STUDIES ON GUT PATHOLOGY OF CHICKEN WITH SPECIAL REFERENCE TO PROVENTRICULITIS/ PROVENTRICULAR DILATATION

Thesis

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Roll No. P-1723

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF

Doctor of Philosophy
(Veterinary Pathology)

2017

Dedicated To...

My Beloved Family-

and

Guide

भा.कृ.अ.प.–भारतीय पशु चिकित्सा अनुसंधान संस्थान
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Dated: 9/8/ **2017**

Certificate

This is to be certified that the research work embodied in this thesis entitled **"Studies on gut pathology of chicken with special reference to proventriculitis/proventricular dilatation"** *submitted by* **Dr. Pradeep. M, Roll No. P-1723,** *for the award of* **Doctor of Philosophy** *Degree in* **Veterinary Pathology** *at Indian Veterinary Research Institute, Izatnagar, is the original work carried out by the candidate himself under my supervision and guidance.*

It is further certified that **Dr. Pradeep. M, Roll No. P-1723,** *has worked for more than 30 months in the Institute and has put in more than 300 days attendance under me from the date of registration for the* **Doctor of Philosophy** *Degree in this Deemed University, as required under the relevant ordinance.*

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We the undersigned members of Advisory Committee of **Dr. Pradeep. M,** *Roll No. P-1723, a candidate for the degree of* **Doctor of Philosophy** *with the major discipline in* **Veterinary Pathology**, *agree that the thesis entitled* "**Studies on gut pathology of chicken with special reference to proventriculitis/ proventricular dilatation**" *may be submitted in partial fulfillment of the requirement for the degree.*

We have gone through the contents of the thesis and are fully satisfied with the work carried out by the candidate, which is being presented for the award of **Doctor of Philosophy** *Degree of this Institute.*

It is further certified that the candidate has completed all the prescribed requirements governing the award of **Doctor of Philosophy** *Degree of the Deemed University, Indian Veterinary Research Institute, Izatnagar.*

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(Pradeep, M.)

Date:
Place: *ICAR-IVRI, Izatnagar*

ABBREVIATIONS

%	:	Per cent
/	:	Per
°C	:	Degree Celsius
3'UTR	:	3' UnTranslated Region
5'UTR	:	5' UnTranslated Region
ALV	:	Avian leuocosis virus
ANV	:	Avian nephritisvirus
AvRtV	:	Avian rotavirus
AvRV	:	Avian reovirus
Bp	:	Base Pair
CAstV	:	Chicken astrovirus
CAV	:	Chicken infectious anaemia virus
cDNA	:	Complementary Deoxyribonucleic Acid
ChPV	:	Chicken parvovirus
CPNV	:	Chicken proventricular necrosis virus
DEPC	:	Diethyl Pyrocarbonate
DNA	:	Deoxyribonucleic Acid
DNase	:	Deoxyribo nuclease
dNTP	:	Deoxyribonucleoside triphosphates
DPI	:	Days post inoculation
DPR	:	Directorate of Poultry Research
DW	:	Distilled water
FAdV1	:	Fowl adenovirus1
FAT	:	Fluorescent Antibody Technique
gm	:	Gram
hrs	:	Hours
IBDV	:	Infectious Bursal Disease Virus
IBV	:	Infectious Bronchitis Virus
ICAR	:	Indian Council of Agricultural Research
IVRI	:	Indian Veterinary Research Institute
Kb	:	Kilobase pair
M	:	Molar
MCT	:	Microcentrifute tube
MDV	:	Marek's Disease Virus
mg	:	Milligram
min	:	Minutes

mL	:	Milliliter
mM	:	Millimolar
MNCs	:	Mononuclear cells
NCBI	:	National Cerntre for Biotechnology Information
NDV	:	New Castle Disease Virus
ng	:	Nanogram
nm	:	Nanometre
NS	:	Non Structural
ORF	:	Open Reading Frame
PBS	:	Phosphate Buffered Saline
PCR	:	Polymerase Chain Reaction
REV	:	Reticulo endothelial virus
RNA	:	Ribo nucleic acid
RNase	:	Ribo nuclease
RPM	:	Rotations per minute
RSS	:	Runting- stunting syndrome
Rt-PCR	:	Reverse Transcriptase Polymerase Chain Reaction
sec	:	Seconds
Sp	:	Species
TBE	:	Tris borate EDTA
Temp	:	Temperature
UV	:	Ultra Violet
W/V	:	weight per volume
µg	:	Microgram
µl	:	Microlitre

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Introduction

1 INTRODUCTION

Poultry industry is a fast growing sector in India. India is the 6th largestchicken meat producer and 3rd largest egg producer in the world (FAOSTAT rankings, 2011). Efforts are made to maximize production by genetic selection and maximizing feed conversion ratio (FCR). For improving FCR, gut health remain to be one of the important factors along with good proportion of quality feed.

