Sudan University of Science and Technology

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A Field Study of Infectious Bursal Disease in Khartoum, Sudan

دراسة ميدانية لمرض الجراب المعدي في مزارع الدجاج البياض بولاية الخرطوم

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Preface

The work described in this thesis was carried out in the department of Pathology, Faculty of Veterinary Medicine, University of Sudan, under supervision of Dr. Hind El Rayah Osman. The material presented is original and has not been submitted to any other university.

Dedication

To our Mothers, Fathers, Sisters, Brothers and Friends with sincere love and loyalty.

Acknowledgement

We thank Allah who gave us the strength and patience to conduct and finish this work.

We are grateful to Dr. Hind El-Rayah Osman for her support, supervision of this work and motherly compassion.

Our deep thanks and gratitude to doctors and the technical staff in Labchek Veterinary Diagnostic Laboratories, in which the study was conducted, for their permission to work and for their help during the whole period of study.

We thank the authorities of the private farm, from which the data and observations were recorded, for generously gave us the chance to study the field infection of Infectious Bursal Disease and for supporting in turn the veterinary education in Sudan and animal well fare in the regional area.

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Finally we are very grateful to our parents for their love and support all over the period of the study.

Abstract

Key words: infectious bursal disease - Gumboro disease – bursa of Fabricius.

The study is focusedthe gross lesions and pathological changes, the mortality rate caused by IBD virus and serological profile during infection caused by infectious bursal disease IBD virus. The main pathological changes are oedema, in Bursa and haemorrhages in different organs.

The Mortality of IBD infected layer flocks were evaluated and recorded, the authors found that the total mortality in the infected flock was 22494 which contributes about 28% of the flock.

The bursa of Fabricius showed decreased size at the early stage of disease and increased gradually. The mean of the size of bursa of Fabricius throughout the infection was 0.9 cm.

Serological profiling during IBD infection, Elisa test showed change at the end of the infection only. Agar Gel Immunodiffusion (AGID)test was found positive for the pooled bursa of Fabricius samples. Immunochromatography gave positive result for the all examined samples.

IBD virus causes severe economic losses of the poultry in horn of Africa east Mediterranean country.

The previous studies showed same age of susceptibility of birds to the infection and lesions of the post-mortem examination of different organs as the authors found. The mortality reaches a peak after five days which is slightly longer duration than that some other studies

المستخلص

الدراسة تركز على العلامة الظاهرة والتغيرات المرضية، معدل الوفيات بسبب فيروسمرض الجراب المعدي والاختبارات الفصلية أثناء الاصابة بمرض الجراب المعدي.

التغيرات المرضية الرئيسية هي تجمع السوائل في الجراب ونزيف بمختلف الأعضاء .

معدل وفيات الإصابة بالمرض مقيمة ومسجلة في قطيع الدجاج البياض، المؤلفون وجدوا أن معدل الوفيات الكلي في القطيع المصاب يقدر ب 22494 ويمثل حوالي 28% من القطيع.

غدة فبريشيس تظهر نقصان في الحجم أثناء المراحل الأولى من المرض وتزيد تدريجية. الحجم الأساسي للغدة خلال العدوى 0.9 سم .

الإختبارات المصلية خلال المرض هي إختبار اليزا ويضهر تغييره في المرحلة النهائية من المرض فقط و Agar Gel Immunodiffusion وجد نتيجة موجبة العينات غدة فابريشيس .Immunochtoma tography أعطى نتيجة موجبة لكلعينات الاختبار .

فيروس الجراب المعدي يسبب خسائر إقتصادية حادة من الدواجن في القرن الإفريقي شرق بلاد البحر الأبيض المتوسط.

أظهرت الدراسات السابقة نفس العمر لحساسية الطيور الى العدوى آفات الفحص بعد الوفاة من الأعضاء المختلفة كما وجد المؤرخون .

يصل معدل الوفيات الى القمة بعد خمسة أيام وهي فترة أطول قليلا من بعض الدراسات .

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Introduction

Infectious bursal disease (IBD) is caused by avirus that is classified as a member of the genus Avibirnavirus of the family Birnaviridae (Leong, *et al.*, 2000), is an acute highly contagious viral infection of young chickens that has lymphoid tissue as its primary target with special predilection for the bursa of Fabricius (cloacal bursa) it was first recognized as specific disease entity by Cosgrove, (1962) in 1962 and was referred to as (avian nephrosis) because of the esxtreme kidney damage found in bird (Saif, *et al.*, 2008) It was first diagnosed in Belgium in 1974 (Meulemans, *et al.*, 1974). Since first outbreak occurred in area of Gumboro, Delaware, "Gumboro disease" was synonym for this disease (Saif, Y. M. *et al.*, 2008).The disease is an acute, highly contagious viral disease of young chickens (Hafez, *et al.*, 2003). It may be manifested as subclinical form in chicks of age 0-3 weeks with imunosuppression or also as clinical form depending on the age of the bird (Sellaoui, *et al.*, 2012). Chicken is the only host known to develop clinical disease and distinct lesions following exposure to IBDV (Raj, *et al.*, 2009). It is most often found in highly concentrated poultry producing areas throughout the world.

Viral diseases are major causes of severe economic losses in poultry worldwide. During the 63rd General Session of the Office International des Epizooties, it was estimated that IBD has considerable socio-economic importance at the international level(OIE,1995),The disease is present in more than 80% of the surveyed countries (Thiery, P. *et al.*, 2000)to 95% of Member Countries of OIE (Eterradossi, 1995).

Infectious bursal disease (IBD) has been a great concern for the poultry industry (Thiery, *et al.*, 2000).In many countries, including Sudan, the immergence of new variant and vvIBD strain, failure of vaccination, wide variation in control of a disease caused by resistant virus and the socioeconomic effect of this disease may constitute the major significance factors of the disease.

The aim of this study is to provide more information about field IBDinfection.

Objectives

General Objective

A Field Study of Infectious Bursal Disease in Khartoum, Sudan

Specific objectives

1. To describe the gross lesions caused by the disease.

2. To determine the mortality caused by IBD.

3. To determine the serological profile during infection with IBD.

Chapter I

Literature Review

1.1. Introduction

Infectious Bursal disease, also known as Gumboro, is a highly contagious acute viral disease of young chickens of 3-6 weeks old that causes fatality or immunosuppression by damaging bursa of Fabricius of affected chickens (Islam, 2005**).** The bursa of Fabricius (BF) is a lymphoid organ, of lympho-epithelial structure. It is a site of B lymphocyte repertoire differentiation and maturation, located in the dorsal terminal part of the cloacae of the birds (Toivanen, *et al.*, 1987; Alloui and Sellaoui, 2012). The BF is the essential (primary) target of Gumboro disease virus (IBDV) (Sellaoui, *et al.*, 2012).

IBD virus that is classified as a member of the genus Avibirnavirus of the family Birnaviridae (Leong, *et al.*, 2000;He, *et al.*, 2012), has a bi-segmented dsRNA genome (Moller,*et al.*, 1979; Kibenge,*et al.*, 1988; Jackwood, *et al.*, 1984). IBD viruses belong to one of two distinct serotypes, designated 1 and 2 (McFerran, *et al.*, 1980). Only serotype 1 viruses are virulent for chickens, replicating in and eventually destroying maturing B lymphocytes in the bursa of Fabricius (Cheville, 1967), inducing immunosuppression (Faragher, *et al.*, 1974). Serotype I strains can be subdivided into classical and variant strains, with some of the classical strains inducing low mortality rates (Faragher, 1972).Serotype 2 viruses are avirulent for chickens. The different strains of the IBD

virus (IBDv), are antigenically similar to the classic virulent strains (Eterradossi, *et al*., 1997; 1999; Zierenberg, *et al*., 2000, 2001).

Until the late 1980s, classical strains were successfully controlled by vaccination. In 1987, a pathogenic variant, termed very virulent (vv) IBDV, emerged in The Netherlands, characterized by acute disease and high levels of mortality throughout Europe, the Middle East, Asia, Africa and South America (Chettle, *etal*., 1989; van den Berg, *et al*., 1991; Nunoya, *et al*., 1992; Cao, *et al*.,1998; Chen, *etal*., 1998; Pitcovski, *et al*., 1998; DiFabio, *et al*., 1999; Eterradossi, *et al*., 1999; Kwon, *et al*., 2000; Zierenberg, *et al*., share a common ancestor or are of independent origin (Lin, *et al*., 1993; Eterradossi, *etal*., 1999: Zierenberg, *et al*., 2000). Most geographically diverse vvIBDV isolates have identical amino acid sequences within the hyper variable region (HVR) of the viral protein 2 (VP2) and share three unique amino acid residues at positions 222(Ala), 256(Ile) and 294(Ile) that differentiate them from classical IBDV strains. Only two vvIBDV strains (88180 and Dl 1-2) have been identified that differ at these positions; 88180 has two unique substitutions at 222(Ala +Glu) and 294(Ile + Leu) (Eterradossi, *et al*., 1999).

Due to the high mutation rate in the VP2 variable domain (vVP2) sequence, comparison of this region among strains offers the best evolutionary clue for IBDVs. These studies, together with epidemiological observations and mortality studies, clearly suggest that vvIBDV strains belong to the same genetic lineage (Brown, *et al.*, 1994, Van den Berg, *et al.*, 1991; Yamaguchi, *et al.*, 1997; Eterradossi, *et al.*,

1997). The first published sequence, strain UK661, is now considered as the reference strain for European vvIBDVs (Brown and Skinner, 1996). The Asiatic very virulent strains were probably derived from Europe and then spread throughout Asia in an extremely explosive and conserved manner (Lin, *et al.*, 1993; Yamaguchi, *et al.*, 1997; Cao, *et al.*, 1998; Chen, *et al.*, 1998; To, *et al.*, 1999). Moreover, some recent phylogenetic analyses performed on the vVP2 sequences of vvIBDV strains isolated in Africa in the late 1980s (Eterradossi, *et al.*, 1999; Zierenberg, *et al.*, 2000) demonstrated that they belong to the common very virulent lineage. There are, however, significant distances between these strains and the European and Asiatic ones, indicating independent evolution. Taken together, all these data might indicate the possible emergence of all vvIBDV from an unique event and, hence, a common ancestor. However, comparison of total viral genome sequences should be performed for a more detailed analysis of the spatio-temporal relationships among strains. Changes in vVP2 have to be considered as a common evolution, not as a virulence marker, and the occurrence of new and diverging lineages of vvIBDVs should not be excluded in the future. Origin and phylogeny,the question of the origin of vvIBDV is still open. Phylogenetic analyses performed on segment A of vvIBDVs (Brown and Skinner, 1996; Yamaguchi, *et al.*, 1997; Pitcovski, *et al.*, 1998) confirm that they constitute a specific cluster and that they are more closely related to classical virulent strains, e.g. 52/70, than to other lineages. On the other hand, the topology tree performed on segment B is quite different, indicating that a genetic re-assortment from an unidentified reservoir (wild birds, fish or insects) might have played an important

role in the emergence of hyper virulent strains (Howie and Thorsen, 1981; Lasher and Shane, 1994; Yamaguchi, *et al.*, 1997). Moreover, although no data on viral shedding have been reported, serological surveys in wild birds (Wilcox, *et al.*, 1983; Gardner, *et al.*, 1997; Ogawa, *et al.*, 1998) suggest their possible role as a reservoir. Finally, the possible existence of asymptomatic carriers or latently infected birds should also be considered. (Thierry, P. 2000)Two serotypes of IBDV exist, namely: serotype 1 which is pathogenic for poultry, and serotype 2, which is a pathogenic and has been isolated from chickens and turkeys. The two serotypes are differentiated in vitro by the absence of cross-neutralisation, and in vivo, by the absence ofcross-protection (Becht,*et al.*, 1988;Ismail, *et al.*, 1988;Jackwood,*et al.*, 1982;Jackwood, *et al.*, 1984;McFerran, *et al.*, 1980).In addition to serological classification, the viral strains maybe classified according to virulence (mortality and bursallesions). Thus, strains of IBDV may be considered a pathogenic, attenuated (vaccines), classical virulent, variant, or hypervirulent (vvIBDV). Serotype 2 strains cause neither mortality nor bursal lesions in SPF chickens and are thus a pathogenic for chicks. Within serotype 1, a great deal of confusion can be found in the descriptions of virus virulence. In particular, the term 'hypervirulent' has been used to describe hypervirulent strains from Europe as well as the variant strains from the USA, although the latter are responsible for less than 5% specific mortality.

