5.7%) were identified with the presence of hemorrhages and swollen with gelatinous edema and 2 (5.7%) were found with swollen with gelatinous edematous which identified by incision (**Figure 5C**). On the other hand, out of 35 examined spleens, 5(14.3%) were found to have gross lesions. Of these, 4 (11.4%) were evidenced by the presence of congestion and swollen and 1 (2.9%) was observed with hemorrhagic and abnormal enlarged size with discoloration (**Figure 5D**).

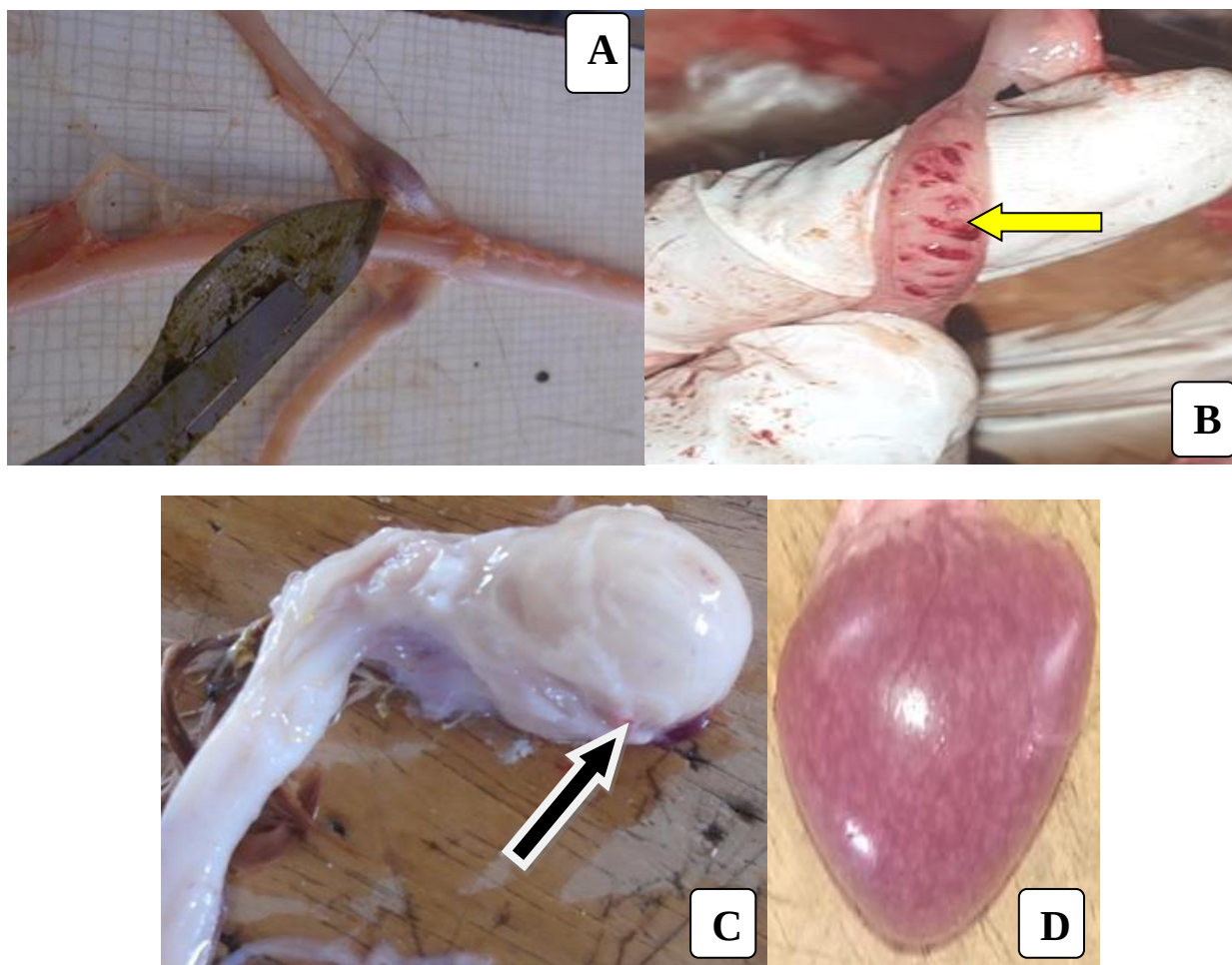


Figure 5: Various gross pathological lesions found in the immune organs of chickens.

Keys: (A and B) Swollen and hemorrhagic caecal tonsil, respectively; (C) Hemorrhagic and swollen bursa of Fabricius and (D) Hemorrhagic and enlarged spleen

Necropsy findings of Urogenital system: Out of examined 35 kidneys, 8 (22.8%) were observed with different gross lesions. Among these gross pathological lesions, 4 (11.4%) were hemorrhage and swollen and 4 (11.4%) were discolored and swollen with urine accumulation. In addition to this, out of the 27 female chickens (21 from clinically sick and 6 from dead cases) examined, 11(40.7%) ovaries were found with gross lesions, of these, 4(14.8) were found with mild to severe hemorrhages, 5(18.5) were atrophied ovaries and 2(7.4%) were discolored to yellowish (**Figure 6A**).

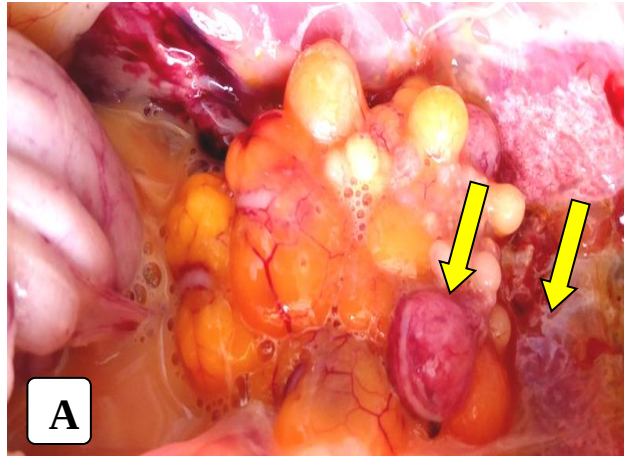


Figure 6: Gross hemorrhages of ovaries in the chickens (A).

Necropsy findings of Musculoskeletal and Head regions: Among the examined bones and joints of each case, 2 (5.7%) of femoral head were found with sloughed /necrosis. 6(17.1%) of the muscles/carcasses out of the all cases were evidenced with the presence of abnormal condition. Of these, 4 (11.4%) were observed to have hemorrhages on the thigh, breast and pectoral muscles, 2 (5.7%) were observed by the presence of carcass emaciated/pale in color in addition to hemorrhages (**Figure 7A**). In addition to this, some of the muscles were discolored to almost pale in color. Furthermore, 2 (5.7%) of the 35 heads, were observed to have cheesy exudate in the infra orbital sinus (**Figure 7B**).



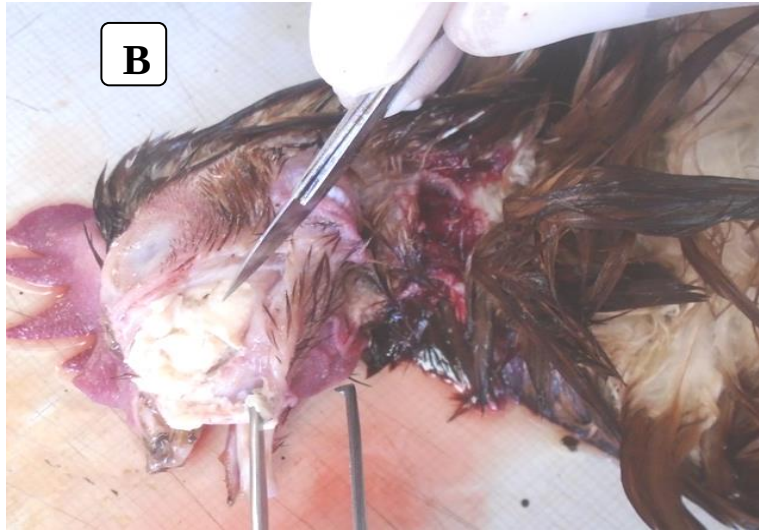


Figure 7: Hemorrhagic foci on the thigh muscle and cheesy exudate in the infra orbital sinus of chickens (**A** and **B**, respectively).

Abdominal ascites: Abnormal fluid accumulation in abdominal cavity was found in 8 (22.9%) of the cases. In some dead cases these fluids was abundant.

Frequency differences of gross lesions observed: Even if the numbers of the involved chickens in both clinically sick and dead chickens were not equal, the types and frequencies of the lesions observed were most similar within the same organs/tissues by considering their difference in numbers (25 and 10, respectively) with few differences as summarized in by table (**Table 3**). The different gross lesions demonstrated in both cases had little variations in lesions frequencies and severity within the same affected organs. For instance, out of the examined caecal tonsils: 14 (64%) and 9(90%), livers: 8(32%) and 6 (60%), ovaries:5 (20%) and 6 (60%), kidneys:5 (20%) and 3 (30%) and presence of abdominal ascites: 2 (8%) and 6 (60%) ,were observed with gross lesions in the clinically sick and dead cases of chickens, respectively. This is indicative of that caecal tonsil, livers, ovaries and kidneys were frequently and severely affected in dead cases than clinically sick chickens. In addition to this, abdominal ascites was highly frequent in dead cases with abundant fluids.

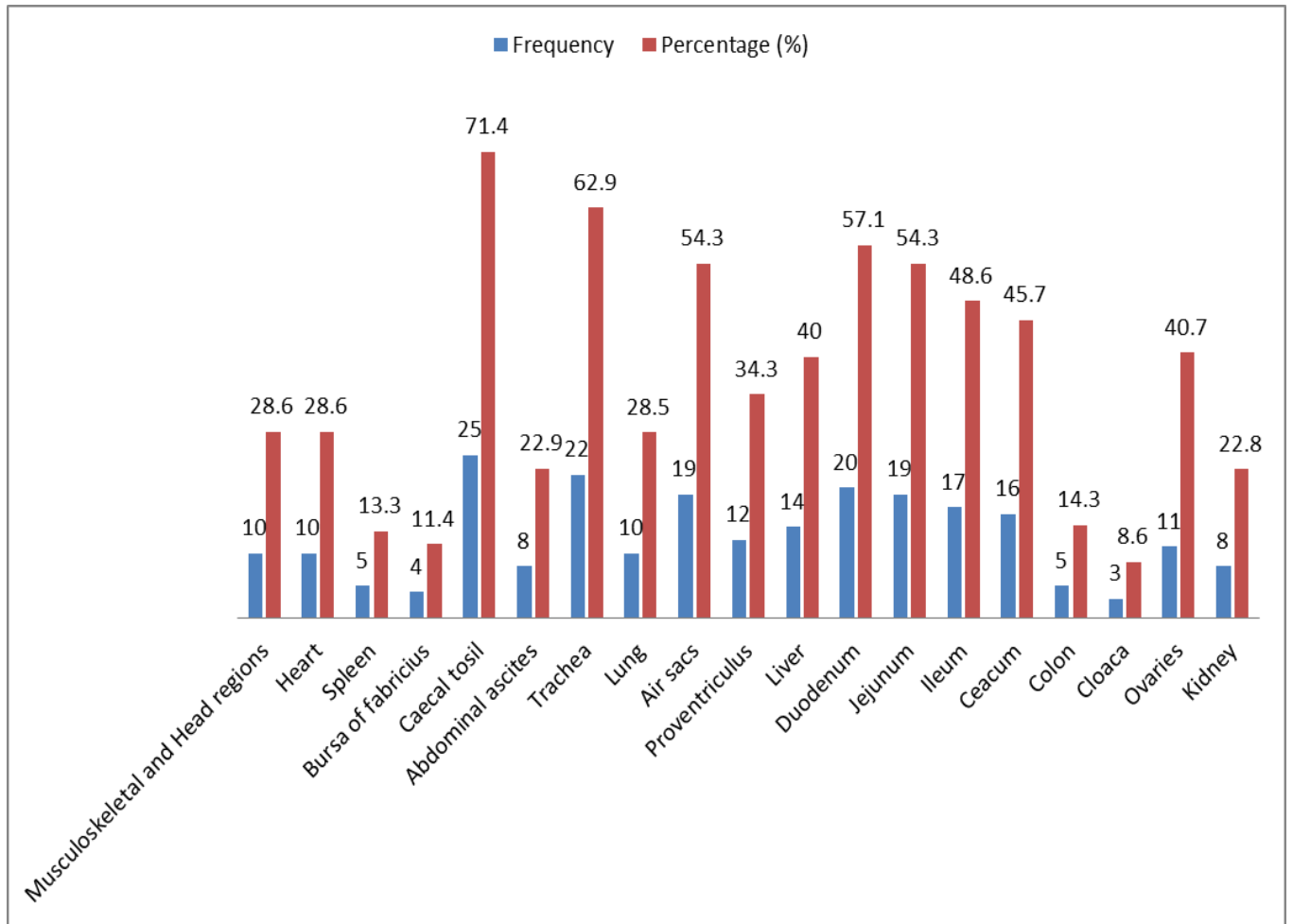


Figure 8: General necropsy findings of the frequently affected organs in clinically sick and dead chickens with their frequencies and percentages, N=35.