The gut, which includes stomach and intestine, is the site of digestion and absorption of feed ingested. Unlike mammals, birds' stomach has two parts, glandular (proventriculus) and muscular (ventriculus/ gizzard) stomach. These parts help in enzymatic digestion and grinding of feed respectively. The small intestine consists of duodenum, jejunum and ileum, is the major site of chemical digestion and nutrient absorption. Caecum with its large microbial community breaks down indigestible plant materials. (Clench and Mathias, 1995)

Gastrointestinal (GI) tract has the most extensively exposed mucosal surface in the body and is constantly exposed to a wide variety of potentially harmful substances (Yegani and Korver, 2008). Anatomically and functionally, the GI tract has many selective barriers against the invading pathogens preventing them from entering bird's body. Physical, immunological and microbiological barriers are the major barriers present in the GI tract. Change in diet, toxins, adverse environment and infections negatively affect gut health keepers, and deleteriously affect the health of the bird and its production performance. The microbes of the intestine include both potentially harmful organisms like *Clostridium* species and beneficial organisms that help in production of vitamin and inhibition of multiplication of pathogenic microbes like *Salmonella* (Jeurissen *et al*., 2002). Antibiotics have been used widely as growth promoters

in poultry feed. These antibiotic growth promoters were helping to reduce the occurrence of necrotic enteritis in chicken (Prescott, 1979; Williams, 2005). Incidence of infectious diseases like necrotic enteritis is increased in countries that have stopped use of antibiotics as growth promoters (Van Immerseel *et al.,* 2004). European Union has banned the use of growth promoting antibiotics in poultry in 2006 and many countries are following the path (Cogliani *et al*., 2011). This may alter the microbial profile of the gut environment in the commercial poultry, making it more susceptible for diseases.

The aetiology of the gut diseases is often multifactorial. A combination of non-infectious and infectious agents may be involved in the development of gut diseases (Pantin-Jackwood, 2013;Hafner and Guy, 2013). In the proventriculus, haemorrhages in the mucosa are seen variety of disease conditions like Newcastle disease, Infectious bursal disease and avian influenza. Proventricular conditions like proventriculur dilatation and proventriculitis especially Transmissible Viral Proventriculitis (TVP) is being reported from developed countries (Hafner and Guy, 2013). Proventricular dilatation is characterised by thinning of the wall without any histological lesion. Proventriculitis is a microscopic diagnosis characterised by inflammation in the proventriculus. The causes of proventriculitis can be non-infectious like toxin, biogenic amines, copper sulphate, mycotoxin ((Brugh and Wilson,1986; Jensen *et al.,*1986), or infectious. An infectious type of proventriculitis known as transmissible viral proventriculitis (TVP) has been reported from different parts of the world which is characterised grossly by thickened proventricular wall with pale serosa and histologically by specific microscopic changes that include glandular epithelial cell necrosis, ductal epithelial cell hyperplasia, and inflammation where lymphocytes predominate (Bayyari *et al.,* 1995; Goodwin *et al.,* 1996). Proventriculitis is mainly noticed at the slaughter house without showing clinical signs. Proventriculitis associated with runting and stunting syndrome is reported in broilers (Kouwenhoven *et al.* 1978b; Noiva *et al.,*2015). Proventriculitis were reported in young commercial broilers and recently in adult birds such as broiler parents and layer hens (Marusak *et al.*, 2012).

The primary etiologic agent of the proventriculitis was not clear. Some workers has isolated Infectious Bronchitis virus from the proventriculus of proventriculitis cases (Yu *et al.,* 2001; Ganapathy *et al.,* 2012) but their pathogenicity was not ascertained. Recently Guy *et*

al. (2011) isolated and characterised a novel birna virus called Chicken Proventricular Necrosis Virus (CPNV) from the TVP cases. The team has developed a Reverse Transcriptase –PCR that is highly sensitive and specific for the detection of CPNV. Using the technique CPNV was detected in proventriculus of broiler chickens affected with runting and stunting syndrome (Noiva *et al.*,2015) and in older birds (broiler parents and layer hens) with or without any gross lesions (Marusak *et al.*, 2012).

Thickening of proventricular wall was observed in birds reported of stunted growth and avian nephritis virus infection (Gouthaman *et al.*, 2015) from India.