1.2. Isolation

A filtered homogenate of the bursa of Fabricius is inoculated in nine- to eleven-day-old embryonated eggs originating from hens free of anti-IBDV antibodies. The most sensitive route of inoculation is the CAM; the yolk sac route is also practicable, and the intra-allantoic route is the least sensitive. The specificity of the lesions observed must be demonstrated by neutralising the effect of the virus with a monospecific anti-IBDV serum. Isolation in embryonated eggs does not require adaptation of the virus by serial passages, and is suitable for vvIBDVs. In the absence of lesions, the embryos from the first passage should be homogenised in sterile conditions and clarified, and two additional serial passages should be performed (Hitchner, 1970;Lukert and Saif, 1997; Rosenberger, 1989) Detection of viral antigens Thin sections of the bursa of FabriciusThe viral antigens specific to IBDV may be detected by direct and indirect immunofluorescence (Allan,*et al.*, 1984; Meulemans, *et al.*, 1977) or by immuno peroxidase staining (Cho, *et al.*, 1987) in the bursal follicles of infected chickens between the fourth and sixth day after inoculation. No viral antigen is detectable from the tenth day. However, the virus can be isolated from bursae sampled from the second to the tenth day, with a maximum infectious titre after four days (Vindevogel, *et al.*, 1976; Winterfield, *et al.*, 1972). The use of monoclonal antibodies for detection of the virus enhances the specificity of the test (Cho, *et al.*, 1987).

Suspensions of the bursa of Fabricius,The AGID technique is based on a comparison between the suspension to be tested and a specific antiserum or a monoclonal antibody. The appearance of precipitation lines signals

the presence of viral antigens (Hirai, *et al*., 1974; Snyder, *et al.*, 1992; Takase, *et al*., 1993).

Detection of the viral genome *Deoxyribonucleic acid probes* Deoxyribonucleic acid (DNA) probes labeled with 3 2 P (Davis and Boyle, 1990; Jackwood, 1990; Kibenge, F. 1992), biotin (Jackwood,. *et al*., 1990) or digoxigen in (Hatchcock and Giambrone, 1992) have been used on prints of infected tissues to detect the multiple virus strains of serotypes 1 and 2. No genomic probe enabling differentiation between variant viruses or vvIBDVs has yet been described, undoubtedly owing to the very high degree of genetic resemblance between serotype 1 strains of the virus. *Reverse transcription and genetic amplification by polymerase chain reaction* Reverse transcription-polymerase chain reaction (RT-PCR) allows the detection of viral RNA in homogenates of infected organs or embryos, as well as in cell cultures, irrespective of the viability of the virus present. The choice of amplified genomic zones depends on the objective. When the only objective is to detect multiple strains of the virus, primers are selected in the highly preserved zones (Stram, *et al*., 1994; Tham, *et al*., 1995; Wu, *et al*., 1992; Wu, *et al*., 1997).When the characterisation of the amplified fragment is to allow for identification of the virus strains, the central, so-called variable portion of VP2 is generally chosen (Lin, *et al*., 1993; Liu, *etal*., 1994).The amplified fragment may then be characterized by direct sequencing (Lin,*et al*., 1993), and the analysis of the coded amino peptide sequence. The simultaneous presence of four amino acids (alanine 222, isoleucine 256, isoleucine 294 and serine 299) is

considered as indicative of vvIBDV (Brown,*et al.*, 1994; Cao, *et al.*, 1998; Eterradossi, *et al.*, 1999; Yamaguchi, *et al.*, 1997). The electrophoretic profile of the amplified fragment may also be studied after digestion with different restriction endonucleases (RT-PCR/RE) (Jackwoodand Nielsen, 1997; Liu, *et al.*, 1994). The value of the results obtained will depend on the choice of endonucleases. In agiven virus, the absence of restriction sites for enzymes BstNI and Style, located respectively at codons 222 and 253 of the gene coding for VP2, has been correlated with an a typical antigenicity, such as that found in the variant viruses from the USA (Jackwood and Jackwood, 1994;Jackwood and Nielsen, 1997).

1.3. Host Susceptibility

Host rangeOnly chickens *(Gallus gallus)* develop IBD after infection by serotype 1 viruses (Van den Berg *et al.*, 2000 *)* Anti-IBDV antibodies have been detected in guinea-fowl*(Numida meleagris)* (Adewuyi,*et al.*, 1989.), common pheasants *(Phasianuscolchicus)* (Louzis,*et al.*, 1979) and ostriches *(Struthio camelus)* (Cadman, *et al.*, 1994), which have also been demonstrated to carry serotype 2 viruses (Guittet, *et al.*, 1982).Neutralising or precipitating antibodies have been detected, *inter alia*, in various species of wild duck, goose, tern, puffin, crow and penguin, which may mean that wild birds act as reservoirs or vectors (Allan, *et al.*, 1984; Bayliss, *et al.*, 1991; Ogawa, *et al.*, 1998; Wilcox, *et al.*, 1983).

1.4. Susceptibility factors

The age of maximum susceptibility is between three and six weeks, corresponding to the period of maximum bursa development, during which the acute clinical signs are observed. Infections occurring prior to the age of three weeks are generally subclinical and immunosuppressive. Clinical cases may be observed up to the age of fifteen to twenty weeks (Ley, *et al*., 1979; Okoye, *et al*., 1981). Light strains of laying stock are more susceptible to disease than the heavy broiler strains (Bumstead, *et al.,* 1993; Hassan, *et al.,* 1996).

Natural infections with IBDV have been reported mainly in fowls (faragher, 1972) and more rarely in turkeys (McNulty, *et al*., 1979; Perelman and Heller, 1981; Barnes, *et al*., 1982; Sivanandan, *et al*., 1984; Chettle, *et al*., 1985). However village weavers (*ploceus*

cucullatus) have been found to be positive for IBDV antibody (Nawathe, *et al*., 1978). Coturnix quails *(coturnix coturnix*) and two breeds of turkey were successfully infected with IBDV. Although no clinical signs were observed and neither microscopic lesion found in their bursae nor the virus isolated from cloaqcal swabs , the turkeys developed precipitin and virus neutralizeing antibodies (Weisman and Hitchner, 1978). similar results were obtained when susceptible ducks were challenged (Yamada, *et al.*,1982). IBD has been reported in nine-day to 20 weeks old chickens (Cosgrove, 1962; hanson, 1967; Luthgen, 1969; Onunkwo, 1975; Okoye and Uzoukwu, 1981; Durojaire, *et al*.,

1984) . It is however most common in birds three to seven weeks old (Cosgrove, 1962; Hanson, 1967; Ojo, *et al*., 1973). Clinical sings were not observed in laying birds and chicks less than two weeks old but histopathological lesions were seen in the BF of the chicks (Hitchner, 1970; Hitchner, 1971). The age resistance to clinical IBD is believed to be independent of the ability of the virus to replace and induce lesions (Fadly and Nezerian, 1983). All breed of fowl may be infected but light breeds show a more severe reaction to virus than heavy breeds (Hitchner, 1978). However, no significant difference in mortality rate between the breeds was found (Meroz, 1966).

1.5. Transmission and Epidemiology

Only horizontal transmission has been described, with healthy subjects being infected by the oral or respiratory pathway. Infected subjects excrete the virus in feces as early as 48 h after infection, and may transmit the disease by contact over a sixteen-day period (Vindevogel, *et al*., 1976). The possibility of persistent infection in recovered animals has not been researched. The disease is transmitted by direct contact with excreting subjects, or by indirect contact with any in animate or animate (farm staff, animals) contaminated vectors. Some researchers have suggested that insects may also act as vectors (Howie and Thorsen, 1981). The extreme resistance of the virus to the outside environment enhances the potential for indirect transmission. The virus can survive for four months in contaminated bedding and premises (Benton, *et al*., 1967) and up to fifty-six days in lesser meal worms *(Alphitobius* sp.)

taken from a contaminated building (McFerran, *et al*., 1980). In the absence of effective cleaning, disinfection and insect control, the resistance of the virus leads to perennial contamination of infected farm buildings(Van den Berg, *et al*.,2000). The most likely route of infection is oral ingestion of contaminated feaces or other contaminated organic material (Raj, *et al.*, 2009).

Benton, *et al*., (1967) found IBDV highly contagious. They found houses that had housed infected birds infective for other birds 122 days after removal of the infected birds and that water, feed and droppings taken from infected pens remained infectious for 52 days. A ground suspension of lesser meal worm- Alphitobius disperinus-taken from a poultry house eight weeks after an outbreak of IBD was successfully used to infect susceptible chickens (Snedeker, *et al*., 1967). Mites may play an important role in the transmission of IBDV (Brady, 1970). A strain of IBDV isolated from mosquitoes have been identified (Howie and Theorsen, 1981) experimental infections can be achieved by oral, intramuscular, intrabursal and subconjunctival routes. (Abdu, *et al.*, 1986)

1.6. Incubation period

Incubation period is very short: two to three days (Van den Berg, *et al.*, 2000).

1.7. Pathogenesis

A kinetic study using immunofluorescence (Müller, *et al.,* 1979) has shown that, 4 hour after oral inoculation, the virus is found in the lymphoid tissues associated with the digestive tract, where the first cycle of viral replication occurs. The virus subsequently enters the general circulation via the hepatic portal vein- Aphase of primary viraemia ensues, during which the virus reaches the bursa, 11 h after infection, and a major secondary replication cycle occurs. A phase of secondary viraemia then occurs, and the other lymphoid organs become massively infected (Van den Berg*, et al*.,2000**).**

Mortality commences on the third day of infection, reaches a peak by day four, then drops rapidly, and the surviving chickens recover a state of apparent health after five to seven days. (Van den Berg, *et al*., 2000).

1.8. Clinical signs andlesion

In the infected flocks, the clinical signs are not specific, but include lethargy, ruffled feathers, watery diarrhoea due to increased water intake and lower feed consumption, and sudden death. Typical lesions include haemorrhagic and enlarged or atrophie bursae, degenerative livers, haemorrhages in the thigh muscles or brownish kidneys with swollen tubules (Van den Berg, *et al.,* 2000**).** In acute cases, the animals are exhausted, prostrated, dehydrated, suffer from watery diarrhoea, and feathers are ruffled. Mortality commences on the third day of infection, reaches a peak by day four, then drops rapidly, and the surviving

chickens recover a state of apparent health after five to seven days. Disease severity depends on the age and breed sensitivity of the infected birds, the virulence of the strain, and the degree of passive immunity. Initial infection on a given farm is generally very acute, with very high mortality rates if a very virulent strain is involved. If the virus persists on the farm and is transmitted to successive flocks, the clinical forms of the disease appear earlier and are gradually replaced by subclinical forms. Nonetheless, acute episodes may still occur. Moreover, a primary infection may also be in apparent when the viral strain is of low pathogenicity or if maternal antibodies are present. The clinical signs of IBD vary considerably from one farm, region, country or even continent to another. Schematically, the global situation can be divided into three principal clinical forms, as follows: a classical form, as described since the early 1960s, is caused by the classical virulent strains of IBDV. Specific mortality is relatively low, and the disease is most often subclinical, occurring after a decline in the level of passive antibodies (Faragher, 1972).the immunosuppressive form, principally described in the USA, is caused by low-pathogenicity strains of IBDV, as wellas by variant strains, such as the Delaware variant E or GLS strains, which partially resist neutralisation by antibodies against the so-called 'classical' viruses (Jackwoodand Saif, 1987; Snyder, 1990).the acute form, first described in Europe, and then in Asia, is caused by 'hypervirulent' strains of IBDV, and is characterized by an acute progressive clinical disease, leading to high mortality rates on affected farms (Chettle, *et al*., 1989;Stuart, 1989;Van den Berg, *et al.,* 1991).