Table 3: Necropsy findings in different organs of clinically sick and dead chickens with their frequencies and percentages (%) of the lesions, (N=25 and 10, respectively). N=35.

Organs	Gross Lesions	Diseased cases (N=25)		Dead cases (N=10)		Total (N=35)	
		Frequency	%	Frequency	%	Frequency	%
Muscle/carcass	Hemorrhagic thigh, breast and pectoral muscles	3	12	1	10	4	11.4
	Hemorrhagic and emaciated/pale in color	1	4	1	10	2	5.7
Heart	Surfaced by nodules	7	28	3	30	10	28.6
Spleen	Congested and Swollen	2	8	2	20	4	11.4
	Hemorrhagic and enlarged	1	4	0	0	1	2.9
Bursa of	Hemorrhagic and swollen with gelatinous edema	2	8	0	0	2	5.7
Fabricsius	Swollen with gelatinous edema	1	4	1	10	2	5.7
Caecal tosil	Swollen and hemorrhagic	13	52	8	80	21	60
	Hemorrhagic with necrotic foci	3	12	1	10	4	11.4
Ascites	Occurrence of abdominal ascites	2	8	6	60	8	22.9
Trachea	Hemorrhagic	14	56	3	30	17	48.6
	hemorrhagic with mucoid exudate	2	8	3	30	5	14.3
Lung	Hemorrhagic	2	8	0	0	2	5.7
	Consolidation with black spot	5	20	3	30	8	22.8
Air sacs	Cloudy (thicken)	13	52	4	40	17	48.6
	Cloudy with hemorrhagic spots	1	4	1	10	2	5.7
Proventriculus	Hemorrhagic	9	36	3	30	12	34.3

Liver	Congested, discoloration and highly enlarged	6	24	5	50	11	31.4
	Hemorrhagic	2	8	0	0	2	5.7
	Necrotized with necrotic foci	0	0	1	10	1	2.9
Intestines							
a) Duodenum	Hemorrhagic	13	52	5	50	18	51.4
	Hemorrhagic and ulcerated	1	4	1	10	2	5.7
b) Jejunum	Hemorrhagic	14	56	3	30	17	48.6
	Fibrinous inflammation	0	0	2	20	2	5.7
c) Ileum	Hemorrhagic	11	44	6	60	17	48.6
d) Ceacum	Hemorrhagic	9	36	6	60	15	42.8
	Harbored by adult parasite	1	4	0	0	1	2.9
e) Colon	Hemorrhagic	4	16	1	10	5	14.3
f) Cloaca	Hemorrhagic	3	12	0	0	3	8.6
Ovaries	Discolored	1	4	1	10	2	7.4
	Atrophied	3	12	2	20	5	18.5
	Hemorrhagic	1	4	3	30	4	14.8
Kidney	Hemorrhagic and swollen	3	12	1	10	4	11.4
	Swollen with discoloration	2	8	2	20	4	11.4
Bones and Joints	Necrotized/sloughed femoral head	2	8	0	0	2	5.71

5.2.2. Gross lesion findings in the slaughtered chickens

Among the accessed organs of the slaughtered chickens, most of the gross lesions were observed within the livers and some of the heart and spleens were also found to have different gross lesions with different frequencies and percentages (**Figure 11**). In general, 100 positive chickens were included in this investigation among the examined chickens in the Alema chicken slaughtering abattoir and the types and frequencies of the lesion found in each of the affected organ were described in the **Table 4**.

Livers: Among the organs examined in the slaughtered chickens, livers were the most affected organ observed with different gross lesions with high frequency and percentage. Of the 100 livers examined, 92 (92%) of them were found to have various gross lesions. Acute and chronic form of severe and diffused necrosis with white necrotic foci were found in the 28 (28%) and 22 (22%) of the affected livers, respectively (**Figure 9A and B**). In addition to this, 26 (26%) of the livers were highly congested and enlarged in size and mild hemorrhages were observed within 2 (2%) of the affected livers (**Figure 9C**). Fibrinous perihepatitis was also observed within 6(6%) of the affected livers (**Figure 9D**). In general, due to all these disorders i.e mainly due to necrosis with necrotic foci, severe congestion and deposition of fibrin over the surface, almost all the affected livers were characterized by discoloration to either gray to pale, brownish, dark brown, greenish (green bronze) or brownish to greenish and abnormal consistency such as rough/hard surfaces and very soft with degeneration signs in the affected liver. Some bacteria were isolated from lesions and intestinal swabs of such cases as etiological agent following the observed gross lesions as suspicion of bacterial infections.

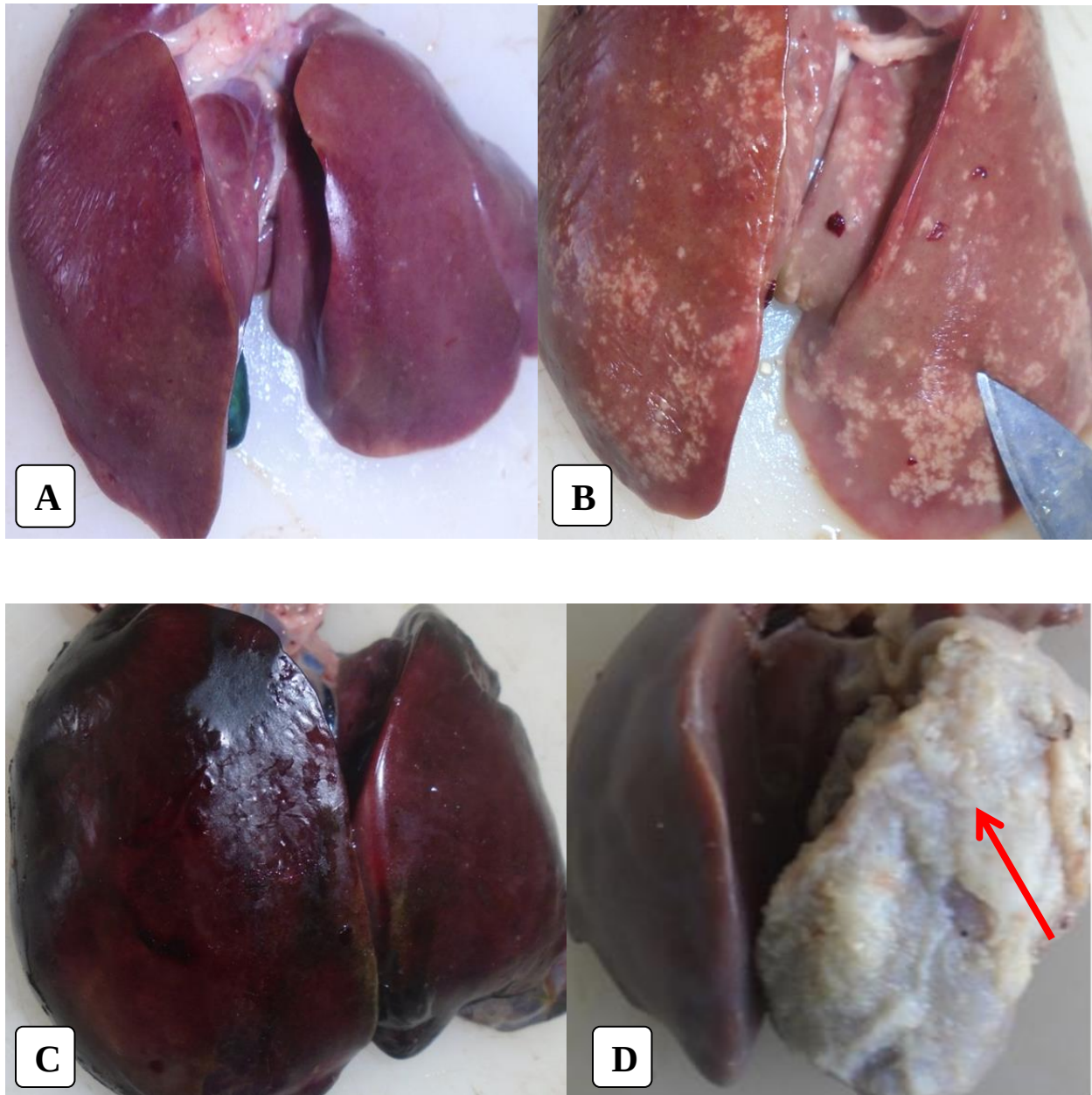


Figure 9: Various gross pathological lesions found in the livers of slaughtered chickens.

Keys: (A) Minute necrotic foci on the liver surface with enlargement; (B) Diffused necrotic foci with enlargement; (C) Highly congested, enlarged and discolored liver; (D) Fibrinous perihepatitis (left lobe indicated by red arrow).

Heart: Out of the 100 examined of positive chickens, 4 (4%) of the hearts were found to have gross lesions which were fibrinous pericarditis. Due to the over accumulation of the fibrin over the all surface of the hearts, they were misshaped, discolored to almost white, enlarged in size and became very hard in consistency (**Figure 10A**).

Spleen: Among the examined 100 spleens, 6 (6%) of them were found with gross lesions characterized by severe congestion and enlarged in size than normal (**Figure 10B**).



Figure 10: Gross pathological lesions found in the heart and spleen of slaughtered chickens.

Keys: (A) Fibrinous pericarditis and (B) Severe congestion and enlargement of spleen.