Enteric diseases are economically important disease in poultry and its impact may vary from insignificant to disastrous. Enteric diseases occur in all age groups, though predominates in younger age groups of chicken. Pathologic and economic impact of enteritis is by increasing susceptibility to other diseases, decreasing feed conversion efficiency, prolonging the time to market and mortality (Mettifogo *et al.,* 2014). Genetic improvements especially in broiler chickens to increase feed conversion have made intestinal health particularly important, because fast-growing broilers have a tendency to be hyperphagic and can be severely affected by decreased feed absorption (Dekich, 1998). Conditions of intensively bred other chicken breeds are also not different. Even an imbalanced microflora could result in decreased vitamin production, immunosuppression, and increased growth of harmful bacteria in the intestines of chickens (Yeganiand Korver, 2008). Enteric affections in the early age can result in runting and stunting syndrome (Pantin-Jackwood *et al.*, 2008; Palade *et al.*, 2011). Many viral, bacterial and protozoal organisms are playing important roles in the cause of enteritis in chicken. Underfield conditions, these intestinal infections are usually complicated by interactions with other infectious agents or non-infectious factors such as age, nutrition, and immune status of the birds as well as the management and environmental conditions, which complicated the evaluation of the role of any specific pathogen alone in the enteric diseases manifestation (Guy, 1998; Shapiro, 1998). In molecular survey conducted by workers like Pantin-Jackwood *et al*. (2008),Palade *et al.* (2011) and Koo *et al*. (2013b) in enteritis cases of broilers detected Avian nephritis virus, Chicken astrovirus, Chicken parvovirus, infectious bronchitis virus, avian rotavirus and fowl adenovirus from the intestine. These viruses are suspected to be associated with enteritis

and runting and stunting syndrome in chickens. Very recently Shah *et al.* (2016) reported and sequenced many of these viruses like Chicken astrovirus, picorna virus and reo virus from healthy chicken.

For maintaining a healthy chicken gut, systematic studies on the major gut affection in different breeds of poultry including the native breeds are essential. As gut diseases are often multifactorial, study on any of the specific pathogen, affecting the gut may not provide adequate rationale for diagnosing the underlying problem. Pathomorphological analysis of gut will help in better understanding of prevailing gut problems of different breeds of chicken. The studies pertaining to the proventriculitis are found to be sparse in India. Hence the current study is planned with following objectives.

1. **To study, the occurrence of various pathomorphological conditions of chicken gut.**
2. **To screen proventriculitis and proventricular dilatation cases for possible viral aetiology using molecular methods and isolation techniques.**

Review of Literature

2 REVIEW OF LITERATURE

Proventriculitis and Proventricular Dilatation

Proventricular dilatation is characterized by an enlarged, but thin-walled proventriculus with no microscopic evidence of inflammation. The lesion is often attributed to low fibre diet (Newberne, 1956; Riddell, 1976; Taylor and Jones, 2004). Proventriculitis on the other hand characterized by inflammatory reactions (Hafner and Guy, 2013). Proventriculitis has infectious and non-infectious causes. Non-infectious causes include ingestion of toxins, biogenic amines, excess of copper sulphate and mycotoxins. Many viruses are been isolated from cases of proventriculitis such as avian rotavirus (Lublin *et al.,* 2004), infectious bronchitis virus (Yu *et al*., 2001) and Chicken proventricular necrosis virus (Guy *et al.*, 2007). Some of the proventricular thickening were found to be infectious in nature and termed it as Transmissible Viral Proventriculitis (TVP). Proventricular enlargement and histological lesions like necrosis of proventricular glandular epithelium, ductal epithelial cell proliferation and lymphocytic infiltration (Bayyari *et al*., 1996; Goodwin *et al*., 1996) characterize this disease. Proventricular enlargement with inflammatory signs were primarily noticed in broiler chickens and associated with proventricular fragility, poor feed conversion and impaired growth (Goodwin *et al.,* 1996; Pantin-Jackwood *et al.,* 2004). Proventricular enlargement may also cause carcass contamination by rupture during the processing of poultry meat (Thayer and Walsh, 1993). In a recent study proventricular enlargement was observed in chicken identified with QX-like type IBV in the intestine (Koo *et al*., 2013b).