Although the other lymphoid organs are affected (Sharma, *et al* 1993;Tanimura, *et al.,* 1995; Tanimura, *et al* 1997), the principal target of the virus is the bursa of Fabricius (Kauferand Weiss, 1980), which is the reservoir of B lymphocytes in birds. Indeed, the target cell is the B lymphocyte in active division, for which the infection is cytolytic (Burkhardt and Müller, 1987). Cell sorting studies have demonstrated that the B lymphocyte is susceptible in the immature stage, during which immunoglobulin M is carried on the surface of the lymphocyte (Hirai, *et al.,* 1981; Nakaiand Hirai, 1981). This accounts for the paradoxical immune response to IBDVin which immunosuppression co-exists with high anti-IBDV antibody titers. The mature and competent lymphocytes will expand as a result of stimulation by the virus whereas the immaturely mphocytes will be destroyed. Macroscopic lesions are observed principally in the bursa which presents all stages of inflammation following acute infection (McFerran, 1993; Vindevogel, *et al.,* 1974). Autopsies performed on birds that died during the acute phase (three to four days following infection) reveal hypertrophic, hyperaemic and oedematous bursas. The most severe cases are characterised by a major infection of the mucous membrane and a serous transudate, giving the bursal surface a yellowish color. This appearance is often accompanied by petechiae and haemorrhages. By the fifth day, the bursa reverts to normal size and by the eighth day becomes atrophied to less than a third of the normal size. The affected animals are severely dehydrated, and many birds have hypertrophic and whitish kidneys containing deposits of urate crystals and cell debris. Haemorrhages in the pectoral muscles and thighs are frequently observed, probably due to

a coagulation disorder (Skeeles,*et al.,* 1980). Certain variants from the USA are reported to cause rapid atrophy of the bursa without a previous inflammatory phase (Lukert and Saif, 1997). Moreover, in the acute form of the disease caused by hyper virulent strains, macroscopic lesions may also be observed in other lymphoid organs (thymus, spleen, caecal tonsils, Harderian glands, Peyer's patches and bone marrow) (Hiraga,*et al.*, 1994; Inoue,*et al.*,1994, Inoue,*et al.,* 1999; Tsukamoto, *et al.,* 1995; Henry,*et al.,* 1980) have developed a system for evaluating microscopic lesions of the affected organs, with a score ranging from one to five according to severity (Henry,*et al.,* 1980). The B lymphocytes are destroyed in the follicles of the bursa as well as in the germinal centers and the perivascular cuff of the spleen. The bursa is infiltrated by heterophils and undergoes hyperplasia of the reticulo-endothelial cells and of the interfollicular tissue. As the disease evolves, the surface epithelium disappears and cystic cavities develop in the follicles. Severe panleukopenia is also observed. These microscopic lesions are exacerbated in the acute forms of the disease. (Van den Berg, *et al*., 2000)

The chicks become anorectic, reluctant to move, and show ruffled feathers with watery diarrhea, trembling and severe prostration. The lesions characteristics of the disease include dehydration of the muscles with ecchymotic hemorrhages, enlargement, and orange discoloration of kidneys (Brugere-Picoux and Vaillancourt, 2015). The BF becomes enlarged and shows pale yellow discoloration. Intra-follicular hemorrhages may be found and pin point hemorrhages on the skeletal

muscles are usually prominent (Madej, *et al*., 2012).The IBD virus (IBDV) spread in the bursa, by deteriorating its lymphoid structure and causing lesions of different importance, depending on the strains and the immunizing state of the affected birds

1.9. Control and prevention

In general, IBDV is resistant to many disinfectants and environmental factors, and remains infectious for at least four months in the poultry house environment. Because of the resistant nature of IBDV, once a poultry house becomes contaminated, the disease tends to recur in subsequent flocks (Lukert and Saif, 1997). Hygienic measures alone are ineffective and vaccination is essential. Several vaccines are available. When they are given correctly, good immunity and protection can be achieved (Van den Berg and Meulemans, 1991). Beside the proper application the major problem with the live vaccination of young chickens with maternally derived antibodies (MDA) is determining the proper time of vaccination, through monitoring of the antibody level in a breeder flock or its progeny (Hafez, *et al*., 2003).

Moreover, there is a wide variation in disease control procedures that seldom conform to a specific or standard plan. These features justified the elaboration of a specific resolution, Resolution XVIII in 1995 (Thierry, 2000)

1.9.1 Vaccines

The development of safe vaccines that could either transmit a high passive immunity which could protect broilers during the whole growing period or prime an immune response before or at hatching in the presence of passive immunity might be established in the near future. In this context, recombinant vaccines and virus-neutralizing factor technology might have an advantage over other approaches**.** (Thierry, 2000)

Newcastle disease (ND) "LaSota" and infectious bursal disease (IBD) vaccines batch numbers 4/2001 and 7/2001 respectively, used in the vaccination of the experimental birds were obtained from National Veterinary Research Institute (NVRI) Vom, Nigeria. The following five live commercial vaccines were given by intra-conjunctiva instillation at 14 days of age: T2-Lukert1 (intermediate strain), T3-Lukert2 (intermediate plus strain), T4-228E, T5-V877 and T6- Winterfield 2512 ("hot" strains). Groups T1 and T7 were not vaccinated against IBD. (Nishizawa, M. *et al.,* 2007) A commercially available recombinant HVT vaccine expressing virus protein (VP) 2 of IBDV was used at the recommended dose of the manufacturer. The following commercially available vaccines were used all at one dose/bird in accordance to the guidelines of the manufacturers: a cell-associated Marek's disease vaccine based on the CVI988/Rispens; an infectious bronchitis (IB) live vaccine based on the strain CR88121; an IB live vaccine based on H120; a ND live vaccine based on the VG/GA strain; an intermediate as well as

an intermediate plus IBD vaccine based on the strains D78 and 228E, respectively; and a ND, IB, Egg-drop syndrome (EDS) inactivated vaccine based on the Newcastle disease virus (NDV) Ulster 2C, Mass41 and V127 strains, respectively, as a water-in-oil emulsion (Francesco, *et al.,* 2016).

Immunization of chickens is the principle method used for control of IBD in chickens. The vaccine must be safe, pure and efficient (Mardassi, *et al.,* 2004). There are many choices of available live vaccine based on virulence such as classical vaccine (D78) that gave protection against mortality ranging between 30-40% during the first 48 house post vaccination but the acute problem for disease control is still due to interference of maternally antibodies in the establishment of the vaccination schedule (Hsieh, *et al*., 2010). Maternal antibodies interfered with the development of satisfactory protection in commercial broiler chicks and vaccination at 2 weeks of age resulted in better immune response in vaccinated group with intermediate 228E strain and gave 90% protection (Azhar, 2000). In spite of vaccinations against IBD, some flocks suffered from immunosuppression due to IBD**.** As well as some flocks up to 3 weeks (unsusceptible age of classical IBD) were immunosuppressed with atrophied bursa indicating the possibility of infection with the variant form of IBDv(Susan *et al.*,2013**)**

1.9.1.1 Living Infectious bursal disease (IBD) vaccines

Seven IBD commercial imported live attenuated vaccines were used: Three Intermediate: IZO IBD2 .Batch No. (0335G); Nobilis Gumboro 228E.Batch No (A065A1J01) &INDOVAX-Georgia strain Batch No (BG 2911). Two Intermediate plus: IBD Xtreme. Batch No (B045611); & Gumboro L. Batch No (3106Z341A) .One Invasive intermediate INDOVAX- Bursa B2K Batch No (GP 3311) and Classical Intervet D78 Batch No (12601LJ01) (Susan, *et al* 2013**).**

Classical serotype 1 vaccines still induce good protection, but the actual problem for control of the disease has became the interference of maternally derived antibody in the establishment of the vaccination schedule.(van den Berg, *et al.*, 2000).

1.10. Immunosuppression

Although the other lymphoid organs are affected (Sharma, J. M. *et al.*, 1993) (Tanimura, *et al., 1995*,Tanimura, *et al* 1997), the principal target of the virus is the bursa of Fabricius (Kauferand Weiss, 1980), which is the reservoir of B lymphocytes in birds. Indeed, the target cell is the B lymphocyte in active division, for which the infection is cytolytic (Burkhardt, and Müller, 1987). Cell sorting studies have demonstrated that the B lymphocyte is susceptible in the immature stage, during which immunoglobulin M is carried on the surface of the lymphocyte (Hirai, *et al.*, 1981, Nakaiand Hirai, 1981). This accounts for the paradoxical immune response to IBDV, -in which immunosuppression co-exists with

high anti-IBDV antibody titers. The mature and competent lymphocytes will expand as a result of stimulation by the virus whereas the immature lymphocytes will be destroyed. (Van den Berg, *et al*.,2000). The destruction of immature B lymphocytes in the bursa creates an immunosuppression, which will be more severe in younger birds (Faragher, *et al.*, 1974). In addition to the impact on production and role in the development of secondary infections, this will affect the immune response of the chicken to subsequent vaccinations which are essential in all types of intensive animal production (Giambrone, *et al.*, 1976). The most severe and longest-lasting immunosuppression occurs when day-old chicks are infected by IBDV (Allan, *et al.*, 1972, Allan, *et al*., 1984, Azad, *et al.*, 1987, Adewuyi, *et al.*, 1989, Allan, *et al.*, 1984, Allan, *et al.*, 1972, Sharma, *et al.*, 1994). In field conditions, this rarely occurs since chickens tend to become infected at approximately two to three weeks, when maternal antibodies decline. Evidence suggests that the virus has an immunosuppressive effect at least up to the age of six weeks (Gardner, *et al.*, 1997, Lucio, and Hitchner, 1980, Wyeth, 1975). Immunosuppression is most often demonstrated using experimental models based on the measurement of humoral responses induced by different antigens such as *Brucellas abortus* (Hopkins, *et al.*, 1979), sheep red blood cells, or Newcastle disease vaccines (Allan, *et al*., 1972, Faragher,*et al.*, 1974, Giambrone, *et al.*, 1976). The best assessment is clearly the measurement of vaccinal protection against a challenge infection by the Newcastle disease virus, as described in the OIE *Manual of Standards for Diagnostic Tests and Vaccines* (OIE, 2000), since this constitutes a measurement of both humoral and cellular

immunity. Unfortunately, these techniques are time-consuming, tedious, costly, and require the use of animals. Thus, they are usually confined to IBD vaccine registration procedures. (van den Berg, *et al.,* 2000). Besides their immunosuppressive effects of IBD has been well documented (Lukert, 1992; Trautwein, 1992) (El-Yuguda, 2007).

In 1987, we diagnosed the first cases of IBD caused by highly pathogenic strains. The course of the disease is around 6 days (van den, Berg, *et al*., 2000). The disease reached Europe in the years 1962 to 1971 (Faragher, 1972). From 1966 to 1974, the disease was identified in the Middle East, southern and western Africa, India, the Far East and Australia (Faragher, 1972; Firth, 1974; Jones, 1986; Lasher, *et al.*, 1994; Provost, *et al.*, 1972). (Van den Berg, *et al*., 2000; van der Sluis, 1999).Infectious bursal disease is currently an international problem: 95% of the 65 countries that responded to a survey conducted by the Office International des Epizooties (OIE) in 1995 declared cases of infection (Eterradossi, 1995),including New Zealand which had been free of disease until 1 993 (Jones, 1986).

1.11. Morbidity and mortality

Infectious bursal disease is extremely contagious. In infected flocks, morbidity is high, with up to 100% serological conversion, after infection, whilst mortality is variable. Until 1987, the field strains isolated were of low virulence and caused only 1% to 2% of specific mortality. However, since 1987 an increase in specific mortality has

been described indifferent parts of the world. In the USA, new strains responsible for up to 5% of specific mortality were described (Rosenberger and Cloud, 1986). At the same time, in Europe and subsequently in Japan, high mortality rates of 5 0% to 60% in laying hens and 25% to 30% in broilers were observed. These hypervirulent fields trains caused up to 100% mortality in specific-pathogen-free (SPF) chickens (Nunoya, *et al*., 1992; Van den Berg, T. P. *et al*., 1991). Mortality rates varying from 5 to 15% with occasional extremes of 0 to 25% have been reported. In laying pullets mortality rates of up to 60% are observed, suggesting a difference in susceptibility between layer and broiler chickens. (Van den Berg,*et al*., 2000).

1.12. Clinical signs and diagnosis

1.12.1. Clinical and differential diagnosis

The clinical diagnosis of the acute forms of IBD is based on disease evolution (a mortality peak followed by recovery in five to seven days), and relies on the observation of the symptoms and post-mortem examination of the pathognomonic lesions, in particular of the bursa of Fabricius. The conditions most liable to be clinically mistaken for IBD are avian coccidiosis, Newcastle disease in some visceral forms, stunting syndrome, chicken infectious anaemia, mycotoxicoses and Nephropathogenic forms of infectious .bronchitis. In all acute cases, the presence of bursal lesions allows for a diagnosis of IBD. In subclinical cases, an atrophy of the bursa may be confused with other diseases such

as Marek's disease or infectious anaemia A histological examination of the bursa will allow differentiation between these diseases (Lukertand Saif, 1997).

1.12.2. Histological diagnosis

Histopathology of the bursa from both flocks showed different degrees of necrosis of lymphocytes in the medullarly area of bursal follicles, haemorrhagic areas and interfollicular heterophilic infiltration indicating an inflammatory reaction. Additionally, the 30-day-old flock showed follicular and interfollicular oedema.

The ability to cause histological lesions in the non-bursal lymphoid organs, such as the thymus (Inoue,*et al.*, 1994), the spleen or bone marrow (Inoue, *et al.*, 1999) has been reported as a potential characteristic of hypervirulent IBDV strains. The histological approach has the advantage of allowing for diagnosis of both the acute and chronic or subclinical forms of the disease.