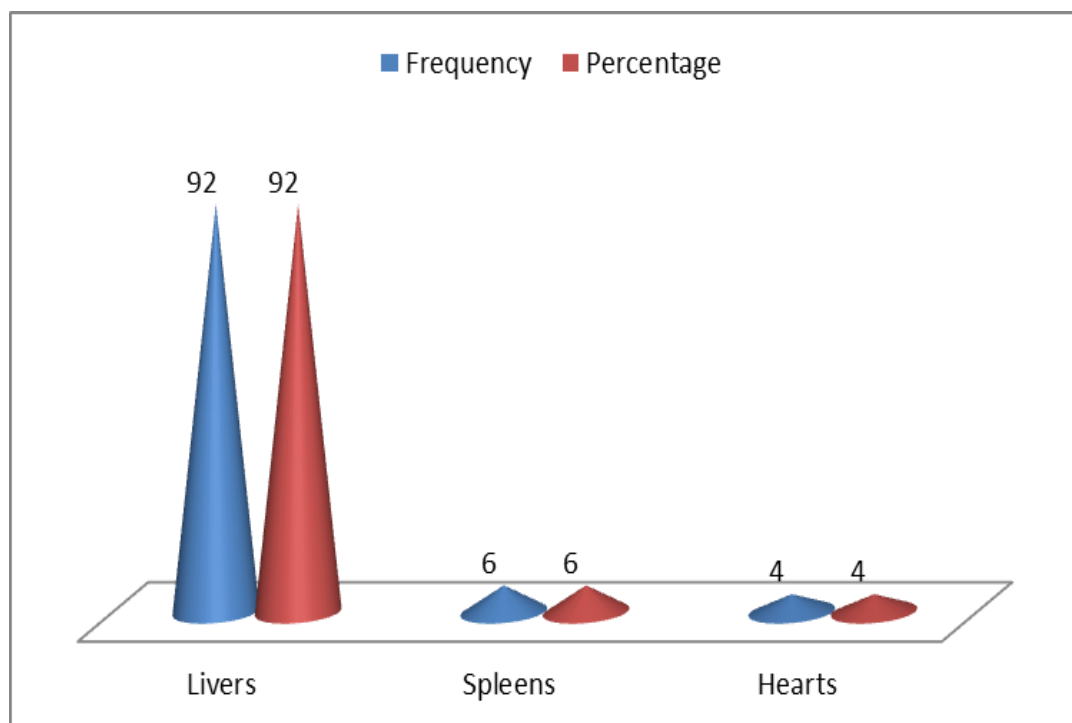


Figure 11: General necropsy findings of the frequently affected organs in slaughtered chickens with their frequencies and percentages, N=100.

Table 4: Necropsy findings in different organs of slaughtered chickens with their frequency and percentages of the lesions, N=100.

Organ	Gross lesions	Frequency	Percentage	Remark
Heart	Fibrinous pericarditis	4	4	
Spleen	Congested and swollen	6	6	
Liver	Severe, acute and diffused necrosis with white necrotic foci	28	28	Salmonellosis,
	Severe, chronic and diffused necrosis with white necrotic foci	22	22	Staphylococcus
	Congested and highly enlarged	26	26	and
	Hemorrhagic	2	2	
	Discoloration with abnormal size and consistency	6	6	
	Fibrinous perihepatitis	6	6	Collibacillosis

5.3. Histopathological Findings

5.3.1. Histopathology of digestive system

a) Intestines: Microscopically, the characters of lesions observed in the intestines taken from both sick and dead chickens were almost similar as in the gross lesions. In both cases, the segments of the intestines were presented by sloughed/removal of mucosal epithelium (desquamation of epithelial cells and villi), moderate to severe infiltration of leucocytes which were mostly heterophils and eosinophils in some cases, enteritis (severe inflammatory cells mainly in the caecum) and also few leucocytes in lamina propria, congestion which extended to the muscular region in some cases and hemorrhages as well as there were the necrosis in some area of the intestine, especially in the small intestines of some cases, in addition to compressed caecal glands (**Figure 12a**).

b) Proventriculus: The proventriculus were characterized microscopically by the presence of mucosal fold and loss of mucosal epithelium (sloughed mucosal epithelium), congested, diffused type of hemorrhages, edema and mild to severe infiltration of leucocytes which were mostly heterophils in epithelium and muscular part (intramuscular infiltration) (**Figure 12b and c**).

c) Livers: i) Livers from clinically sick and dead chickens: The results of histological examination of the liver tissues sampled from both clinically sick and dead chickens were similarly characterized by severe infiltration with aggregation of heterophils around the blood vessels, mild to severe congestion and hemorrhages as well as there were degenerative changes like hydropic degeneration microscopically.

ii) Livers from slaughtered chickens: these liver tissues were highly infiltrated than that of clinically sick and dead birds by leucocytes (hepatitis), there were severe, focal and diffused types of congestions, hemorrhages and necrosis with hydropic degeneration (**Figure 12d-f**). Diffused and severe necrosis and congestion was the principal microscopic lesion appreciated in the liver tissues. The microscopic lesions observed in the liver tissues were mostly corresponded with the gross lesion found and the lesions observed here were severe than the lesions identified in the clinically sick and dead chickens.

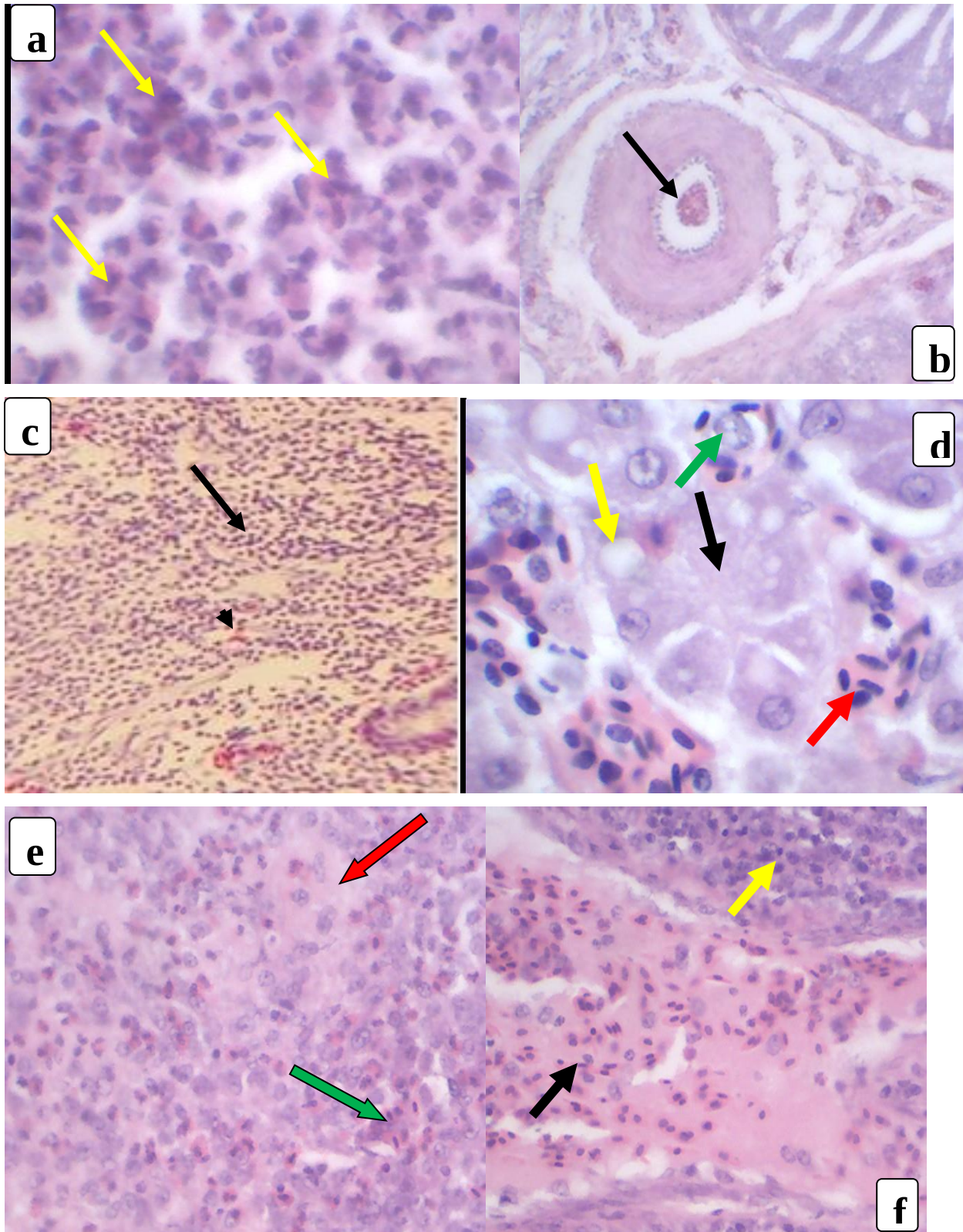


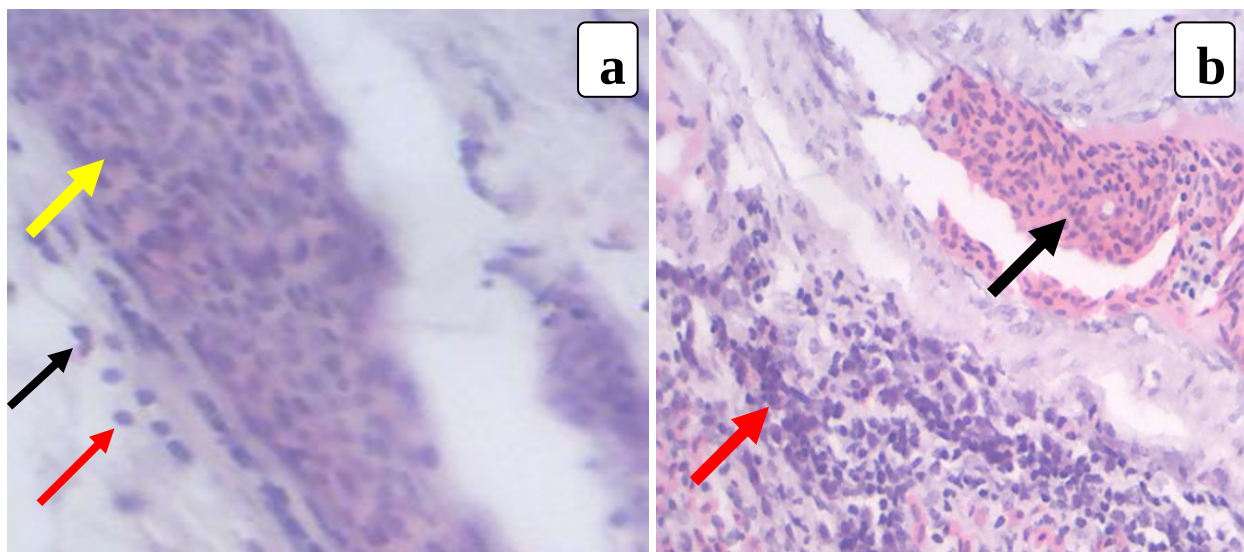
Figure 12: Different microscopic lesions found within the digestive system (H and E).

Keys: (a) Diffuse and severe infiltration of intestine (Caecum) mostly by eosinophils (yellow arrows) (100x); (b) Proventriculus with congestion and enlarged blood vessels (10x); (c) Proventriculus with severe infiltration and moderate hemorrhages (black arrow and arrow head, respectively) (40x); (d) Liver with karyolysis, hemorrhages, hydrophic and fatty degeneration (black, red, green and yellow arrows, respectively) (100x); (e) Liver with severe and diffused necrosis and hemorrhages (red and green arrows, respectively) (40x) and (f) Congestion and inflammatory cells infiltration of liver (black and yellow arrows, respectively) (40x).

5.3.2. Histopathology of respiratory system

a) Trachea: The microscopic lesion of the trachea sampled from clinically sick and dead chickens were characterized by the presence of the sloughed mucosal epithelium, submucosal congestion and diapedesis, treachitis (hemorrhagic inflammation) and infiltration by inflammatory cells specially in the submucosal part (**Figure 13a**).

b) Lungs: The lungs collected from clinical sick and dead chickens were found to have the mild to severe congestion and hemorrhages, alveoli filled by red blood cells and leucocytes (mostly hetrophils and few lymphocytes) as well as fluids in the alveoli surrounded by inflammatory cells and severe interstitial pneumonia with pulmonary edema (**Figure 13b and c**).