Proventriculitis was described as early as 1950s (Newberne, 1956) in experimental chicks fed with purified ration. Later, reports were found in the commercial broilers. Induced

pathological thickening of proventriculus with inflammation by feeding of biogenic amines (Shifrine,1960) and excess copper sulphate (Poupoulis and Jensen, 1976) in poultry were reported. Dorner *et al.* (1983) showed that mycotoxin can also induce inflammatory type proventricular thickening. Kouwenhoven *et al.*, (1978b), Page *et al.* (1982) noticed that infectious proventriculitis causes runting and stunting syndrome. Goodwin *et al.* (1996) identified intranuclear 60-70 nm, adenovirus-like virus (AdLV) in the glandular epithelium of priventruculi of chickens affected with proventriculitis. Similar viruses were later reported by Huff *et al.* (2001) and Pantin-Jackwood *et al.* (2005). Huff *et al.* (2001) orally inoculated bacterial and viral isolate from homogenate of affected proventriculus to chicks and suggested proventriculitis is infectious and has a complex aetiology possibly of both viral and bacterial. Yu *et al.* (2001) isolated infectious bronchitis virus from proventriculitis affected chicken from China.

Pantin-Jackwood (2005) identified the presence of infectious bronchits virus, infectious bursal disease virus using molecular methods like PCR and Rt-PCR from the proventriculus of the experimentally inoculated chicks. They could not isolate any bacteria. They found approximately 120 nm size virus-like particle within the cytoplasm of proventricular cells using electron microscopy. Due to the consistent finding of virus particle in the cases of proventriculitis, it was termed as transmissible viral proventriculitis (TVP). Guy *et al*. (2005) demonstrated the etiological role of virus in TVP. They partially characterized the virus and successfully propagated through the amniotic root of the chicken embryo. The homogenate from the proventriculitis tissue was sieved through 0.2 um filter and was treated with chloroform to exclude the bacteria and infectious bronchitis virus. Even though they termed the isolated virus as adino like virus (AdLV), they could not recognize the virus in PCR from the DNA isolated from the affected proventriculus. Guy *et al.* (2007) experimentally reproduced TVP in SPF and commercial broiler chickens by eye drop inoculation of AdLV. Considering proventriculus as the principal site of replication and cell damage, the virus was named as chicken proventricular necrosis virus (CPNV). At the latest, physical and genomic characteristic of CPNV was done by Guy *et al.* (2011) and identified it as a novel Birna virus, deeply divergent from other birna virus. They developed Reverse Transcriptase-PCR method for detection of CPNV in experimental as well as natural cases (Guy *et al.*, 2011).

Natural occurrence of infectious type of proventriculitis was mainly reported from commercial broilers from regions like US, Holland, UK and Australia (Kouwenhoven *et al.*, 1978a and b; Goodwin *et al.,* 1996; Reece, 2002). Age of affection generally is 3- 8 weeks with most common between 4-5 weeks old chickens in these reports. Recently proventriculitis has been reported in older birds such as broiler breeders and layer hens (Marusak *et al.*, 2012). A possible association of picornavirus in TVP was reported by Kim *et al.* (2015) in a metagenomic study of proventriculitis cases.

Proventricular thickening by *Macrorhabdus ornithogaster* (previously known as megabacteria), a yeast, opportunistically colonize proventriculus in chicken and other birds were reported (Pennycott *et al.,* 2003). It mainly multiplies in isthmus region. Its effects in commercial chicken not well studied. Proventricular enlargement with thickened wall and microscopically moderate to marked lymphoplasmacytic and heterophilic infiltration in the proventriculus and gizzard were observed with the infection of *Macrorhabdus ornithogaster* (Martins *et al*., 2006).

Candida albicans infection may cause swollen proventriculus with glossy appearance of serosa and haemorrhage in mucosa often covered with catarrhal or necrotic exudate (Dykstra *et al.,* 2013).

Proventriculitis observed in experimental study with Fowl adenovirus1 that caused gizzard erosion, but the virus was not detected in the proventriculus (Lenz *et al*., 1998).

Dilatation of proventriculus with thin wall and small gizzard was described by Riddell (1976) in chicks fed with low fibre diet

Experimental studies of infectious proventriculitis

Oral Inoculation of day old chicks with affected proventriculus developed proventricular enlargement at 7th and 14th day of observation (Pantin-Jackwood *et al.,* 2005). Bayyari *et al.* (1995) observed that field birds with severe proventriculitis were shown to have increased body weights compared with birds without proventriculitis or with milder lesions. They also observed that the intestinal weakness was not associated with proventriculitis and field birds with the most severe proventriculitis had stronger intestines.

Experimental eye inoculation of chicks at 15 days of age, with filtered homogenate of affected proventriculus resulted in proventricular enlargement at 14th day of post inoculation (Guy *et al.,* 2005).

Experimental inoculation and propagation of CPNV was successful through amniotic route at 15 days of incubation of chick embryo (Guy *et al.,* 2005) and proventricular enlargement of the hatched chick at 2 days of age was evident only in the 4th passage. Clear cut and consistent microscopic changes noticed only on 8th passage. Histologically all the proventricular glands were not affected in the study and the proventricular mucosa remained normal.