Complementary examinations using direct immunofluorescence (Meulemans *et al.*, 1977) are sometimes necessary to differentiate IBD from nephritis caused by infectious bronchitis virus (Van den Berg, *et al.*, 2000**)**

Particularly, the absence of known markers to easily characterize very pathogenic viral strains is a serious hindrance, preventing early detection and application of specific prophylactic measures as soon as they appear

The European picture has been dominated for a decade by the emergence of very virulent (vv) IBDV strains of infectious bursal disease. These strains have now spread all over the world. Therefore, this review will focus on the acute form of the disease, referring to outbreaks due to vvIBDV, as proposed by Stuart (1989) in his letter. (Thierry, 2000)

1.12.3. Elisa and Serological diagnosis

The ELISA technique was as used by Owoade (1999).In areas contaminated by IBDV, most broiler flocks have anti-IBDV antibodies when leaving the farm. Current serological tests cannot distinguish between the antibodies induced by pathogenic IBDV and those induced by attenuated vaccine viruses, so serological diagnosis is of little interest in endemic zones. Nonetheless, the quantification of IBDV-induced antibodies is important for the medical prophylaxis of the disease in young animals, in order to measure the titre of passive antibodies and determine the appropriate date for vaccination (DeWit, 1999; Kouwenhoven and van den Bos, 1994; Muskett, *et al.,* 1979) or in laying hens to verify success of vaccination (Lucio, 1987; Meulemans, *et al.*, 1987). Serology is likewise essential to confirm the disease-free status of SPF flocks. Each serological analysis must include a sufficient number (at least twenty) of individual serum samples representative of the flock under study. A kinetic study requires at least two serological analyses separated by an interval of three weeks (paired sera).The most widely used quantitative tests are the detection of precipitating antibodies by

agar gel immunodiffusion (AGID) (Cullen and Wyeth, 1975; Hirai, *et al.*, 1972), enzyme-linked immunosorbent assay (ELISA) (Marquardt, *et al.*, 1980;Meulemans, *et al.*, 1987), and SN in cell culture (Weisman and Hitchner, 1978). Agar gel immunodiffusion is the simplest, but least sensitive technique. Results are obtained after an incubation period of 48 h. Variability in results may be due to the investigator, as well as the nature of the viral strain used as an antigen (Nicholas, *et al.*, 1985; Van den Berg, *et al.*, 1991; Weisman and Hitchner, B. 1978; Wood, G. W. *et al.*, 1979; Wood, G. W. *et al.*, 1984).Serum neutralisation presents the disadvantages that specialised equipment and five days incubation are required. The technique is much more sensitive than AGID and correlates better with the level of protection of the subjects tested (Jackwood and Saif, 1987; Roney and Freund, 1988; Weisman and Hitchner, 1978). The ELISA is the most rapid and sensitive method, and presents the fewest variations due to the viral strain used as an antigen (Roney and Freund, 1988). Considerable inter- and intra-laboratory variability can occur with certain commercial kits (Kreider, *et al.*, 1991). Although the correlation between results obtained using SN and ELISA is high, ELISA remains less sensitive, and does not detect low neutralizing titers which are sufficient to block vaccine administration (residual maternal antibodies). Enzyme-linked immunosorbent assays which use are recombinant VP2 protein as the sole antigen may be better correlated with protection (Jackwood, *et al.*, 1999; Van den Berg, *et al.*, 1997).

1.12.4.Virological Diagnosis

Infectious bursal disease virus may be detected in the bursa of Fabricius of chicks in the acute phase of infection, ideally within the first three days following the appearance of clinical signs (van den Berg, *et al.*, 2000).

1.13. Economic significant

This disease is one of the most economically important diseases that impair growth of young chickens which results in significant economic losses in the poultry industry (Hussain, *et al.*, 2004). A previous study comparing the mortality of infected and non infected flocks had shown that IBD related mortality rates was found to beraging from 2.9%-4.5%, a slight reduction in daily weight gain as well as in production number were also observed. (Hafez,*et al.*, 2003).

According to van den Breg, *et al*. (2000) it is difficult to assess the economic impact of IBD due to the multi-factorial nature of the losses involved. In addition to direct losses related to specific mortality (which in turn depends on the dose and virulence of the strain, the age and breed of the animals and the presence or absence of passive immunity), indirect losses also occur, due to acquired immunodeficiency or potential interactions between IBDV and other viruses, bacteria or parasites. Further losses may occur as a result of growth retardation or the rejection of carcasses showing signs of haemorrhages.

Significant economic losseswere attributed to the “reemergence” in variant or highly virulent forms. Vaccination failure in 1986-1987 were described in different parts of the world The disease by itself usually causes mortality of 5-10% but this rate can reach up to 30- 40% (OIE, 2004). In Malaysia, IBD has been a serious acute disease of the poultry industry since 1990, with high mortality being reported in several poultry farms (Hair- Bejo, 1993b). The effect of the IBD is largely dependent on the strain and the amount of the virus, age and the breed of chickens, the route of inoculation, the presence or absence of neutralizing antibodies, inter current primary and secondary pathogens and environmental and management factors (Muller, *et. al.*, 2003).

According to Raj,*et al.,* (2009) in Pakistan, IBD causes 20 percent mortality per annum by destroying immune system despite vaccination. Until 1987, the strains of virus were of low pathogenicity, causing less than 2% mortality and satisfactorily controlled by vaccination(Meulemans, *et al.,* 1980). But in 1986 and 1987, vaccination failures were described in different parts of the world. In the US, it was demonstrated that the new isolates had been affected by antigenic drift against which classical IBD virus (IBDV) vaccines were not satisfactorily protective (Jackwood and Saif, 1987; Snyder, *et al.*, 1992), where as in Europe, the first cases of acute IBDV were described (Chettle, *et al.*, 1989; van den Berg, *et al.*, 1991). Some of these first acute outbreaks occurredat farms where all the hygienic and prophylactic measures had been taken, at the end of the fattening periodof the broiler which was indicativefor a dramatic change in the

field situation and consequently strains of increased virulence were identified.

Fortunately, there isno evidence of transmission of IBDV to humans exists (Petersen, *et al.*, 1990); the disease thus has no direct impact on public health. In contrast, "variant strains" with a different antigenic profile were described in the USA .Vaccination failure was incriminated to be responsible for the emergence of antigenic Variation (Rosenberger and Cloud, 1986). While unfortunately, a questionnaire survey in Nigeria results revealed a 34%level of awareness and economic impact of the diseases amongst the respondents (Sadiq and Mohammed 2017).

Chapter II

Material and Methods

2.1. Area of study

Visits to a private poultry farm during infectious bursal disease outbreak were done. The farm is located in Omdurman beside Alfateh city between latitudes 15.6476º N, 32.4807º E.

2.2. Study design

A descriptive observational study of infectious bursal disease in vaccinated pullets in a closed system layer farm was performed. The pullets are of Lohman LSL breed.

2.3. Duration of study

Birds were observed for 15 hours per day during 12 consecutive days from the onset of mortality to the end of the outbreak,pathological changes and mortality were recorded.

2.4. Laboratory work

Laboratory work was done in Sudan University of Science and Technology and Labchek, a private veterinary diagnostic laboratory and the Central Veterinary Laboratory, Soba.

2.5. Problem of study

IBD is an endemic poultry disease in Sudan that causes losses in poultry farms.

2.6. Question of study

What are the economical significance and pathological picture of field IBD infection in pullets at age 40 days.

2.7.Post-mortem examination

Post-mortem examination wasdone for Forty three dead or sacrificed birds. The birds were examined externally and then a careful examination of the medial aspect of the skin and the internal organs was performed for presence of gross lesions and pathological changes. Gross lesion in affected organs were recorded and photographed.

2.8. Mortality

Dead birds were counted daily at the same time. Daily and total mortality of the infected flock were recorded. Two batteries were closely observed for mortality. Each battery was divided into four sections and every section contained 48 cages. The mortality in each section was recorded separately.

2.9. Tissue samples.

During post-mortem, ten samples of affected bursa that showed haemorrhage, exudation and/or edema were takenand groundedfor Immunochromatography and AGID.

2.10. Immunochromatography

Qualitative immunochromatographic assay for the detection of the infectious bursal disease virus (IBDV Ag) in avian bursa of Fabricius was done usingRapid IBDV Ag test kit (Lillidale Diagnostics, UK).

2.10.1. Test Procedure

The kit components and specimen were allowed to reach room temperature prior to testing. The test card was removed from the foil pouch and the pipette was filled with the supernatant form the assay tube,containing the sample inthe assay buffer, and three drops were taken to the sample well in the test card. Interpretation of test results was made within ten minute.

2.10.2. Interpretation of the result

The presence of two color bands T and C (control) within the result window- no matter which band appears first indicates a positive result. The presence of only one band at C line within the result window indicates a negative result. If the control band is not visible within the result window the result is considered invalid.

2.11.Agar Gel Immunodiffusion (AGID)

AGID is an immunological technique used in the detection, identification and qualification of antibodies and antigens. Plates were Prepared 24 hours to seven days before use. The agar was dissolved by placing in a steamer or boiling water bath after which it waspoured into each of the required number of nine cm diameter plastic petridishes and

laid on a level surface. The plates were covered, the agar wasallowed to set, and then stored at 4°C.

2.12. Collection of blood samples

Blood samples were collected from the wing vein using one ml syringes with 27 g×5/8″ needle. Collection of blood samples was performed daily at the same time.

2.12.1. Preparation of serum samples

0.5 to 1 ml of blood are left to clot at room temperature. After three to four hours, drops of serum are collected in epindorff tube and centrifuged. Clear serum was then taken to another cleanepindorff using micropipette 30-300 µl. The serum was preserved at 4C for 24 hours waiting for the mortality record to detect the peak of the outbreak or otherwise discarded. For the end of the outbreak sampling, serum samplewere preserved for 48 hours.

2.13. ELISA test

Indirect ELISA diagnostic kit, that is designed to detect antibodies directed against the infectious bursal disease (IBD), is used. It is a quantitative test for the detection of (IBD) specific antibodies in chicken sera. Kit components were preserved and used as indicated by the manufacturer (IDvet, France). Method three Elisa tests were performed during the outbreak, the first at the onset of mortality, the second is at the peak, the last one is after the drop of mortality to its lowest level. Micro wells that have been coated with purified IBDV antigen were

used. Samples to be tested and controls are added to the wellsAnti IBDV antibodies if present form an antigen-antibody complex after washing, an anti-chicken horse radish peroxidase (HRP) conjugate is added to the wells. It fixes to the antibodies forming an antigen-antibody-conjugate-HRP complex.The excess conjugate was eliminated by washing. The substrate solution (TMB) was added. The resulting coloration depends on the quantity of specific antibodies present in the specimen to be tested. In the presence of antibodies a blue solution should appear and become yellow after addition of the stop solution. In the absence of antibodies no coloration should appear. The microplate is read at 450 nm

2.13.1. Sample preparation

In order to avoid differences in incubation times between specimens, the test and control specimens were prepared in a 96-well plat, dilution plate; they were then transferred into an ELISA microplate using a multichannel pipette.

2.13.2. Wash solution and conjugate preparation

Dilutions of the wash solution and conjugate were made according to the manufacturer's instructions(IDvet, France).

2.13.3. Testing procedure

A final dilution of 1:500 in dilution buffer was made for the serum samples using the instructions of the manufacturer(IDvet, France). The test was performed with incubation for one hour and fifteen minutes as described by the manufacturer.

2.13.4. Reading of the test

The optic density was Read and recorded at 450nm usingElisa reader (BioTeck, USA). Analyses for antibody titer, grouping, minimum, maximum, mean and coefficient of variation was performed using IDvet software program.

Chapter III

Results

A study of IBD field infection was performed at a layer poultry farm in Omdurman. Serological profiling of the affected flock, mortality, postmortem of dead and sacrificed birds and immunochromatography and AGID for the virus antigen from the bursa homgenate weredone.

3.1.Post-mortem examination

Post-mortem examination wasdone for Forty three dead or sacrificed birds. The external examination of the birds revealed no pathological change except presence of diarrhea in the vent area. Examination of the skin and internal organs revealed the following: enlargement (Fig.1.), edema (Fig.2.,Fig.3.,Fig.4.), haemorrhage (Fig.3., Fig.4., Fig.5., Fig.6., Fig.7., Fig.8.) and exudation (Fig.8.) of the bursa of Fabrecious. Heamorrhages in the thigh muscles (Fig.9), drum stick (Fig.10.and Fig.11.), breast, proventriculus (Fig.12.,Fig.13.and Fig.14.) subcutaneous tissues(Fig.15.),liver, spleen (Fig.16.) and cecal tonsils. The kidneys were enlarged (Fig.17. andFig.18.) and showed pale color and apparent tubules(Fig.18.). Haemorrhage was also found in the medial aspect of the thorax(Fig.19.).