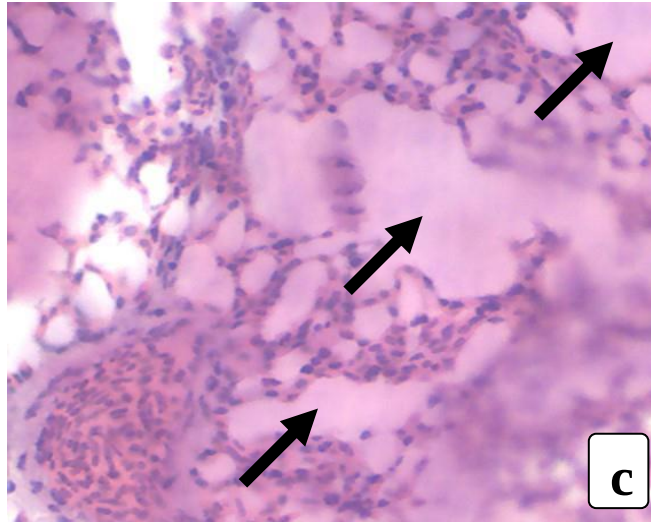


Figure 13: Various histological lesions found within the trachea and Lungs (H and E) 40x.

Keys: (a) Congested and infiltrated trachea (yellow for congestion and red for lymphocytes and black for heterophils arrows); (b) Congested and infiltrated lungs (black and red arrows respectively) and (c) Edematous lung (black arrows).

5.3.3. Histopathology of hearts

Heart: a) Heart from clinically sick and dead cases: The histopathological examination of the heart tissues revealed mild congestion and hemorrhages, infiltration of the leucocytes (Mostly heterophils with few lymphocytes, macrophages and plasma cells) and few erythrocytes as well as myocarditis and pericarditis were identified.

b) Heart from slaughtered chickens: The microscopically investigation of these organs revealed that the hearts were confirmed to have fibrinous pericarditis, infiltrated by polymorphonuclear and mononuclear inflammatory cells which were mostly heterophils with few lymphocytes and macrophages and congestion. In addition to this, mild degenerative changes on myocardium were found microscopically (**Figure 14a and b**).

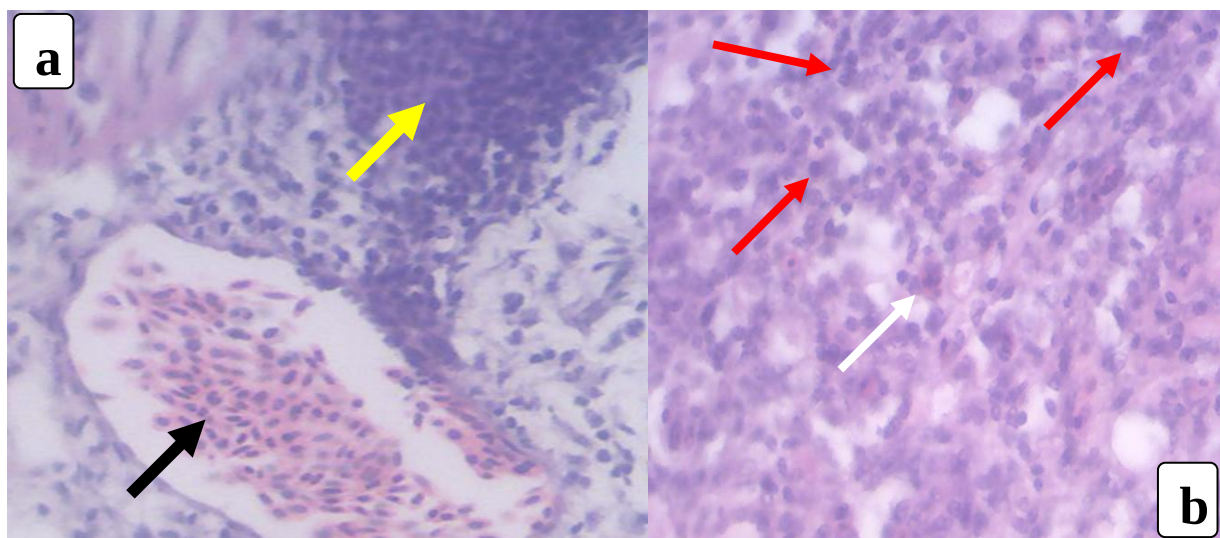


Figure 14: Various histological lesions found within the Hearts (H and E) 40x.

Keys: (a) Heart with congestion and severe infiltration (black and yellow arrows, respectively) and (b) Heart with severe infiltration and some erythrocyte (red and white arrows, respectively).

5.3.4. Histopathology of brains

The brains were collected from both clinically sick and dead cases following the case history of nervous signs of the diseases in some chickens. The results of the microscopic examination of both cases were similar which showed that there were congestion and mild hemorrhages, enlarged Robin's Virchow space due to the presence of perivascular edema surrounding the congested blood vessels and degeneration of the neurons (**Figure 15a and b**).

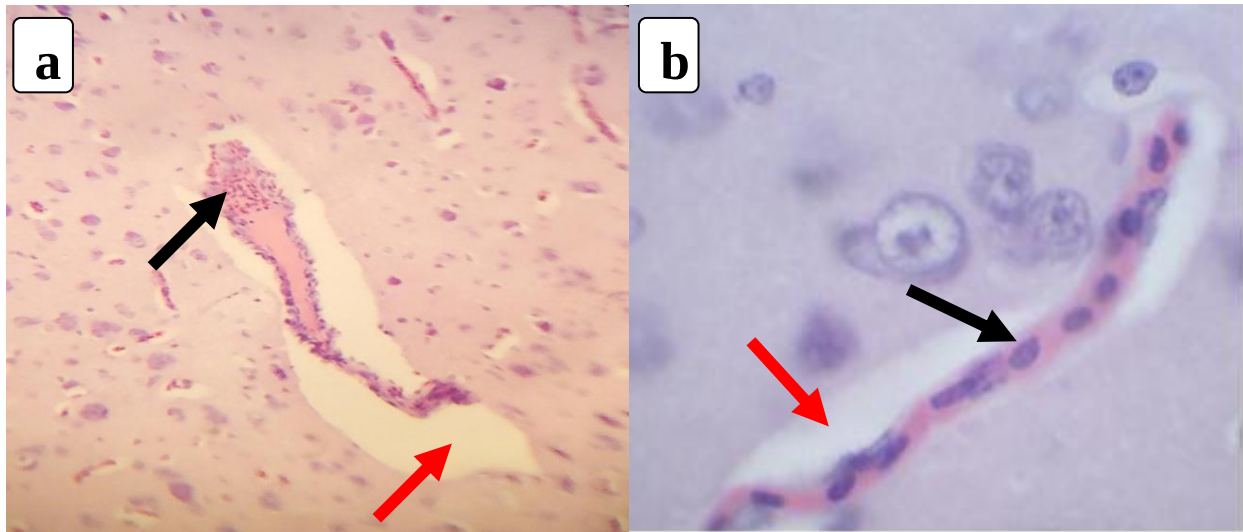


Figure 15: Microscopic lesions found within the Brains (E and H) 40x and 100x, respectively.

Keys: (a) and (b) congestion with enlarged Robin's Virchow space due to the perivascular edema which surrounding the congested blood vessels (black and red arrows, respectively for both figures).

5.3.5. Histopathology of kidneys

The microscopic lesions of the kidney tissues were similarly characterized by the presence of severe and diffuse congestion and hemorrhages which extended to all parts of kidney, degenerative changes and severe glomerulitis in both clinically sick and dead cases. The kidneys were also observed to have vacuolation in the epithelial cells of the renal tubules (**Figure 16a** and **b**).