Characters of CPNV

CPNV was identified as birnavirus (icosahedral, 75nm in diameter, non-enveloped) having bisegmented, double stranded RNA genome. The larger A segment of birna virus ranges in size from 3- 3.4 kilo base pairs (kb) and encodes for capsid protein VP2 and VP3 and Segment B ranges from 2.7- 3.2 kbp and codes for VP1. But phylogenetic analysis showed deep divergence with other birna viruses (Guy *et al.,* 2011).

Attempt to propagate the virus in cell culture is not been successful and propagation of the virus with the use of embryonated chicken eggs resulted only in low concentrations of infectious virus (Guy *et al.,* 2005)

Clinical signs of proventriculitis and proventricular dilatation

Proventricular dilatation is usually not associated with any signs (Taylor and Jones, 2004). Proventriculitis may be associated with impaired growth, poor feed conversion and digestion (Goodwin *et al.,* 1996; Huff *et al.,* 2001; Kouwenhoven *et al.,* 1978b). Outbreaks of TVP associated with runting and stunting syndrome were reported by Marguerie *et al.* (2011) and Noiva *et al.* (2015). Naturally occurring proventriculitis may produce poor flock production data (Thayer and Walsh, 1993).

Gross lesions

Gross lesions of proventricular dilatation due to low fibre diet are thinning of wall of proventriculus with poorly developed gizzard muscles (Riddell, 1976; Taylor and Jones, 2004).

In case of infectious proventriculitis the affected bird may be smaller in size. The proventriculus will be grossly enlarged, occasionally dilated with pale, mottled serosa and thickened wall. Proventricular glands may be distended with white viscous material oozing on applying digital pressure (Noiva *et al.,* 2015). Marusak (2012) has reported TVP in layer hens and broiler parents without any gross lesions in proventriculus and in cases along with Marek's disease.

Proventricular enlargement by excessive feeding of copper sulphate showeds thickened wall and flattened and stippled brown black mucosal lining in chicken (Jensen *et al.,* 1991). Mucosal erosions were seen in feeding of mycotoxin (Dorner, 1983) and histamine (Shifrine *et al.,* 1960).

Microscopic lesions

In TVP, the microscopic lesions are primarily seen in proventricular glands. Glandular epithelium undergoes degeneration and epithelial hyperplasia, replacement of glandular epithelium with ductal epithelium and infiltration of lymphocytes (Bayyari *et al.,* 1995; Goodwin *et al.,* 1996). Microscopical lesions may be patchy often involving many glands or sometimes isolated glands in a locally extensive zonal pattern (Noiva *et al.,* 2015). Collecting ducts are dilated and filled with necrotic cells. Regeneration of glandular epithelium by cuboidal to low columnar duct like epithelium are often seen.

Proventriculitis due to histamine feeding shows mucosal erosion and oedema. In proventricular dilatation distinct microscopic lesion are not evident

Electron microscopy: Intracnuclear, icosahedral virus particles of size approximately 70 nm diameter were seen in the proventricular epithelial cells (Guy *et al.,* 2005) in TVP cases. Intracytoplasmic and larger (100 nm diameter) virus particles were reported by Pantin-Jackwood *et al.* (2005).

Diagnosis: Infectious proventriculitis is diagnosed mainly by characteristic microscopic changes of the proventriculus. Guy *et al.* (2011) made genomic characterization of the Chicken Proventricular Necrosis Virus, as a novel birna virus and developed Reverse transcriptase-PCR procedure for the identification of the virus from natural and experimental TVP. The Rt-

PCR is more sensitive and specific than the indirect immunofluorescent technique used by the Guy *et al.* (2007).

Diagnosis of proventricular thickening by megabacteria was done by finding gram variable, PAS positive in faecal smear or in gastric mucus (Behnke and Fletcher, 2011).

OTHER AFFECTIONS OF PROVENTRICULUS AND INTESTINE

Haemorrhage and necrosis of proventriculus especially haemorrhage at the tip of the proventricular gland is a lesion of vNDV infection but not pathegnomonic (Miller and Koch, 2013).