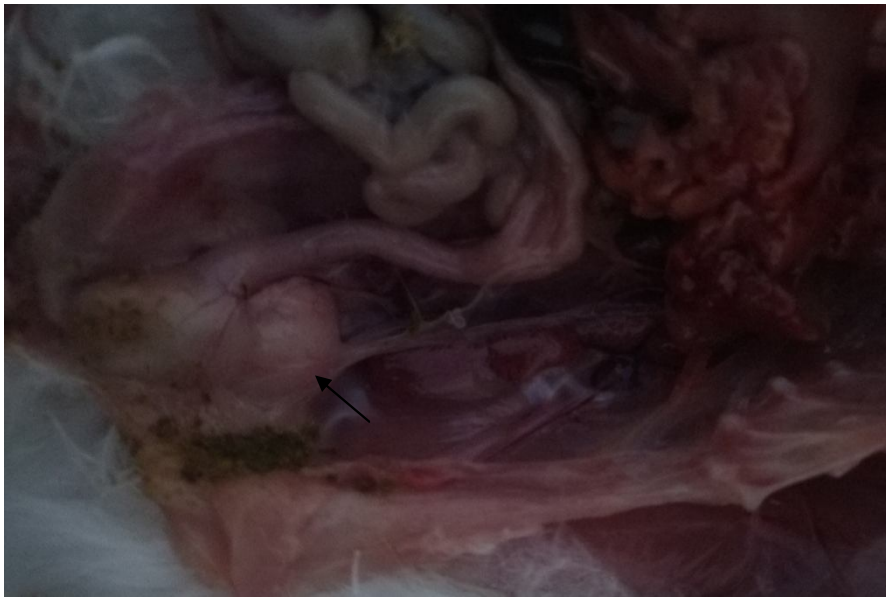

Fig.1. IBD infected pullets at age 6-8 weeks, bursa of Fabrecious: enlargement.

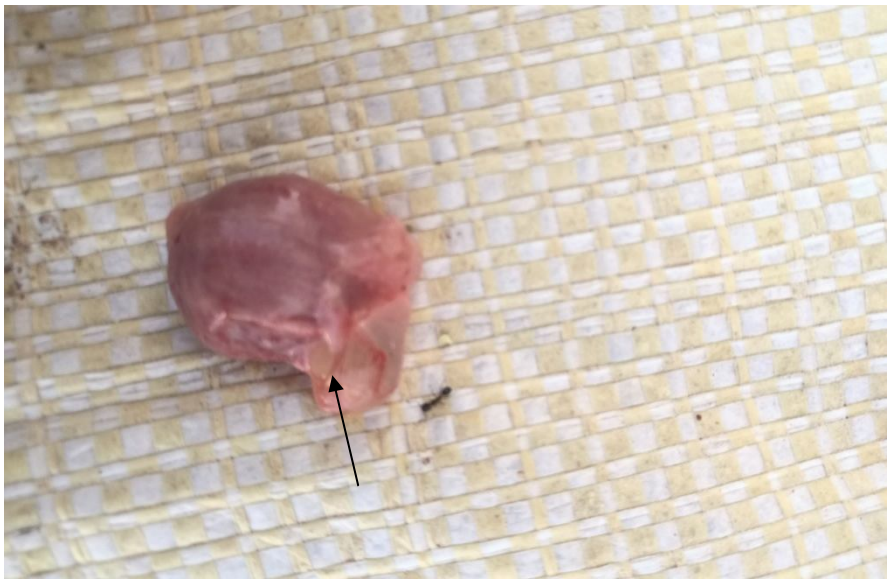

Fig.2. IBD infected pullets at age 6-8 weeks, bursa of Fabrecious: edema.

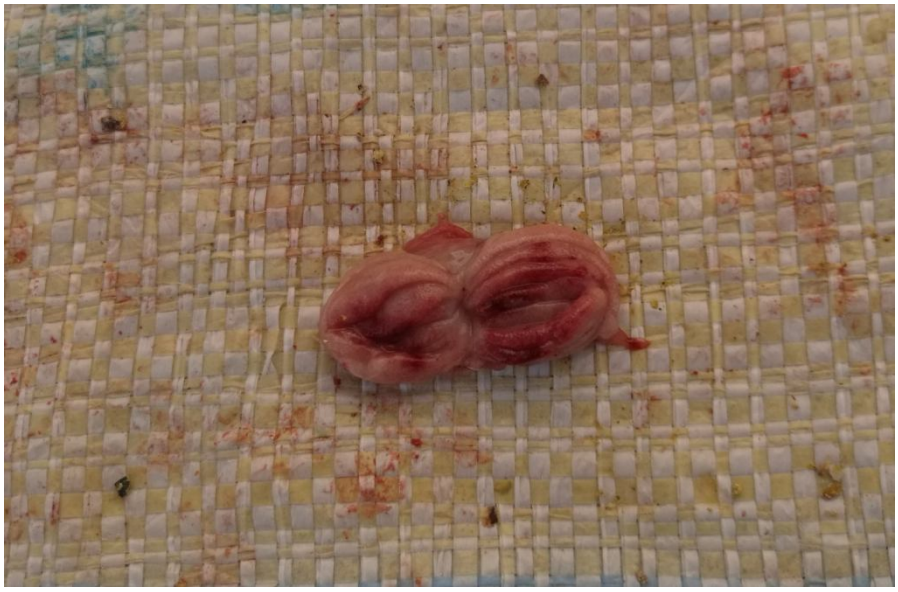

Fig.3. IBD infected pullets at age 6-8 weeks, bursa of Fabrecious: edema and haemorrages.

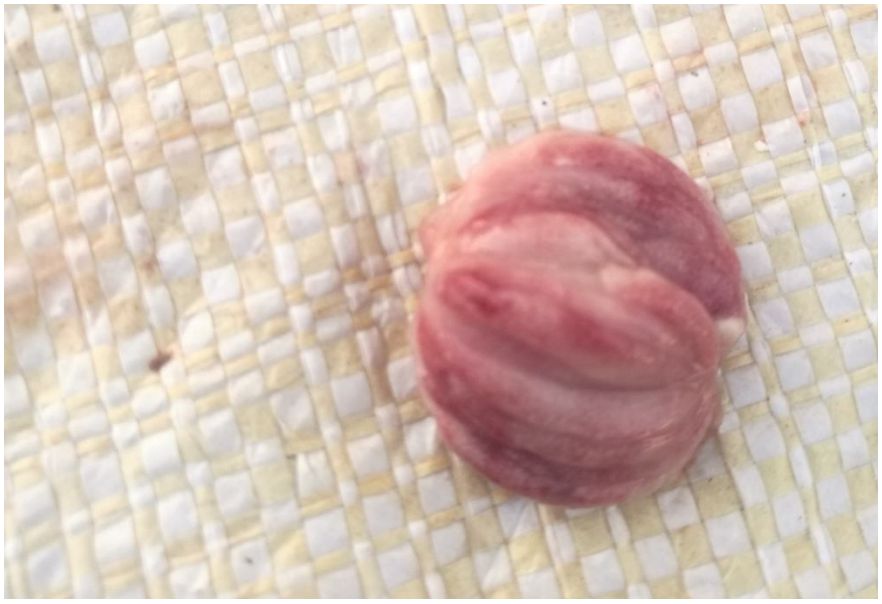

Fig.4. IBD infected pullets at age 6-8 weeks, bursa of Fabrecious: edema and slight to moderate haemorrages.

Fig.5. IBD infected pullets at age 6-8 weeks, bursa of Fabrecious: moderate haemorrhage.

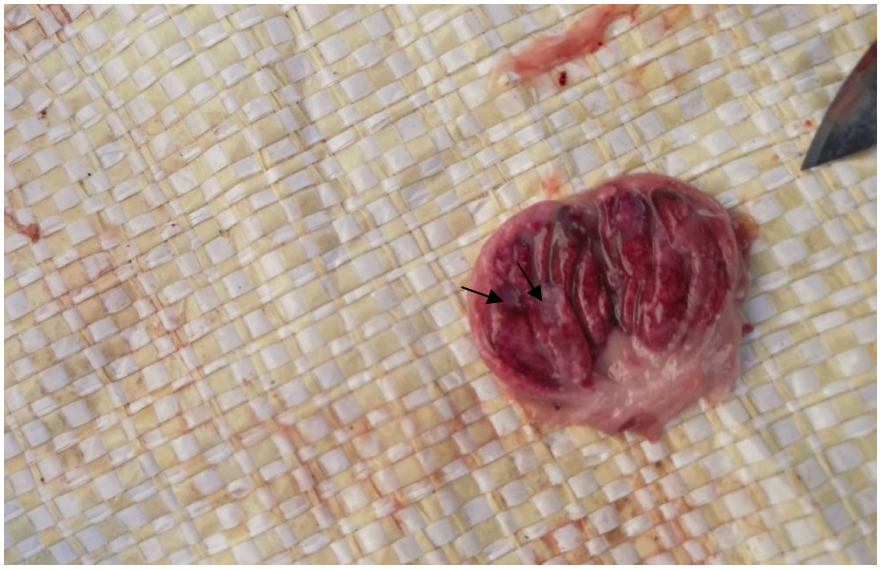

Fig.6. IBD infected pullets at age 6-8 weeks, bursa of Fabrecious: moderate haemorrhage, focal atrophy (arrows) and exudation

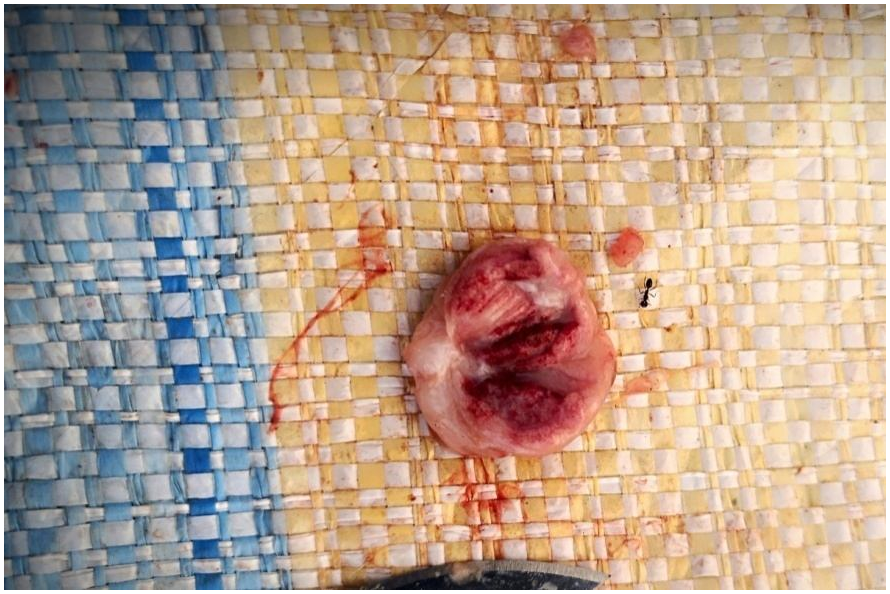

Fig.7. IBD infected pullets at age 6-8 weeks, bursa of Fabrecious: pinpoint haemorrage.

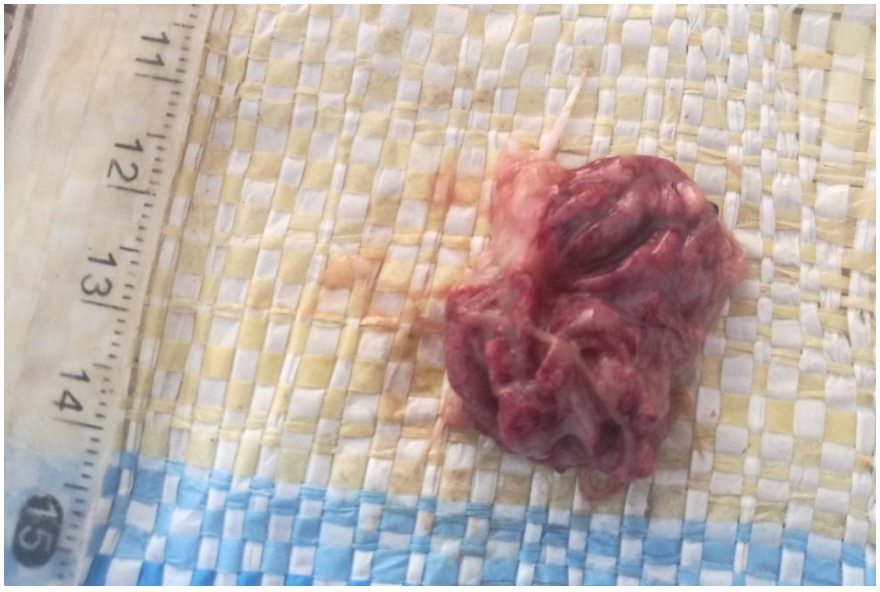

Fig.8. IBD infected pullets at age 6-8 weeks, bursa of Fabrecious: exudation and sever haemorrage.