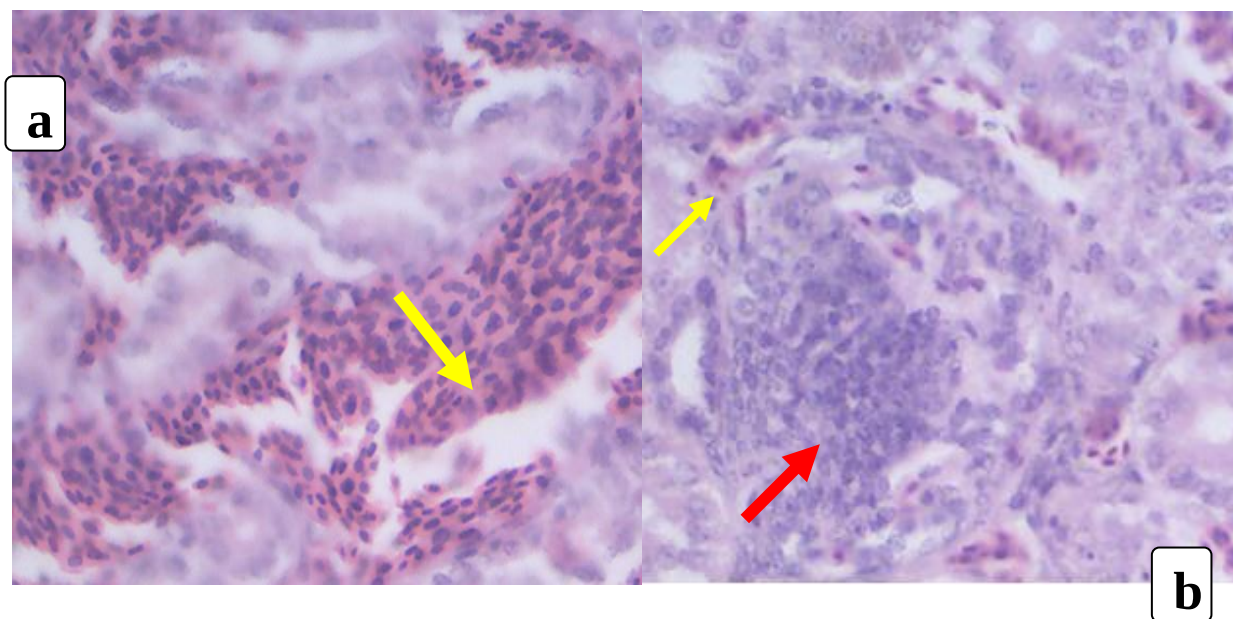


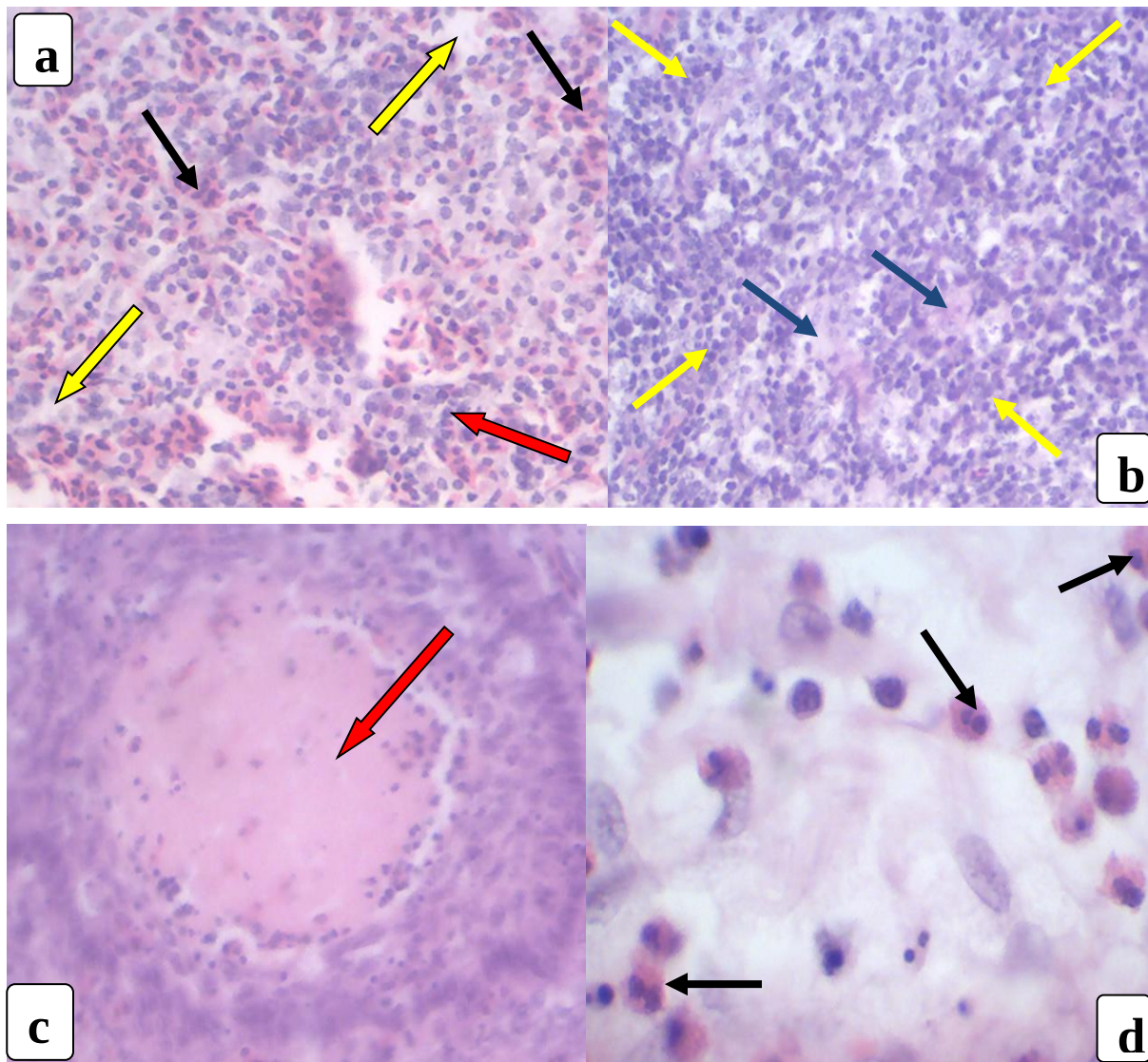
Figure 16: Microscopic lesions found within the Kidneys (H and E) (100x and 40x, respectively).
Keys: (a) Severely congested kidney (yellow arrow) and (b) Glomerulitis and hemorrhages (red and yellow arrows, respectively).

5.3.6. Histopathology of lymphoid organs

a) Spleens: The microscopic examination of the spleens revealed that there were excess red pulps with scanty white pulp showing there were no reaction, severe and diffused congestion and hemorrhages as well as the necrosis of the lymphoid tissues with mild to moderate degenerative changes and severe infiltration mostly by heterophils and lymphocytes (**Figure 17a**).

b) Caecal tonsils: The microscopic examination of the caecal tonsils collected from both clinically sick and dead case of the chickens were characterized by sloughed mucosal epithelium, depletion of the lymphoid tissue, congestion, hemorrhages, moderate to severe leucocytes infiltration (mostly heterophils and few lymphocytes and few macrophages), intact cells in the glandular part which is surrounded by heterophils and with necrosis of the caecal tonsils (**Figure 17b**).

c) Bursa of Fabricius: The main lesions revealed by histological examination of this organs were massive depletion of the lymphoid tissues in the bursal follicles which develop fibroblasts/surrounded by fibroblasts, presence of cystic space in the lymphoid follicles due to massive depletion, extensive necrosis of bursal lymphoid follicles, necrotized mass of bursal follicles surrounded by inflammatory cells, severe infiltration mostly hetrophils with a few lymphocytes and macrophages as well as eosinophils in cases complicated by Coccidiosis, severe hemorrhages specially around the bursal follicles, edema and heterophils in connective tissues for both clinically sick and dead cases (**Figure 17c - e**).



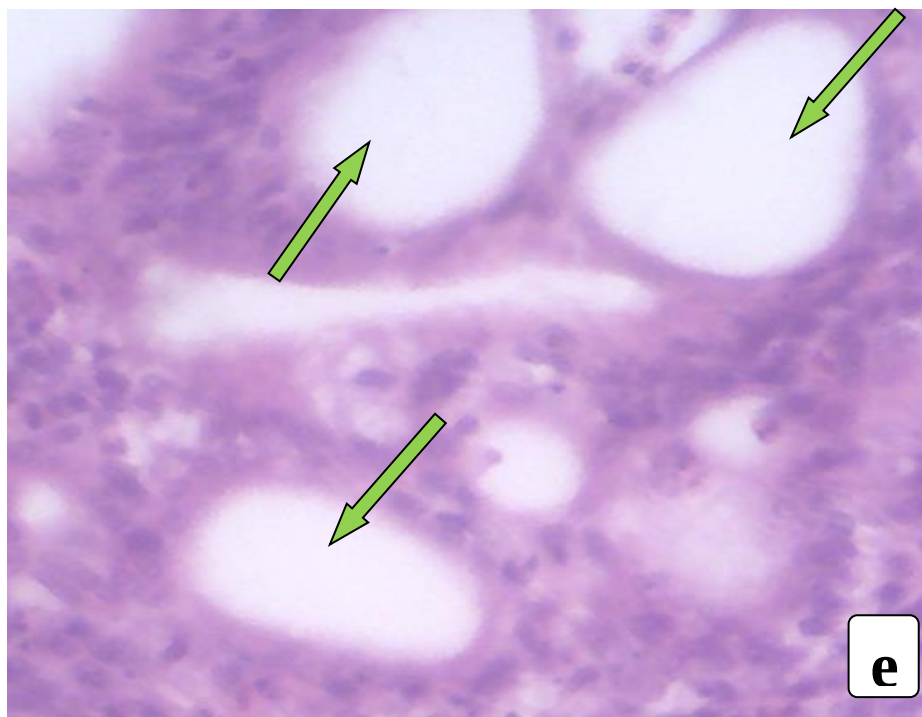


Figure 17: Microscopic lesions found within lymphoid organs (E and H).

Keys: (a) Severe and diffused hemorrhagic and infiltrated spleen with necrosis (black, red and yellow arrows respectively) (40x); (b) Severe and diffusely infiltrated caecal tonsil with necrosis (yellow and black arrows) (40x); (c) Massive depletion of the lymphoid tissues in the bursal follicles (red arrow) (40x); (d) Severe infiltration by eosinophils (black arrows) (100x) and (e) Cystic space developed in the lymphoid follicles of bursa of Fabricius (green arrows) (40x).

5.4. Bacteriological Isolation

Purposively suspected 30 chickens with different clinical signs and gross lesions, of which 8 from the clinically sick chickens with green and white diarrhea, in addition various gross lesions and 22 from slaughtered chickens observed with acute and chronic with severe and diffused types of liver necrosis were involved in this bacterial isolation process. The main bacterial isolated were *Escherichia coli*, *Salmonella species* and *Staphylococcus aureus* in which all the selected cases were positive at least for one of this bacterial species (**Table 5**).

Table 5: Bacteria isolated from chickens with different clinical signs and gross lesions, N=30.

Bacterial isolates	No. of isolates	Percentages (%)
<i>Escherichia coli</i>	18	60
<i>Salmonella species</i>	14	46.7
<i>Staphylococcus aureus</i>	8	26.7

5.5. Microscopic Examination for Coccidia

All the cases suspected for the coccidiosis due to the presence of the bloody feces (blood mixed feces and severely hemorrhagic caecum) within the caecum, revealed by necropsy findings, were examined microscopically for the confirmation of this problem. For this purpose, both mucosal scrapings and caecal feces were used according to the procedures described by Conway and McKenzie (1991) and Dinka *et al.* (2017) (**Annex IV**). For this coccidian oocytes examination, 12 cases among the all 35 chickens involved were done based on the tentative diagnosis, of which, 7 (58.3) were positive for coccidiosis.

6. DISCUSSIONS

In this study, 135 chickens, (25 clinically sick, 10 dead and 100 slaughtered), were examined and characterized by clinical signs, gross and microscopic lesions. In addition to this, isolation of some bacteria was also performed during this study period. The livers and lymphoid organs were the most commonly affected among in the examined chickens as the results of the present study which agree with the results of different authors reported previously (Supartika *et al.*, 2006; Dolka *et al.*, 2012). The overall results of this study revealed the presence of infectious diseases which are the main problems affecting the productive performance of chickens in the study area.

All of the clinically sick chickens were physically examined at clinic before euthanized and characterized by the manifested clinical signs. Unfortunately, 100% of them were found with at least two of the clinical signs recorded. Accordingly, 24 (96%) and 17 (64%) were observed with mild to severe depression and dropping their wings and anorexia (complete cease of feeding in some cases), respectively, 16 (64%) of examined chickens were found with different types of diarrhea and thick and pasting of vent, 5 (20%) were observed with torticollis, paralysis of legs and wings which were either unilateral or bilateral. 9 (36%) of diseased birds were found with signs of huddling together/reluctant to move. 2 (8%) were found with swelling of eye lids, 12 (48%) of them with respiration problem, 4 (16%) were observed with different discharges, 8 (32%) were observed with retarded growth and reduced body condition, 3 (12%) with swelling of infraorbital sinus, 3 (12%) with closure/blinded of the affected eye as well as 4 (16%) with swollen joint.

These all clinical signs recorded in the present study were reported as one of the clinical signs of different poultry diseases such as Avian Salmonellosis, Avian Colibacillosis, Infectious coryza, Avian Mycoplasmosis, staphylococcus, Newcastle disease, Marek's diseases, Gumboro disease, Coccidiosis and other infectious and non-infectious diseases by various authors in different time (Mohan *et al.*, 2000; Gimeno *et al.*, 2001; McDougald, 2003; Witter and Schat, 2003; Lee and Maurer, 2005; Le Nouen *et al.*, 2006; Alexander and Senne, 2008; Shivaprasad and Barrow, 2008; Jackwood, 2015).

In addition to the physical examination performed on the clinically sick chickens, all involved chickens were also examined carefully for the presence of any gross lesion by necropsy findings in terms of consistency, texture, shape, size and color as much as possible of each and individual organ of the body in all systems. Accordingly, the examined chickens were presented with different gross lesions which were suggestive of different poultry diseases which were agree with previous reports of various investigation.

The general, the gross lesions observed, identified and recorded in the present study were characterized by the presence of hemorrhages and ulceration in the intestines, hemorrhages of the trachea, proventriculus, caecal tonsils and carcasses (breast, thigh and pectoral muscles), black spotted lungs, air sacculitis characterized by cloudy/whitish thick, discoloration of livers, kidneys and spleen with congestion and abnormal size and consistency, nodules on the heart, fibrinous pericarditis and prehepatitis, swollen bursa with gelatinous edema, nephrosis, severe and diffused necrosis of the livers which were acute/chronic, moderate to severely congested livers and spleens and abnormal accumulation of fluids in the abdominal cavity.

These necropsy findings were followed by histological examinations of the representative tissues and the major microscopic lesions recorded and identified were characterized by mild to severe infiltration of inflammatory cells, mild to severe congestion, hemorrhages and necrosis, massive depletion/necrosis of lymphoid tissues specially in caecal tonsils, spleens and bursa of fubricius, development different cysts, sloughed mucosal epithelium mainly in the intestines, proventricriculus and trachea, edema and different degenerative changes/hydropic degeneration mainly in the kidneys and livers. The details of the macroscopic and microscopic findings of the present study corresponding to the previous investigation and reports were discussed as follows for each and individual organs of the system.