Infectious bronchitis virus (IBV) found to multiply in the proventriculus and intestine of poultry (Bhattacharjee and Jones, 1997). But the gross and histological changes in proventriculus and intestine due to IBV is very mild unless there is no secondary infection except in rectum where desquamation of the cells from the tips of villi, congestion, and focal infiltration with lymphocytes, macrophages, and some heterophils can be found (Ambali and Jones, 1990). Escorcia *et al.* (2002) reported proventricular and gizzard lesions in experimentally infected chicken embryos with IBV. They reported thin walled proventriculus and gizzard in the chick embryos on 7 days PI (8^{th} passage). Microscopically in the proventriculus, there was a decrease in the gland papillary branching, while the gizzard showed a significant reduction in mucosa thickness and tubular-to- proliferative cell ratio, as well as an absence of hyaline secretion in the lumen. Infectious bronchitis virus was isolated from proventriculus, in proventriculitis cases (Yu *et al*., 2001; Ganapathy *et al.*, 2012) but was not established as the primary cause of proventriculitis. Toffan *et al.* (2013) observed proventriculitis along with congestion and oedema of lungs, fibrinous deposits in airsac and swollen kidney in IBV- Q1 strain infected, clinically ill chickens in Italy. The gross lesions in the proventriculus were thickening of the wall at times associated with congestion at the tip of the glands. Histologically multifocal necrosis and erosion in the mucosal and glandular layer were observed along with fibroplasia of the muscularis mucosa.

In infectious bursal disease (IBD) haemorrhages are occasionally seen in isthmus region of proventriculus and may cause melena (Eterradossi and Saif, 2013).

Chicken infectious anaemia affected chicken may show punctuate haemorrhages in the mucosa of proventriculus, mostly in the distal part (Schat and van Santen, 2013). Microscopically there will be depletion of cells of lymphoid foci of proventriculus, duodenum and caecal tonsil.

Whitish areas (due to masses of infiltrating lymphocytes) in the muscularis of the gizzard are seen grossly in avian encephalomyeilitis (Suarez, 2013). Microscopically the mild lymphocytic aggregates normally seen in the muscular wall become dense and are pathognomonic for the avian encephalomyelitis. Similar lesions may also occur in gizzard muscle in Marek's disease. In Marek's disease proventriculus become thickened and firm as a result of focal lymphocytic areas within and between the glands (Schat and Venugopal, 2013). Necrotizing lesions were observed in duodenum, jejunum, and proventriculus of one week old unvaccinated chicks died of Marek's disease, and large intranuclear inclusion bodies were a striking feature in tissues with lesions (Carvallo *et al.,* 2011).

In neonatal colicepticemia proventriculus develop a dark colour which increases with increasing the interval between death and necropsy time (Nolan *et al*., 2013).

A few serosal haemorrhages in proventriculus are seen in case of spirochaetosis of chicken especially caused by *Borrelia anserine.* In the mucosa, haemorrhage observed in the isthmus region. Marked enlargement and mottling of spleen is typical for the spirochaetosis except in infection with low-virulent strains or in early stages of the disease (Abdul-Aziz and Barnes, 2013).

Mucosal necrosis of proventriculus and gizzards are seen in mycotoxins like T2 toxins, cyclopiazonic acid while erosion and bleeding caused by rubratoxins and patulin (Hoerr, 2013).

Yellow nodules in the serosa of gizzard is seen *Salmonella pullorum* infection (Shivaprasad and Barrow, 2013)

ENTERIC DISEASES

Bacterial disease

Necrotic enteritis- It is a disease primarily of young chicken. Outbreaks of necrotic enteritis are mostly seen in broilers of 2-6 weeks of age. Kwatra and Choudhari (1976) has

reported outbreak in 9 month old birds. The disease is caused by infection with, and toxin production by, *Clostridium perfringens* type A and type C. Alpha toxin produced by the *C. perfrengens* type A (CPA) is considered to have major role in the pathogenesis of the disease (Engstrom *et al.,* 2003; Lovland *et al.,* 2003). Gholamiandekhordi *et al.* (2006) reported that there is no quantitative difference of alpha toxin production between healthy and diseased birds. Keyburn *et al.* (2006) reported their experiment with CPA null mutant concluding that the alpha toxin was not critical for disease production. But findings of Coursodon (2010) reconfirmed the importance of alpha toxin by demonstrating the production of the toxin in intestine of birds infected with CPA null mutant where the intensity of lesion increased with the amount of toxin. A few investigators (Keyburn *et al.,* 2008) reported the role of necrotic enteritis toxin B (NetB) in the production of the disease but not clearly confirmed. Beta 2 toxin of *Clostridium perfringens* type C (CPB2) has also been suggested to have role in the pathogenesis of the disease (Van Immerseel *et al.*, 2004) but not yet proved conclusively.