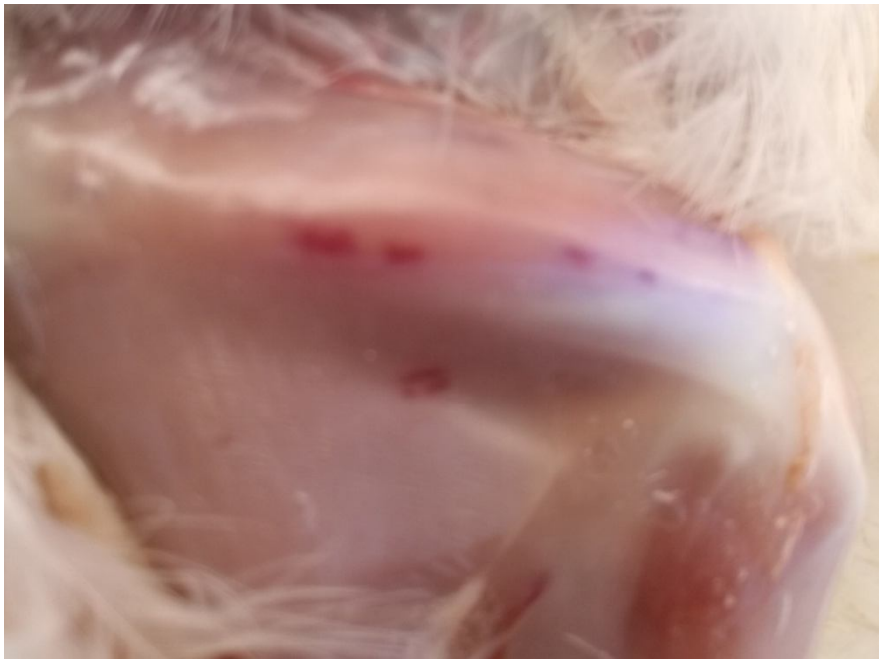

Fig.9.IBD infected pullets at age 6-8 weeks, thigh muscles: Haemorrhage.

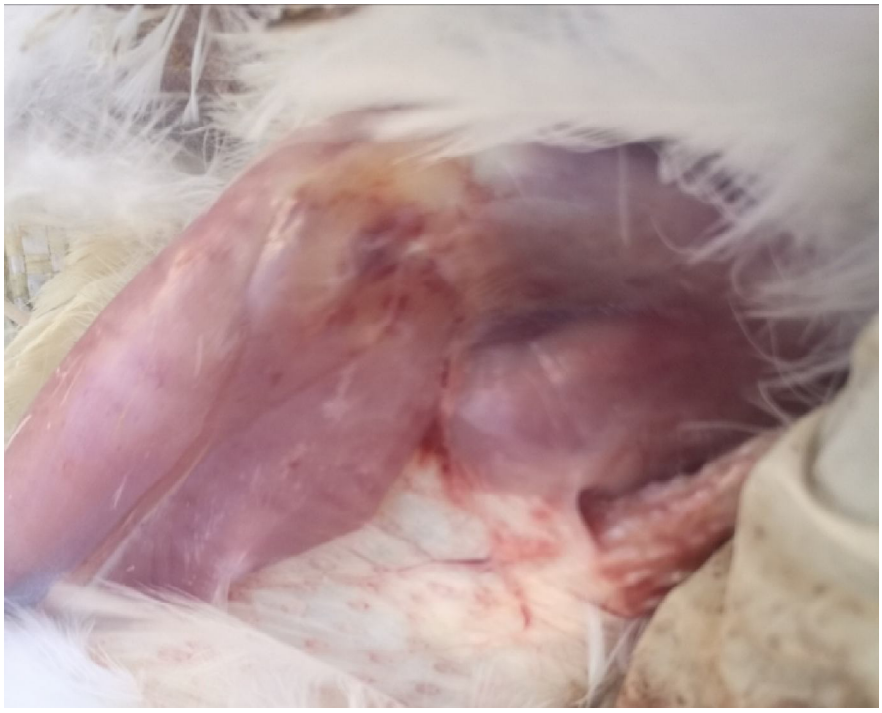

Fig.10.IBD infected pullets at age 6-8 weeks, drum stick muscles: haemorrhage.

Fig.11. IBD infected pullets at age 6-8 weeks, drum stick muscles:heamorrhage.

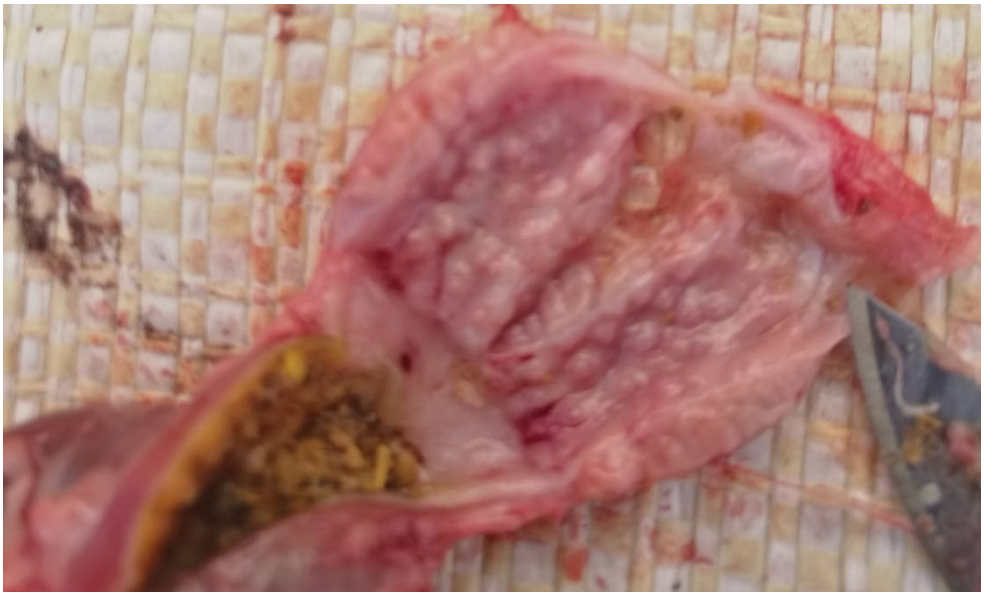

Fig.12.IBD infected pullets at age 6-8 weeks, proventriculus: slight haemorrhage.

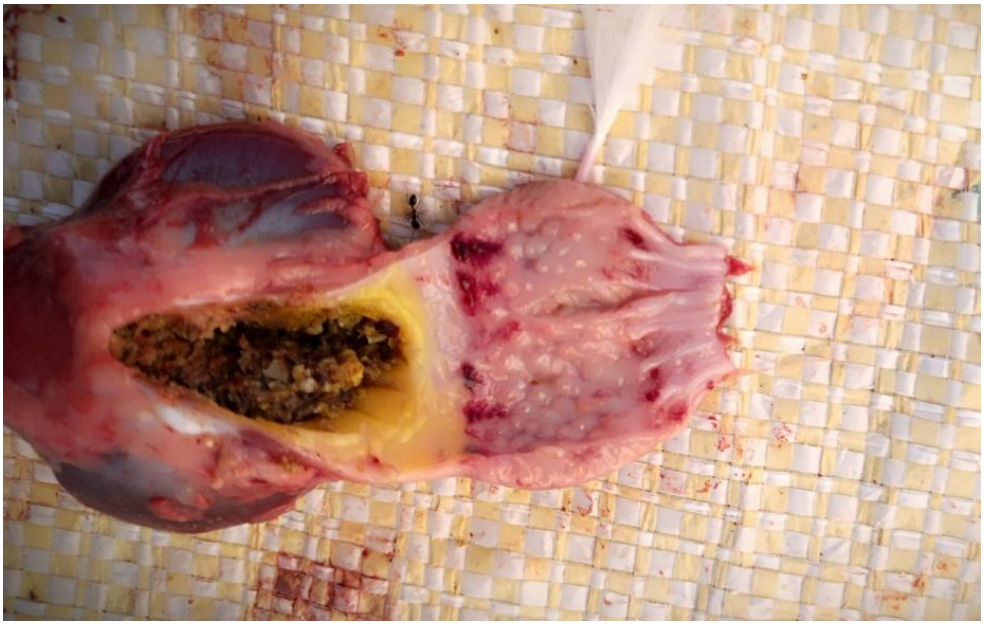

Fig.13.IBD infected pullets at age 6-8 weeks, proventriculus: moderate haemorrhage.

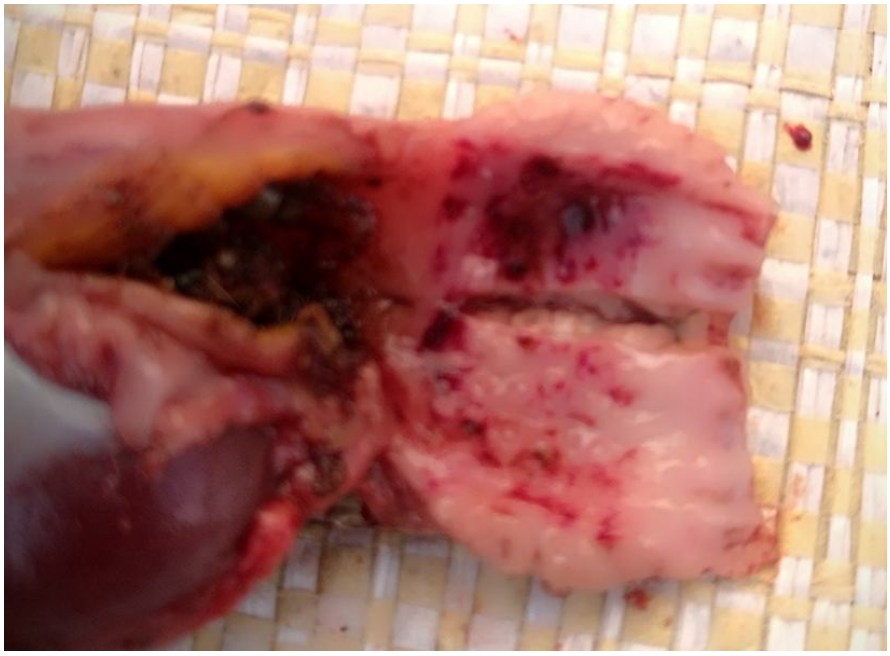

Fig.14.IBD infected pullets at age 6-8 weeks, proventriculus: sever haemorrhage.

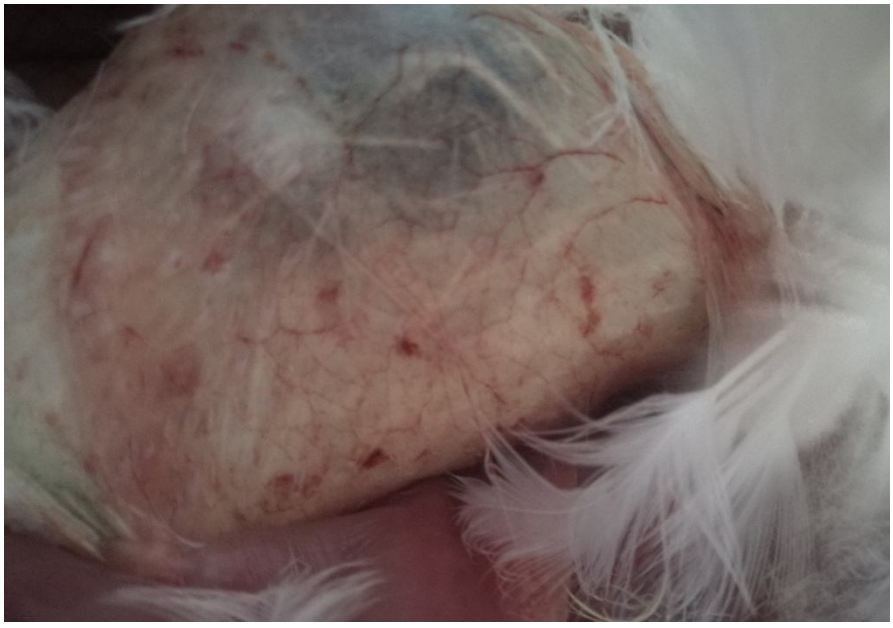

Fig.15. IBD infected pullets at age 6-8 weeks, subcutaneous: haemorrhage.