In the organs of digestive system (proventriculus and intestines as well as livers), different gross and microscopic lesions were observed and identified. Among the examined proventriculus, 12 (34.3%) of them were found to have mild to severe hemorrhages grossly and they were characterized by the presence of sloughed mucosal epithelium, congestion, diffused type of hemorrhages, edema, and mild to severe infiltration of leucocyte which were mostly heterophils

microscopically. Out of the examined intestines by considering each segment, 20 (57.1%) of the duodenum were observed with gross lesions revealed as moderate to severe hemorrhages and ulceration grossly and 19 (54.3%) of the jejunum were presented with gross lesions such as mild to moderate hemorrhages and fibrinous inflammations. In addition this ileum, caecum, colon and cloaca were demonstrated with the presence of mild to severe hemorrhages grossly.

Microscopically, all segments of intestines were similarly characterized by presence of sloughed mucosal epithelium, moderate to severe infiltration of leucocytes which is mostly polymorphonuclear cells (heterophils) and few mononuclear cells (especially lymphocytes), congestion and hemorrhages as well as there were the necrosis in some area of the intestine in addition to compressed caecal glands.

These gross and microscopic lesions recorded in the proventriculus and intestines in the present study were corresponded with the findings of different authors reported in various poultry diseases. For instance, Alexander and Jones (2001) reports marked hemorrhages in the proventriculus which was resulted from necrosis of the intestinal wall or lymphoid tissues in the cadavers of birds dead because of virulent NDV. Proventriculitis diagnosed in some IBD cases by Pantin-Jackwood *et al.* (2004) was in accordance with the present study in addition to the work of Cheville. (1967) in IBD which revealed the presence of hemorrhages in the mucosa at the juncture of the proventriculus and gizzard. The findings of McDougald. (2003) in the chicken infected by Coccidiosis with the thickening of the intestinal mucosa were also similar with present result. Furthermore, focal necrotic lesions were reported in the mucosa of the small intestine in Avian Salmonella (especially in Paratyphoid type) by Gast. (1997) and Roy *et al.* (2001) which agree with the present study.

Coccidiosis is the most common parasitic disease of the gastrointestinal tract of poultry (Adamu *et al.*, 2009; Dolka *et al.*, 2012; Guyonnet, 2015). As in the other investigations, suspected cases of chickens for coccidiosis were first examined using both caecum scraping and parasitological test of microscopic examination in the present study and 7 (58.3%) examined caecum among the 12 suspected cases were being positive for the oocytes of the Coccidial based on microscopic

examination. Similar result was recorded within the caecum by Soomro *et al.* (2001) based on the histopathological study conducted.

On the other hand, out of the examined 135 livers grossly, (35 from clinically sick and dead and 100 from slaughtered chickens), 106 (78.5%) were found to have gross pathological lesions such as severe congestion with enlargement in size, acute and chronic form of severe and diffused necrosis with white necrotic foci frequently, mild hemorrhagic and bulged cut surface (in some cases), fibrinous perihepatitis, discoloration characterized as dark brown, gray to pale, brownish, greenish (greenish bronze) or brownish to greenish and abnormal consistency such as rough/hard surfaces and softness with degeneration signs in some of the affected liver. By using additional diagnosis method, some bacteria such as *Salmonellae*, *Escherichia coli*, and *Staphylococcus aureus* were isolated from such cases as etiological agents based on these revealed gross lesions.

Histologically, the same progressions of lesion development were observed as gross lesions. The main microscopic lesions of these livers were characterized by the presence of highly infiltration by leucocytes (both polymorphonuclear and mononuclear cells) with aggregation of heterophils around the blood vessels, mild to severe congestions, hemorrhages and necrosis with hydrophic degeneration of the hepatocytes cells.

The gross and microscopic lesions identified in the present study in respected to these organs (livers) were corresponded with the findings of different authors reported previously. In the Avian Salmonella (Pullorum Disease and Fowl Typhoid), enlarged and congestion with multiple white foci of necrosis, green discoloration in the liver grossly and multifocal necrosis of hepatocytes, congestion with interstitial fibrosis and infiltration of heterophils mixed with a few lymphocytes and plasma were reported microscopically by Nakamura *et al.* (1986) and Barnes *et al.* (2008). Almost similar gross and microscopic lesions of the affected livers were also reported by Pierson *et al.* (1996) in the systemic forms of Colibacillosis and the report of Andreasen. (2008) in Staphylococcosis in relation to the affected liver consisted of foci and discoloration to green is also agreed with the present study. In general the present results and those reported by other authors indicate that most of the affected organs in the chickens' diseases were associated with liver failure (Singh *et al.*, 1996; Supartika *et al.*, 2006; Dolka *et al.*, 2012).

Even if the microscopic lesions observed in the liver tissues collected from the abattoir were presented with severe (highly severe hemorrhages, congestion and necrosis mainly) than in the samples collected from clinically sick and dead chickens, the lesions were more or less similar in correspond to their gross lesions demonstrated. This may be due to the reason that all the liver tissues collected from the slaughtered chickens were purposively taken from the livers with gross lesion at the time of data collection.

In the necropsy and histological examination of different organs of respiratory system, various gross and microscopic lesions were observed, identified and recorded in the present study. Accordingly, out of the examined tracheas, 22 (62.9%) were found with gross lesions revealed as mild to severe hemorrhages with mucoid exudates in some cases. The microscopic lesions revealed in these organs were characterized by the presence of the sloughed mucosal epithelial cells, submucosal congestion and diapedesis and leucocytes infiltration of submucosal. Among the examined lungs, 10 (28.6%) were observed with gross pathological lesions which includes mild hemorrhages and consolidation of the lungs with black spot in some cases. These lungs were characterized by the presence of severe congestion and hemorrhages, alveoli filled by red blood cells and leucocytes (mostly heterophils) and severe intestinal pneumonia with pulmonary edema by microscopic examination. Additionally, out of the examined air sacs, 19 (54.3) were observed with gross lesions like the presence of the cloudiness with hemorrhages and whitish spot on surfaces in some cases.

These gross and microscopic lesions recorded in the present study in case of these respiratory organs were also reported in the findings of different authors in some poultry diseases. Predominant hemorrhages and congestion of the trachea as well as air sacculitis as the gross lesions and congestion, edema, and dense infiltration of inflammatory cells at upper respiratory tract as the microscopic lesions were reported by Chuhhan and Roy (1998) and Alexander and Senne (2008) in Newcastle diseases problem. Tracheas with sloughing of epithelium, edema with infiltration, pneumonia and air sacculitis were reported by microscopic examination of the lessons in Coryza Infectious by Mohan *et al.* (2000); Shivaprasad and Droual (2002) and Ali *et al.* (2013) which were similar with the statement of present study. In addition to this the macroscopic and microscopic lesions reported in respiratory-originated Avian Colibacillosis by

Ginns *et al.* (1998) and Ask *et al.* (2006) were similar with the present study result in trachea and lungs particularly. Similar results were also recorded perilously by the work of Radkowski *et al.* (1996) and Chacón *et al.* (2007) in the respiratory tract.

Out of the examined hearts, 14 (10.4%) were observed with gross lesions which were presented by gray to white small nodules, fibrinous pericarditis, misshaped and discolored to almost white which may be due to the over accumulation of the fibrin over the all surface, enlarged in size and became very hard in consistency. The microscopic examination of these central organs of circulation revealed that fibrinous pericarditis, myocarditis; infiltration of inflammatory cells (mostly heterophil, lymphocytes and few macrophages) and few erythrocytes, congestion of the pericardium, mild congestion and hemorrhages and also mild degenerative changes on myocardium.

These both gross and microscopic lesions recorded in this investigation were similar with the findings of Shivaprasad (2000) and Shivaprasad and Barrow (2008) in the Avian Salmonellosis in which the presence of large nodules in the heart grossly with congestion and interstitial fibrosis and necrosis of myofibers with infiltration of heterophils mixed with lymphocytes and plasma cells were reported as histopathologic lesion. In addition to this, endocarditis and pericarditis were reported in Coryza Infectious by Talha *et al.* (2001) in line with the present study. Furthermore, some cases with such gross lesion in the heart were being positive for Salmonellae based on the confirmation done by isolation of bacterial using biochemical tests.

Out of the examined kidneys, 8 (22.8%) were observed with different gross and microscopic lesions. The main gross pathological lesions observed were hemorrhage, enlargement and discoloration. The microscopic lesions observed within these organs revealed as severe and diffuse congestion and hemorrhages which reach all kidney parts, degenerative changes and severe glomerulitis. The present investigations in relation to kidney were similar with some reports of the previous investigators. For example, IBD was described as “Avian Nephrosis” because of the extreme kidney damage found in the affected birds at the first time by Cosgrove (1962). Severe vascular congestion in per acute case and interstitial inflammation and infiltration

in chronic cases in affected kidneys by Avian Salmonella are also reported by Wong *et al.* (1996).

Lymphoid organs were also the most frequently affected organs in the chickens. This is probably due to the fact that the lack of lymph nodes in most poultry species increases the importance of lymphoid organs in resistance to various diseases. Major organs of the immune organs of the chickens were examined grossly and microscopically in the present study. Accordingly, out of the examined caecal tonsils, 25 (71.4%) were identified with different gross lesions which revealed as moderate to severe hemorrhages, necrosis and enlarged/swollen in size. Microscopically, these organs were characterized by the presence of sloughed mucosal epithelium, depletion of the lymphoid tissue, congestion, hemorrhages, moderate to severe leucocytes infiltration, peri-glandular infiltration and necrosis of the caecal tonsils. In line with the present investigation results, similar lesions are reported by the work of Alexander and Jones. (2001) and Withers *et al.* (2005) in which gross marked hemorrhages of caecal tonsils were identified due to virulent NDV and Gumboro disease, respectively, with the same progressions of microscopic lesion development as cellular reaction/severe inflammatory cells infiltration.

Moreover, out of the examined bursa of Fabricius, 4 (11.4%) were found with gross pathological lesions observed as hemorrhages and increased in size with gelatinous edema revealed when incised. These organs were histologically characterized by the presence of massive depletion of the lymphoid tissues in the bursal follicles, presence of cystic space in the lymphoid follicles due to massive depletion, extensive necrosis of bursal lymphoid follicles, necrotized mass of bursal follicles surrounded by inflammatory cells, mostly heterophils and few eosinophils and lymphocytes, severe infiltration, severe hemorrhages and edematous. These macroscopic and microscopic lesions of bursa of Fabricius recorded in the present study were similarly stated by other investigators in their previous work. For instance, Sharma *et al.* (2000); Baxendale (2002); Negash (2004) and Withers *et al.* (2005) clearly stated these lesions in their study on the Gumboro/Infectious Bursal Disease. Microscopically, severe lymphoid depletion of the bursa of Fabricius is also reported by Shivaprasad and Droual (2002). Additionally Khan *et al.* (2009) also obtained similar results with significant bursal damage as in the present study.

On the other hand, out of the examined spleens, 11 (8.2%) were found to have gross and microscopic lesions. These organs were observed with the presence of moderate to severe congestion, hemorrhage and highly enlarged in size with discoloration grossly and excess red pulps with scanty white pulp showing there were no reaction, severe and diffused congestion and hemorrhages as well as the necrosis of the lymphoid tissues with mild to moderate degenerative changes were also identified microscopically.