Necrotic enteritis are mostly sporadic (Riddell and Kong, 1992). Clinical signs of the necrotic enteritis are short and birds die without any symptom or within 1-2 hours after onset of clinical signs (Helmboldt and Bryant, 1971). Depression, diarrhoea and drooling of saliva were also reported (Porter, 1998). Major gross lesions of the disease were are seen in jejunum and ileum portion of the intestine occasionally extending to caecum. Intestine get distended with gas, wall become thin and contain foul smelling fluid. Yellow green or yellow brown pseudomembrane adhering loosely or tightly to the mucosa may be seen. In chronic or subacute cases intestinal wall become highly thickened. Mucosal ulceration can also be seen in subacute cases with partially adherent yellow material (Cooper *et al.,* 2013). Histologically extensive mucosal necrosis is characteristic even extending to muscularis mucosa in extensive cases (Kwatra and Chaudhury, 1976). Heterophils are predominant inflammatory cells. Monocytes are seen in chronic cases. Gram-positive rods with square ends are commonly associated with areas of necrosis. Diagnosis is done mainly by characteristic gross and histological changes. Isolation of the organism along with identification of the type A toxin are helpful in the diagnosis even though they can also be present in the healthy birds. Differential diagnosis need to be made from coccidiosis, histomoniasis and ulcerative enteritis (Cooper *et al*., 2013).

Ulcerative enteritis

Ulcerative enteritis was first reported in quails (Morse, 1907; Shillinger and Morley, 1934) but later found to affect young chicken of 4-12 weeks of age (Graubmann and Grafner, 1971; Davis, 1973) and other avian species. The disease is caused by *Clostridium colinum*. In chicken mortality are only 2% to 10% (Barnes, 1987) and the clinical signs include watery diarrhoea, depression and bloody diarrhoea. Gross lesions are mainly haemorrhagic enteritis affecting duodenum. Ulcers can be developed in any part of the intestine. Ulcers in later stages visible even from serosa and may perforate and cause peritonitis. Necrotic liver lesions and haemorrhagic spleen lesions may also be present. Microscopic lesions in earlier stages include mucosal necrosis, oedema and heterophilic and lymphocytic infiltration (Cooper *et al*., 2013). Clumps of bacteria may be visible at the site of necrosis. Presence of large Gram-positive rods with subterminal spores in liver, spleen, and/or intestinal smears or histological sections strengthens a presumptive diagnosis (Songer and Uzal, 2013). Roussan *et al.* (2012) has developed a duplex PCR for simultaneous detection of *Clostridium perfringens* and *Clostridium colinum.*

VIRAL ENTERIC DISEASES

The economic impact of enteric virus infections on the poultry industry has been evaluated and ranges from insignificant economic effects to those that are severe and cause devastating losses (Mettifogo *et al*., 2014). Enteric diseases related to viruses were firstly reported in the late 1970s and is characterized by growth deficiency, retarded feather development, diarrhoea, and other abnormalities (Kouwenhoven *et al.,* 1978 a). Several viruses were identified in the intestine or stool of chickens with enteric disease. However, none of them could reproduce the disease singly in experimental conditions indicating that more than one virus plays role in enteric diseases. Interaction with other infectious agents or factors like age, nutrition and immune status further complicate the disease. The viruses that were detected in high proportion in enteric diseases include chicken astrovirus (CAstV), avian nephritis virus (ANV), chicken parvovirus (ChPV), infectious bronchitis virus (IBV), avian rotavirus (AvRtV), avian reovirus (AvRV), and fowl adenovirus (FAdV) (Yu *et al.,* 2001; Otto *et al.,* 2006; Pantin-Jackwood *et al.,* 2008; Smyth *et al.,* 2009; Palade *et al.,* 2011, Koo *et al.,*2013b).

Chicken Parvovirus (ChPV) Infections

Parvo viruses are smallest of isometric viruses of about 25 nm diameter that comes under genus *Aveparvovirus*, subfamily *Parvovirinae* of *Parvoviridae* family. The nonenveloped, icosahedral virus contain linear single stranded DNA molecule between 4-6 kb lengths. The virus has 2 major, 5' and 3' open reading frames (ORF) and 1 small ORF. The 5' ORF encodes non-structural (NS1) protein important for viral replication and pathogenesis. This gene is considered to be conserved part of the virion important for identification of the virus by PCR (Zsak *et al*., 2009; Domańska-Blicharz and Minta, 2011). The 3' ORF encodes for the parvovirus capsid proteins VP1, VP2, and VP3. Function of 3rd and small ORF is not clear. Day and Zsac (2010) analysed the full length genome of ChPV and found it to be different from other avian parvovirus such as goose and muscovy duck parvoviruses.