Fig.15. IBD infected pullets at age 6-8 weeks, spleen: haemorrhage.

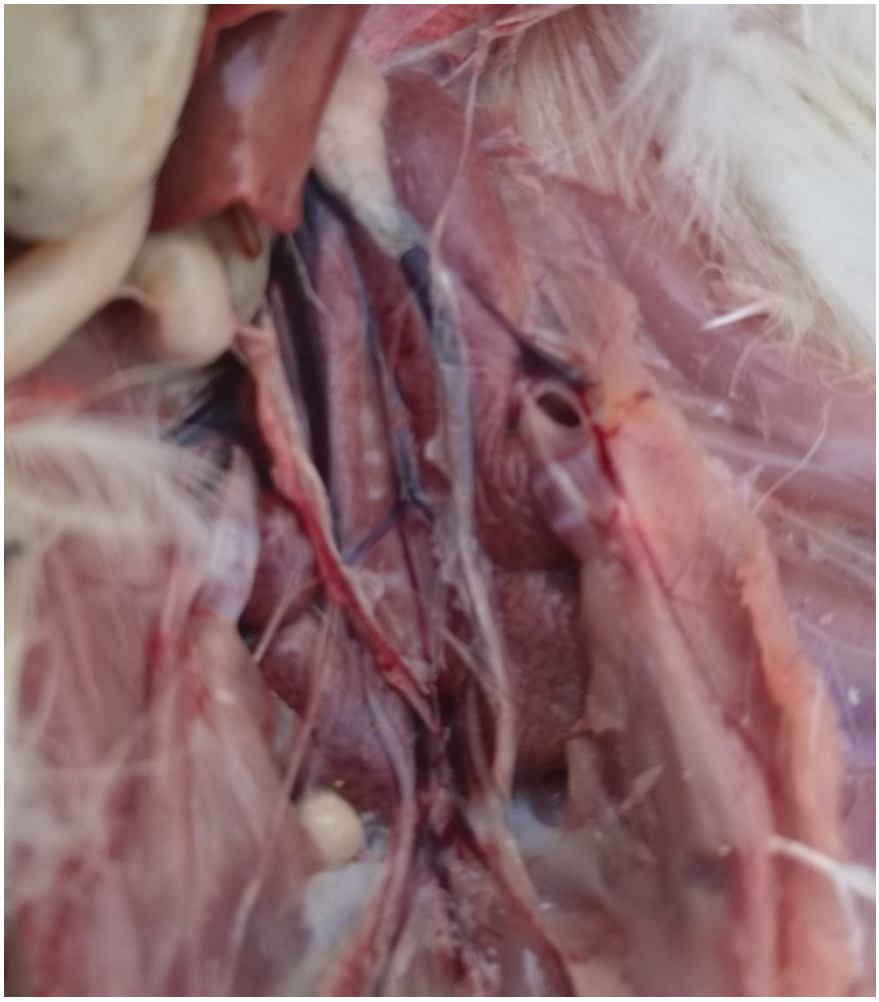

Fig.17.IBD infected pullets at age 6-8 weeks, kidney: enlargement and congested blood vessels.

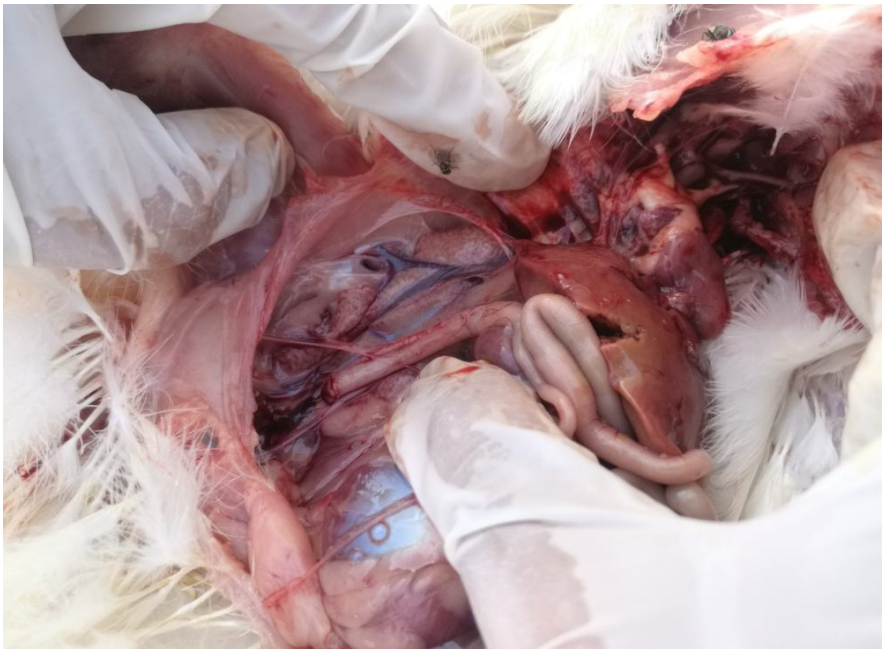

Fig.18.IBD infected pullets at age 6-8 weeks, kidney: enlargement, dilated tubules and pale color.

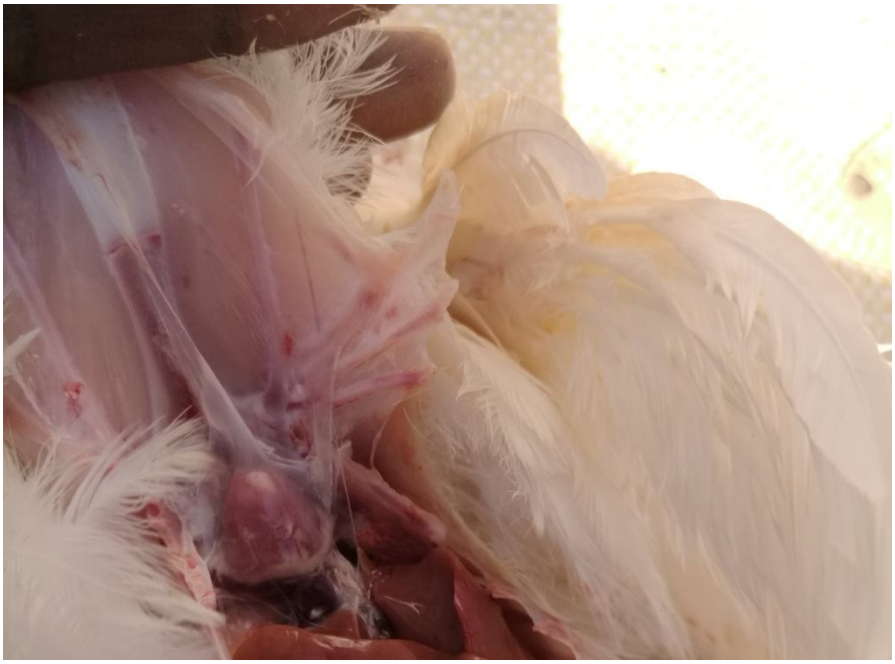

Fig.19. IBD infected pullets at age 6-8 weeks, medial aspect of breast: haemorrhage.

The number of the organs that showed haemorrhage out of the inspected ones is shown in Fig.20.; Thirty one out of forty three inspected bursa were enlarged. The size of the bursa was measured daily, Fig. 21. shows these results.

3.5. Mortality

Eighty thousand birds, housed in battery cages, were infected with IBD. The total mortality in that affected flock was 22494 that constituted about 28% of the flock.

The study of the mortality in the different positions concerning the two batteries revealed different levels of mortality (Fig.22.). Within the two batteries, the four sections revealed different level of mortality (Fig.23.).

3.2. Immunochromatography

Qualitative immunochromatographic assay for the detection of the infectious bursal disease virus (IBDV Ag) in avian bursa of Fabricius gave positive result for the all ten samples examined.

3.3. **Agar Gel Immunodiffusion (AGID)**

AGID test was found to be positive for the pooled bursa of Fabrecious samples.

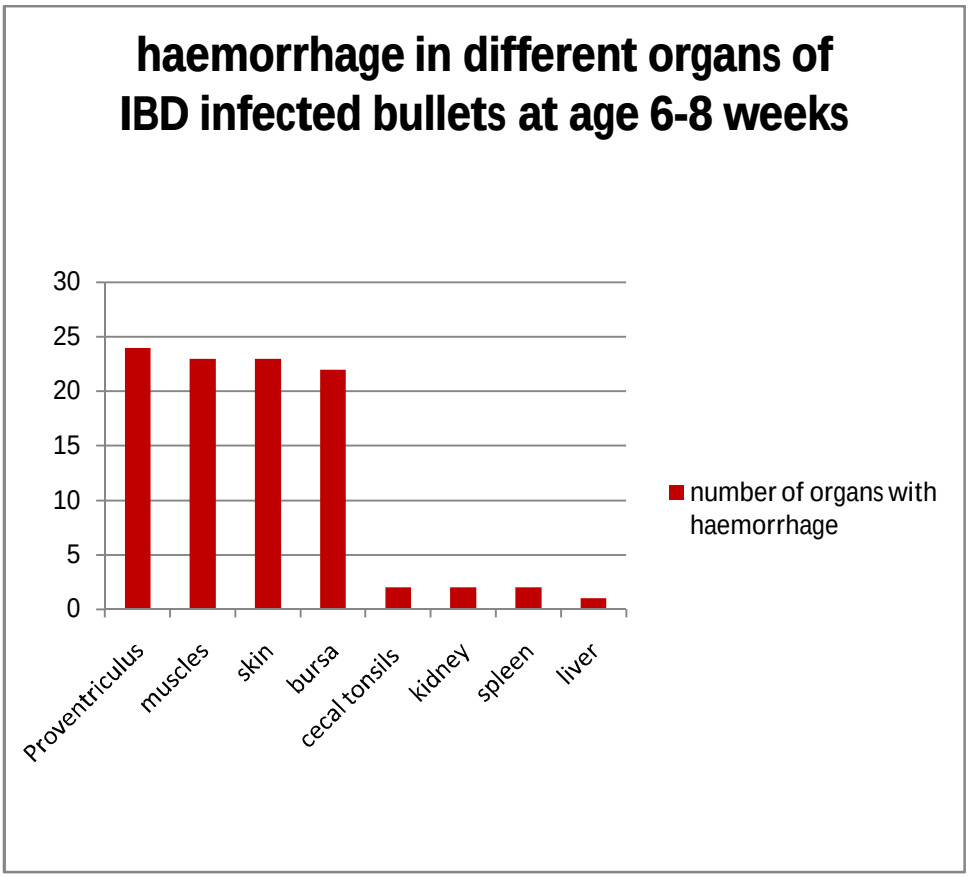

Fig.20.IBD infected pullets at age 6-8 weeks: haemorrhage in different organs.

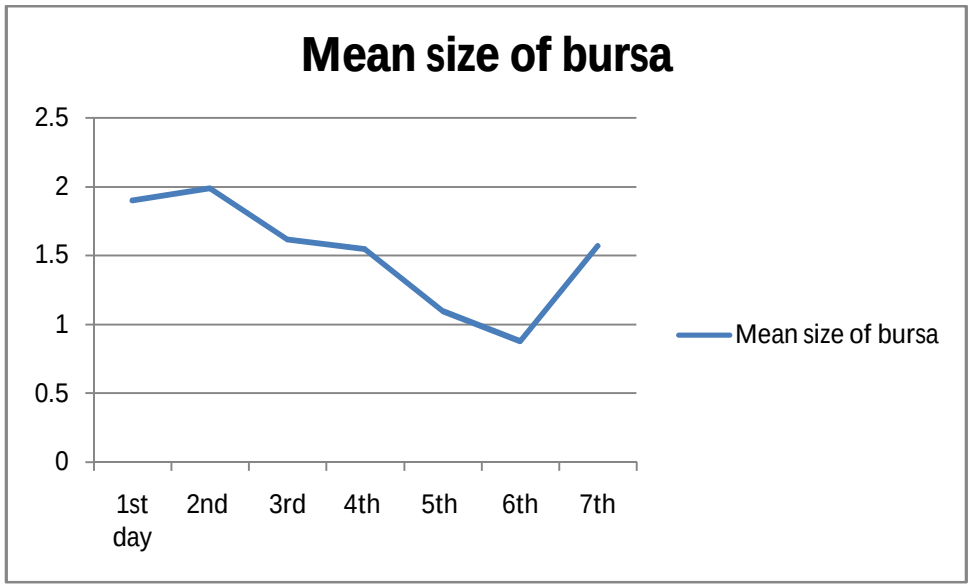

Fig.20. IBD infected pullets at age 6-8 weeks: mean size of the bursa.