Among the previous investigation done on the spleen lesions macroscopically and microscopically, Gast (1997) reports gross enlargement and congestion of spleen in the Paratyphoid Salmonella which is similar with the present findings. Cellular reaction infiltrated with heterophils in the spleen damaged is reported by Eterradossi and Saif (2008) in Gumboro disease as stated in this study. Another report which agrees with the present study is also reported by Newberry *et al.* (1993) and Van den *et al.* (1994) in Enteric-Origin Colisepticemia form of Avian Colibacillosis in which grossly marked enlargement and congestion of spleen in line with the microscopic lesion such as congestion with multifocal necrosis and inflammation of the glomeruli were revealed.

Even if no gross lesion was observed in central nervous system (CNS), the brains collected from both clinically sick and dead were characterized by the presence of congestion and mild hemorrhages, enlarged Robin's Virchow space due to the presence of perivascular edema surrounding the congested blood vessels and degeneration of the neurons microscopically. Previously Al-Garib. (2003) and Alexander and Senne. (2008) done investigation on chickens' CNS (particularly brain) infected by NDV and reports some microscopic lesions such as encephalomyelitis with neuronal degeneration that were similar with the present findings.

Out of the female chickens examined, 11 (40.7%) ovaries were found with gross lesions demonstrated as mild to severe hemorrhage with inflammation on some part, atrophied ovaries and discoloration to yellowish in some of them. The present findings were almost similar with the reports of Murphy *et al.* (1999) in Newcastle disease with virulent NDV and Shivaprasad. (2000) in Avian Salmonellosis specially within Pullorum Disease and Fowl Typhoid in the adult

chickens, those came up with degenerative or regressing ovarian follicle, misshapen, hemorrhages, and discoloration of the other reproductive organs in laying hens affected.

Gross lesions were also found within joints, muscles (carcasses) and head regions of some cases. Among the examined bones and joints of each case, 2 (5.7%) of femoral head were found with slough /necrosis which may be due to dietary deficiency and systemic diseases. Klasing (2008) and Crespo and Shivaprasad (2013) describes the effects of vitamins and minerals on the development and functions of these organs in chickens with the pathology of this deficiencies.

Moreover, out of the examined muscles/carcasses, 6 (17.1%) were demonstrated with the presence of abnormal condition such as presence of hemorrhages on the thigh, breast and pectoral muscles and the carcasses were pale in color in addition to these hemorrhages in some cases which may be due to the IBD. Cheville. (1967) and Eterradosi and Saif. (2008) reported such lesions as the signs of IBD in their investigation previously which was in line with the present study. And also, some of the muscles were discolored to almost paleness which may indicate emaciated. Furthermore, of the examined heads regions, 2 (5.7%) were observed to have cheesy exudate in the infra orbital sinus which was reported by various authors as the main indicator/sign of Coryza Infectious that was similar with the present work (Talha *et al.*, 2001; Mohan *et al.*, 2000).

Finally, the history, clinical signs, gross lesions demonstrated, histological examination done, bacterial isolation performed and microscopic examination for the presence of coccidia infection complicated with other disease revealed the presence of infectious poultry diseases in the study area in the present study. By correlating these all information based on the previous reports and characteristics of some diseases macroscopically and microscopically, the major infectious disease of chickens such as Gumboro disease, Coccidiosis, Avian Salmonellosis, Avian, Staphylococcosis and Newcastle Disease were identified as the most health problem of chickens, in addition to other diseases, in the study area as characterized in this investigation.

7. CONCLUSION AND RECOMMENDATIONS

The fastest growing sector of poultry production in the developing countries including Ethiopia is facing health problem due to viral, bacterial and parasitic diseases which are the first frequently reported as the major constraints. For this reason the present study was focused on the clinical signs, necropsy finding with histological characterization of diseases and isolation of some bacterial etiology in correlating with the problems. Accordingly, various clinical signs and major gross lesions such as mild to severe congestion and hemorrhages, inflammation, discoloration, swelling, and mild to severe necrosis and microscopic lesions such as mild to severe congestion, hemorrhages and necrosis, massive depletion lymphoid tissues, sloughed mucosal epithelium, edema and degenerative changes were recorded in different organs in the corresponding to gross lesion. In addition to this, *E.coli*, *Salmonella spp* *Staphylococcus aureus* and *coccidian* were isolated. The overall results of the present study revealed the presence of the infectious diseases such as, Gumboro disease, Coccidiosis, Colibacillosis and others as the health problems which are the main constraints hampering the productivity and reproductively of the chickens in the study area. This indicates that the community does not getting adequate benefits from chickens as need and the problems needs long lasting solution based on scientific investigation.

In view of the above conclusion, the following recommendations are suggested:

- ✚ Integrated and strategic approach such as strict biosecurity, antimicrobial prophylaxis, vaccination and other managerial measures should be made on regular basis to decrease diseases and mortality of poultry.
- ✚ Government, different professionals' bodies including researchers, veterinarians, animal health service and delivery implementing should give continued attention for poultry sector and its development.
- ✚ Further collaborative researches on specific identification of causative agents of chicken diseases should be done to exactly identify the underlining complexation problems and devices proper preventing strategies in the future.

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9. ANNEXES

Annex I

Chicken Necropsy Protocols (Corrie *et al.* (2008) and Chauhan and Chandra. (2007))

A good necropsy involves paying attention to **ALL** the clues that can be provided, so the routine has to be followed, with attention to detail at every step. The consistent routine entails 6 steps:

Step 1: Obtain the History

This history should include:

- + Bird's age
- + Sex
- + Breed
- + Clinical signs
- + History of trauma or disease
- + History of any treatments administered
- + Any other information that may be relevant to the case such as type of feed and water
- + If the bird is a member of a flock the following is also required:
 - Number of birds in the flock
 - Number of birds in the affected group
 - Number of affected birds
 - Clinical signs of the flock should also be noted

Step 2: Examine the Bird Externally

- ❖ Examine the bird for any signs of trauma and evaluate the bird's general body condition.
- ❖ If possible the bird should be weighed.
- ❖ **Prior to opening the body:**

- If it is planned for additional investigation, you might want to swab the trachea or cloaca. When swabbing the trachea, insert the swab up in the choana
- Dislocate cervical joints of the bird and dip the whole bird/carcass into a bucket of soapy water to thoroughly wet all the feathers.

Step 3: Open the Body

- The body should be placed on its back with its feet facing you and reflect the wings back.
- Cut through the skin between the legs and the breast so the legs can be fully abducted and lie flat against the table.
- Remove the skin from the ventral surface of the bird by cutting across at the caudal edge of the keel and then pulling skin cranially and caudally - peel away from the muscle to expose the muscular body wall.
- Make a small cut into the body cavity using scissors or a scalpel blade just behind the breast bone, and then pull the abdominal muscle caudally to expose some abdominal viscera.
- Extend the cut up through the cervical area and cut open the beak at the angle of the jaw.
- Now oral cavity, esophagus, trachea and crop are all visible
- The keel bone and breast muscle is then removed by incising the pectoral muscles on each side of the keel and cutting through the ribs.
- Use the heavy poultry shears. Remove the keel and breast muscles entirely - you should now be able to see the internal organs from oral cavity to rectum.
- At this point, all internal viscera should be examined in situ for any abnormalities before removing any organs.
- Note the color, position and size of all organs and look for any adhesions.
- Examine the air sacs for increased thickness or cloudiness (caudal thoracic air sac is at the end of the forceps).

Step 4: Remove the Organs and Set Aside For Examination

- ✚ It is probably easiest to remove the abdominal viscera first and then go back and remove the thoracic organs.

- + Remove the liver - in birds the liver takes up a big portion of the abdomen.
- + The spleen can be a difficult organ to find once everything is removed so it is a good idea to locate it now and set it aside.
- + Pull the proventriculus aside and it should pop into view. Take it out now and set it in a clean dry spot.
- + Find the junction of the esophagus and stomach, cut here and pull the digestive tract out, all the way to the cloaca.
- + Cut at the end of the large intestine, but leave the bursa in the bird.
- + Kidneys are nestled up against the body wall - there are three portions - cranial, middle and caudal poles.
- + In females only the left side of the tract persists, the right is vestigial and too small to be identifiable.
- + Pull the heart and lungs away - the lungs are closely adhered to the dorsal body wall, and therefore, careful teasing of the lungs away from the ribs may be necessary to remove them.
- + Open up the nasal cavity to take a close look at the sinuses.
- + Observe the sciatic nerve which is an important location where Marek's disease can be seen.
- + Open some joints to observe the fluid and synovial membranes.
- + The brain can now be removed. Using the smaller scissors (not poultry shears), chip away at the skull, beginning from the foramen magnum, and remove the calvarium covering the cerebral hemispheres and the cerebellum. Take the brain out - use care this organ is quite soft.

Step 5: Examine and Sample the Organs

A) Examining of the organs

- ✓ It is a good idea to go from the “cleanest” organs to the “dirtiest”. Usually this order is: lymphoid tissue, brain, lungs, heart, kidneys, reproductive tract, liver, intestinal tract.

- ✓ Note any abnormalities for each (color, size, shape, consistency, exudates). Be sure to examine both capsular and cut surface.
- ✓ Make several cuts in each organ and collect specimens for further diagnostic work.

B) Sampling of the Organs

- Tissues should be collected in duplicate, with half going into 10% formalin for histopathology and half as unfixed tissues that will be used for bacterial culture etc. if necessary.
- The following tissues should be collected in all cases: lung, liver, spleen, bursa, brain, kidney, intestines.
- Additionally, any other tissue that might have lesions (skin, adrenal, ovary, heart etc.) should also be collected.
- Label the tissues that are collected fresh so that the technician performing the tests can determine what each tissue is.
- Crushing should be avoided by using a sharp blade and a hard surface when taking samples since this may cause artifact histologically.
- When examining any organ with a mucosal surface (trachea, esophagus, intestine, etc.) care should be taken not to damage or destroy the mucosal surface by rubbing the surface with fingers or instruments.
- In order to improve fixation of tissues, samples should not exceed 5mm in thickness and volume of fixative should be at least 10 times the volume of tissue.
- Fresh samples should be packaged so that they remain cool and to minimize possibilities of leaking.

Step 6: Write the Report

- No necropsy is complete until all findings have been recorded in written form.
- The report should include at least the following information:
 - Species, breed, age, sex and history
 - Dead or euthanized?

- Nutritional, hydration status
- Findings from external examination
- Findings by organ system: Lymphoreticular (spleen, bursa), Respiratory, Digestive, Urogenital, Musculoskeletal, Nervous.

Notes: When describing tissues, keep these four qualities in mind - consistency, color, size, and symmetry.