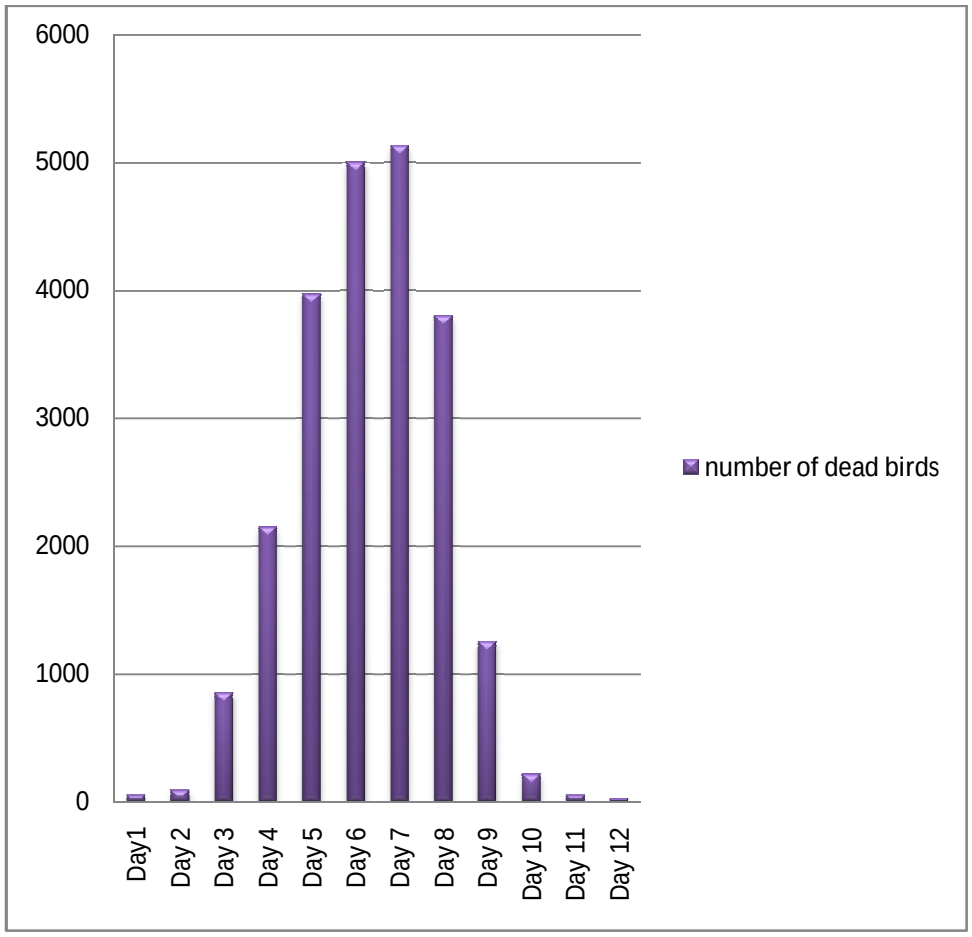

Fig.21. IBD infected pullets at age 6-8 weeks, daily mortality.

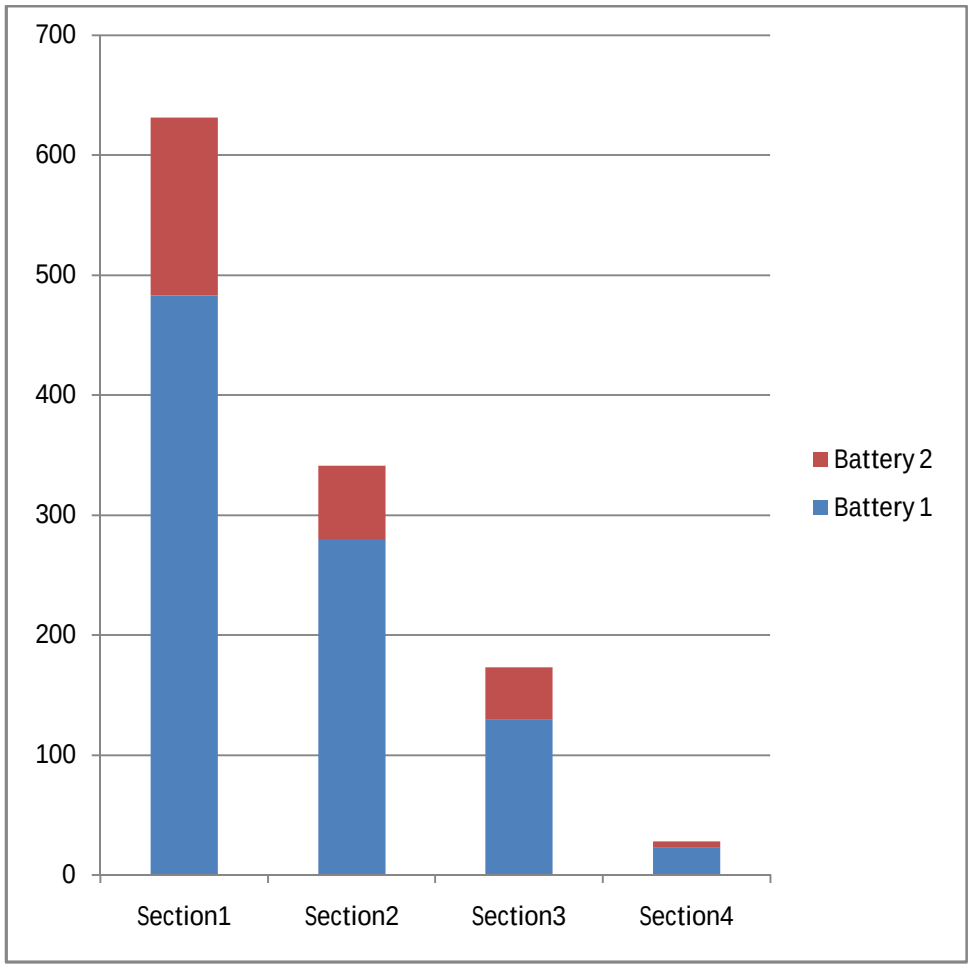

Fig.21. IBD infected pullets at age 6-8 weeks, total mortality at different positions in two different batteries.

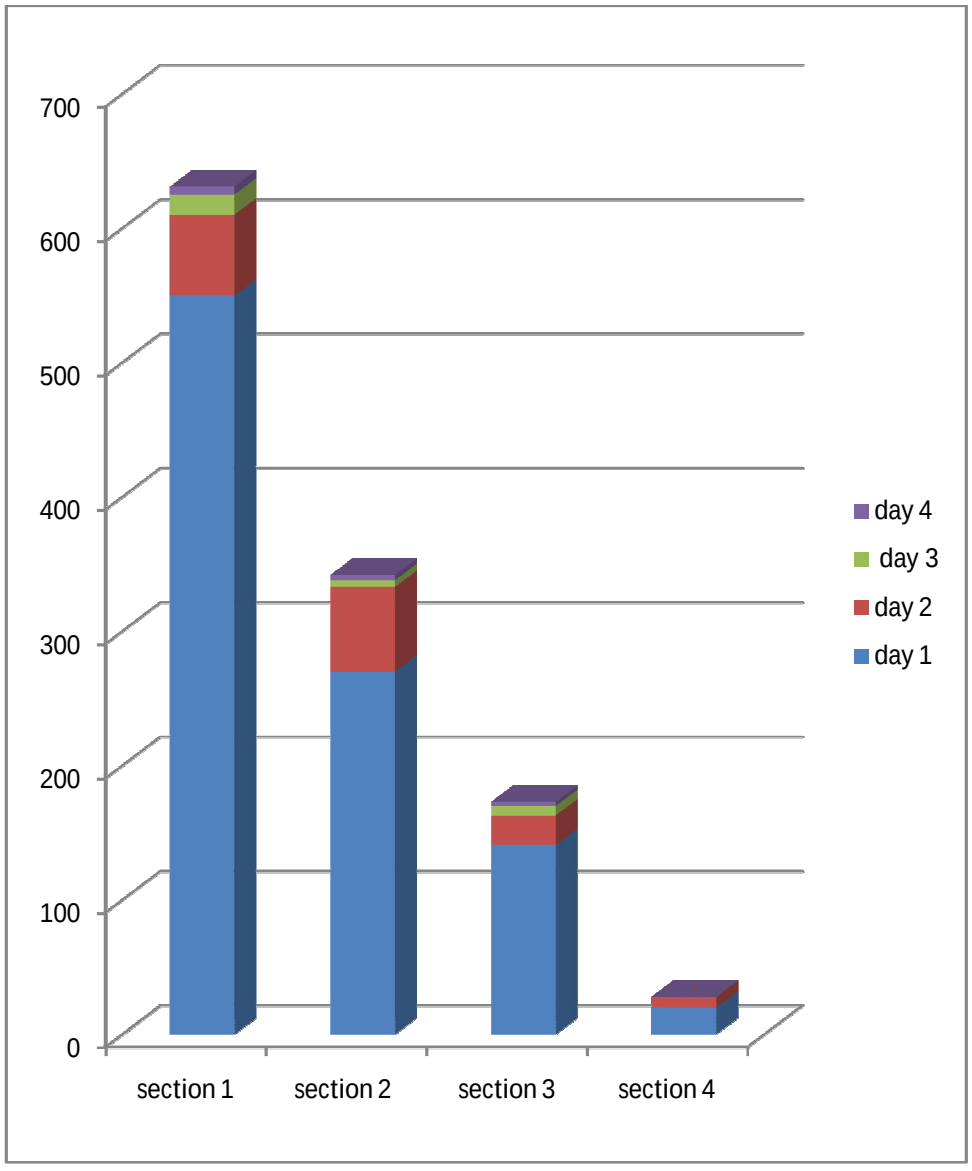

Fig.22. IBD infected pullets at age 6-8 weeks, daily mortality at differentsections of two batteries.

3.4. ELISA test.

The results of Elisa tests during IBD outbreakis shown in table 3.1.and Fig. 23.; Fig. 24. andFig. 25. By the end of the outbreak, there was an obvious change in the antibody titer.

Table.1. Pullets at age 6-7 weeks, Elisa readings for antibody titer to during IBD infection.

titer	Mean titer	Maximum titer	Minimum titer	% CV
Onset of mortality (day1)	37	192	0	168
Peak of mortality (day 5)	10	90	0	260
Least subsiding mortality (day 9)	3985	6275	10	42

Chapter IV

Discussion

Infectious bursal disease is a highly contagious, acute viral disease responsible for considerable economic losses in manycountries. A study of field infection of IBD in 40 days vaccinated pullets was conducted during an outbreak in a layer farm in Khartoum state.

The study of IBD field infection was performed at a layer poultry farm in Omdurman. Serological profiling of the affected flock, mortality, postmortem of dead and sacrificed birds and immunochromatography and AGID for the virus antigen from the bursa homgenate were done.

The birds were infected at about six weeks which is a normal age for susceptibility and assumed to cause clinical diseaseas it was recorded before by Ley,*et al*., (1979) and Okoye, *et al.*, (1981). Hitchner, (1978) recorded thatLayer light strains are more susceptible to IBD than broiler strains and this may explain the losses in the current study.

In the outbreak under investigation, the mortality reaches a peak afterfive days which is slightly longer duration than that recorded by Van den Berg, *et al*., (2000) in which the peak was reachedby day four, that authors described arapiddrop, and recovery of the surviving chickens to a state of apparent health after five to seven days. In this study the mortality took the same period of time to drop to the normal.

Postmortem examination of the IBD infected birds revealed same lesion of haemorrhage atrophy of bursa of Fabrecious, heamorrahages of muscles and swollen kidney tubules described by Van den Berg, *et al.*,(2000**).**

Skeeles,*et al.,* (1980) reported that the most severe cases are characterized by a major infection of the mucous membrane and a serous transudate, giving the bursal surface a yellowish color, the researchers in this study found the same changes in the the IBD outbreak. And in both of the studies petechiae and haemorrhages were reported.

In our study the bursa showed fluctuation in size at the end of the outbreakthat appeared as an increase following the gradual decrease in size this may be attributed to the number of samples.

Recommendations

1. The economic losses due to IBDcreated the need for a better characterization of the circulating strains.

2. Vaccination schedule should accommodate with the current situation in the farm in specific and the state in general.

3. Further studies for better understanding of the current circulating strain and its pathogenesis to improve control.

Chapter V

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