Chickens Necropsy Examination Performing Form Used

Date: _____

Case code (Case No): _____

A) Important Information

Owner Information	Chickens Information		Vaccines/Treatments
Name:	Age:	Number of Chicken/s:	
	Sex:	At Risk:	
Address:	Breed:	Diseased:	
	Submitted Chicken:	Dead:	
Phone:	Types of Chickens:	Place of Examination:	
	Status of Case:		

B) History of the Case

C) Clinical Signs/Ante Mortem Findings By External Examination

D) Postmortem Findings By Internal Examination

Body Systems	Examined Organs	Gross Lesions observed	Sampled Organ/s
Locomotive and Musculoskeletal	Bones and Joints		
	Muscles		
	Footpads		
	others		
Circulatory and Lymphoid	Pericardium		
	Heart		

system	Spleen		
	Thymus		
	Bursa		
	Caecal Tonsils		
	Ascites		
	Others		
Respiratory	Sinus		
	Trachea		
	Lungs		
	Air sacs		
	Others		
Digestive Tract	Beak and Mouth		
	Oesophagus		
	Crop		
	Proventriculus		
	Gizzard		
	Liver		
	Duodenum and Pancreas		
	Jejunum		
	Ileum		
	Caecum		
	Others		
Urogenital	Ovaries		
	Testicles		
	Kidneys		
	Ureters		
	Cloaca		
	Others		
Nervous system	brain		

E) Conclusions

Annex II

a) Histopathological procedures (Takulder, 2007)

1. **Fixation** of tissue by 10% neutral buffered formaldehyde
2. **Trimming** part of the tissue in a way that the lesion we require be included or not missed and to fit standard histological processing tissue cassettes (5mm thickness).
3. **Tissue Specimen Processing:**
 - ❖ This process fixation of tissue by formalin, dehydrating tissue by increasing alcohols concentration, clearing of tissue by xylene, and impregnation of tissue by paraffin wax.
Formalin-I 2hr → Formalin-II 2hr → 70% Alcohol 1hr → 95% Alcohol 1hr → 100% Alcohol-I 1hr → 100% Alcohol-II 2hrs → 100% Alcohol-III 2hrs → Xylene I 1:30hrs → Xylene-II 1:30hrs → Xylene-III 1:30hrs → Paraffin-I 2hrs → Paraffin-II 3hrs.
4. **Embedding of processed tissue**
 - Impregnated tissue is placed in a mould with their labels and then fresh melted wax (54-60°C) is poured and allowed to settle and solidify.
5. **Sectioning**
 - Sectioning of tissue in 3-5 micron thickness and put on water bath to straighten the ribbon, and then adhere on the surface of frost ended and clear slide.
 - Later label and put an incubator overnight.

b) Staining Procedures (Talukder, 2007)

Hematoxyline and Eosine Stain Procedures

1. Deparaffinize slides in 2 changes of xylene for 5 minutes each.
2. Rehydrate slides in 100% alcohol (3times) and 95% alcohol and 70% alcohol respectively for 3 minutes each, and rinse in distilled water until ripples disappear from slides.
3. Place in Hematoxylin for 8 - 15 minutes.

4. Rinse in tap water until water runs clear.
5. Decolorize in 1% acid alcohol, 3 - 6 quick dips. Check differentiation microscopically: Nuclei should be distinct; Cytoplasm should be uncolored.
6. Rinse in tap water until ripples disappear from slides.
7. Stain in Eosin (Counter stain) (3dips).
8. Wash in tap water
9. Dehydrate in 70%, 95%, 100% (3times) alcohol respectively for 3 minutes each.
11. Clear in xylene (3times) 5 minutes each.
12. Mount with cover glass (with canada balsam)
13. Finally examined under microscope starting from the lower to higher magnification (4x, 10x, 40x and 100x) for the presence and identification of the microscopic lesions.

Annex III

Procedures of Bacterial Isolation (Quinn *et al.*, 2004)

Salmonella

1. Lesions and cloacal/caecum swabs will collected by using a sterile wooden cotton swab on the surface of affected organs and insert in the 10ml test tube that contains sterile buffered peptone water (BPW) used as a pre-enrichment media for 24hrs at 37°C and transported to processing area (in this case AAU-CVMA) lab) by using icebox containing ice bag.
2. 1ml of the pre-enriched culture was transferred to 10 ml of Selenite F Broth (SFB) tube and another 0.1 ml portion will be transferred to 10 ml of Rappaport Vassiliadis Soy broth (RVSB) and incubated at 37°C for 24hrs and 48hrs, respectively.
3. Finally one loop of broth culture from the inoculated and incubated SFB and RVSB sample will be inoculated and incubated on to Xylose Lysine Deoxycholate (XLD) at 37°C for 48hrs.

4. When suspected colonies detected (characteristic Salmonella colonies, having a slightly transparent zone of reddish color and a black center on XLD media) will be sub cultured on the nutrient agar media plates.
5. Finally confirmed by using biochemical tests IMViC test (Indole test, Methyl Red-Vogues Proskauer (MR-VP) test, Simon's citrate test) and Triple sugar iron agar (TSI).
 - Atypical biochemical reaction on TSI i.e. alkaline (red) slant, acidic (yellow) butt, H₂S and gas production.
 - Citrate test positive (utilization as a carbon source)
 - Indole test negative
 - M-R test positive and
 - V-P test negative

Escherichia coli

1. Lesions and cloacal/caecum swab will collected by using a sterile wooden cotton swab on the surface of affected organs and insert in the 10ml test tube that contains sterile buffered peptone water (BPW) used as a pre-enrichment media for 24hrs at 37°C and transported to processing area (in this case AAU-CVMA) lab) by using icebox containing ice bag.
2. 1ml of the pre-enriched culture will be transferred and suspended into 9 ml of modified Tryptone Soya Broth and incubated overnight at 41 °C.
3. After selective enrichment, 50µl of product will be streaked onto MacConkey agar for primary isolation and incubated aerobically at 37 °C for 24hrs.
4. The plates will be observed for the growth of *E. coli* (pink colony; lactose fermenter).
5. A single, isolated colony will be picked and sub-cultured on Eosin Methylene Blue (EMB) agar for formation of metallic sheen.
6. Suspected colonies of *E. coli* (pinkish color appearance on MacConkey agar and metallic sheen on EMB) will be then sub-cultured will be inoculated on nutrient agar.
7. Then biochemical tests will be performed to confirm the *E. coli* using catalase test, Indole Production test, Methyl red test, Voges proskaur test, and Simmon's Citrate test on tryptone broth, MR-VP medium and Simon citrate agar respectively.

Annex IV

Microscopic Examination for Coccidia (McKenzie, 1991 and Dinka *et al.*, 2017)

A) By Using Mucosal Scrapings from Caecum

1. Mucosal scrapings will be taken from segments of the caecum with gross lesions
2. The scrapings will be placed on microscopic slides and diluted with a drop of distilled water using Pasteur pipette and mixed thoroughly
3. Then it will be covered with a cover slip and
4. Finally examined under light microscope.

B) By Using Fecal /Intestinal Content Floatation Technique

1. 3 gram of intestinal content will weighed using a digital balance and put into a mortar and mixed with floatation solution of sodium chlorides (NaCl).
2. By using a pistol, it is thoroughly mixed and strained using a sieve (filter paper) into another beaker.
3. The filtrate will poured into test-tube of respective sample number and centrifuged at 1500 rpm for 3 minutes.
4. Then the test tubes will be placed in a test tube rack
5. Each test tube will be filled to the brim with the floatation solution, covered with a cover slip and left to stand for 10 minutes
6. After this these cover slips will gently lifted up and then placed on clean microscopic slides
7. Finally they will be viewed under the microscope.

Annex V

Pictorial Description of the Fields and Laboratory Works

a) Figures describing data collection from clinically sick chickens (Figures 18a-d)



Figures 18: Data collection from clinically sick chickens at clinic (AA- CVMA VTH)

Keys: (a and b) Discussions with undergraduate students on patient's history and physical examination of chicken, respectively; (c and d) Postmortem examination of gross lesion followed by data collection from internal organs.

b) Figures describing data collection from abattoir (Figures 19A-D)



Figures 19: Data collection from slaughtered chickens in Alema abattoir

Keys: (A and B) Removing out internal organs which were ready for examination
(C and D) Gross examination and data collecting from internal organs.

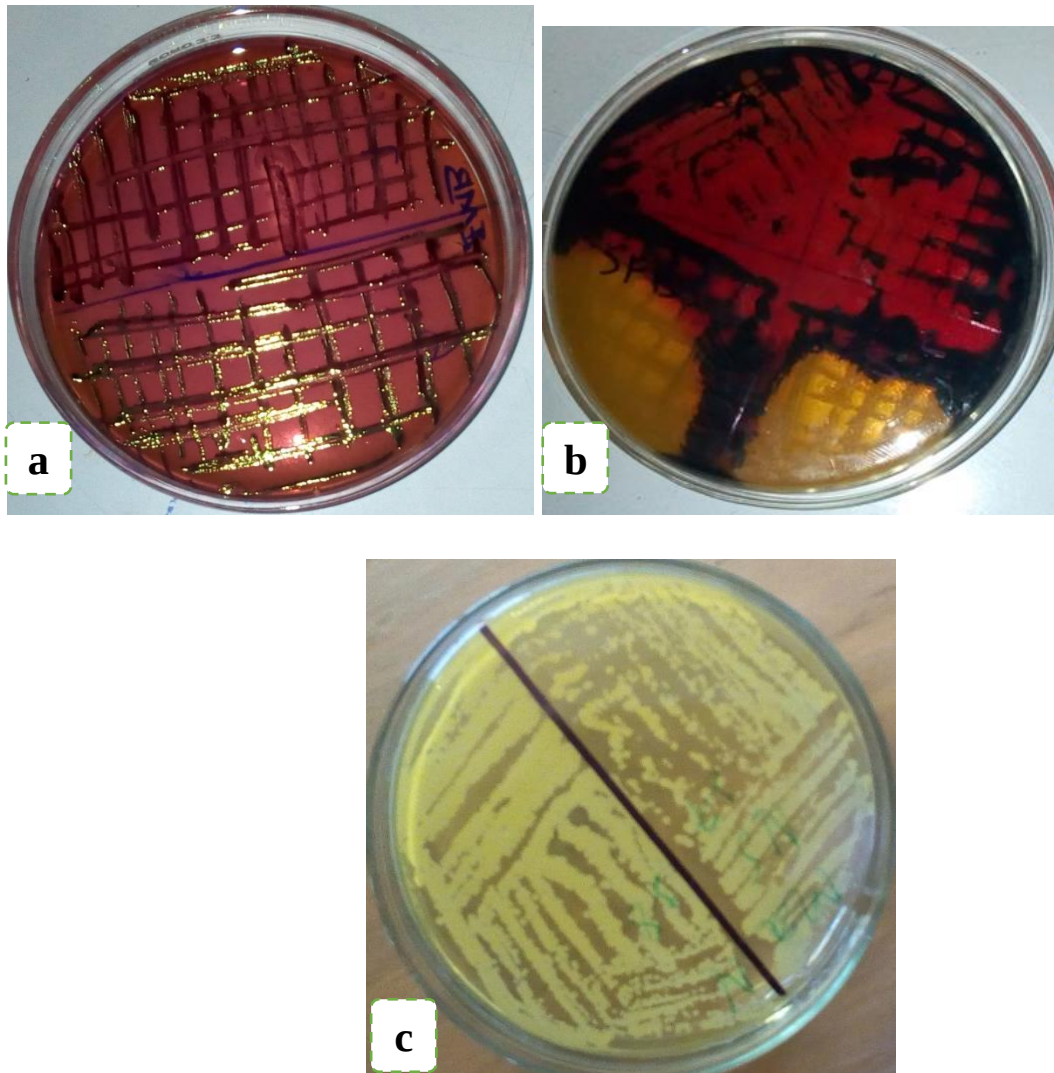
c) Figures describing laboratory works (Figures 20a-c)



Figures 20: Tissue process and microscopic examination of lesions.

Keys: (a and b) Tissue trimming and sectioning in NAHDIC, Sebeta Pathology laboratory respectively; (c) Microscopic examination lesions for processed tissues in AAU CVMA Pathology laboratory.

d) Figures describing bacterial Isolation (Figures 21a-c)



Figures 21: Bacterial isolation at AAU CVMA Clinical Pathology laboratory

Keys: (a) Sample suspected for *E. coli* on the Eosin Methylene Blue (EMB) with formation of metallic sheen seen; (b) Sample suspected for *Salmonellae* on the Xylose Lysine Deoxycholate (XLD) media with zone of reddish color and a black center seen on the media; (c) Sample suspected for *staphylococcus aureus* on the Mannitol Salt Agar (MSA) with formation of yellow colonies. All the suspected samples were followed by secondary biochemical tests and confirmed finally.