Chicken parvovirus was first identified in the intestinal tract of 10 day old chicks and designated as ABU strain by Kisary *et al.* (1984). Thereafter, recent years showed a peak momentum in the investigations on the virus and reports on detection of the virus came from countries like US (Zsak *et al.,* 2009), Hungary(Palade *et al.*, 2011), Croatia (Bidin *et al.,* 2012), Poland (Tarasiuk *et al*., 2012), South Korea (Koo *et al*., 2013a), Brazil (Mettifogo *et al*., 2014) and latest from China (Feng *et al*., 2016). ChPV has been detected in cases of enteric diseases (Zsak *et al.,* 2008; Bidin *et al.,* 2011; Palade *et al.*, 2011; Tarasiuk *et al.,* 2012) and runting and stunting syndrome (Kisary *et al*., 1984; Zsak, 2013) of chicken. Kisary (1985) successfully reproduced RSS experimentally by oral inoculation of purified ChPV viral particles in day old broiler chicken. However, at the same experiment, slow growing white leghorns exhibited minimal effect, even though replication of the virus found in their intestine. In the embryo inoculation through allantoic route by Kisary (1985) the dead embryos showed oedema and haemorrhage all over the body and the hatched one were one or two days late in hatching and weak that survived only one week.

Clinical signs in leghorns most frequently seen in chicks between 7-28 days of age. Infection in older birds produce antibodies but without clinical signs (Zsak and Day, 2010).

Transmission electron microscopy (TEM) studies and immunohistochemistry (IHC) showed that the primary target cells for replication *in vivo* are located in the small intestine (Kisary, 1985; Zsak *et al.,* 2009; Palade *et al.*, 2011). Zsak (2013) reported presence of parvovirus positive cells, 7-14 days of post inoculation, in chicken, causing enteric disease and runting and stunting syndrome. No specific gross lesions connected with the ChPV reported so far except the observation of curving of (J shaped) duodenal loop with pancreatic atrophy in the broilers from which only parvovirus could be detected (Nunez *et al.,* 2016).

Adenovirus infections

Avian adenoviruses are mostly opportunistic pathogen when bird sufferes immunosuppression or concurrent infections. However, some adenoviruses are primary pathogens like turkey haemorrhagic enteritis virus, quail bronchitis virus, and egg drop syndrome virus (Fitzgerald, 2013). Taxonomically the adenovirus has 4 genus which were known as Mastadenovirus, avian adeno virus group I, group II and group III which later rechristened in the Ninth report of International committee on taxonomy of Virus as Mastadenovirus, Aviadenovirus, Siadenovirus and Atadenovirus respectively (Harrach, 2012). The genus aviadenovirus further divided into fowl adenovirus (FAdV), Turkey adenovirus B (Turkey adenovirus 1, 2), Falcon adenovirus A and Goose adenovirus A. The FAdV has five species, A to E with serotype 1 to 10 (Fitzgerald, 2013).

The major primary diseases caused by adenovirus in chicken are inclusion body hepatitis hydropercardium syndrome, gizzard erosion (Aviadeno virus), spleenomegali (Siadenovirus) and egg drop syndrome (Atadenovirus) (Hess, 2013).

Aviadenovirus is considered as one of the causes for gizzard erosion in chicken. Serotype 1 of FAdV- A (FAdV-1) was identified as causative agent in the outbreaks of gizzard erosions reported from Japan and European countries (Okuda *et al.,* 2004; Manarolla *et al.,* 2009; Domanska-Blicharz *et al.,* 2011). In Japan the coexistence of FAdV-1 with different pathogenicity was reported (Okuda *et al.,* 2006). They observed strain difference in pathogenicity that FAdV-99ZH strain was capable of inducing gizzard erosions (pathogenic) in experimentally infected chickens but the OTE strain, the prototype strain of FAdV-1 in

Japan, failed to induce such lesions (apathogenic). Reports of occurrence of the disease in one flocks without affecting other flocks of the same farm indicates possible vertical transmission of the disease and effectiveness of preventing the disease by proper biosecurity measures (Ono *et al.*, 2007). Clinical signs not apparent except mild reduction in weight gain, if present. Gross lesions mostly confined to gizzards, that are distended with haemorrhagic fluid and contain multiple black patchy erosions within the koilin layer. Focal pancreatitis may often associate with the gizzard erosion (Ono *et al.*, 2004). Intranuclear inclusion bodies containing adenovirus antigen in glandular epithelial cells were associated with necrosis of the koilin layer, and infiltration of the lamina propria, submucosa, and muscle layers by macrophages and lymphocytes (Manarolla *et al.*, 2009; Domanska-Blicharz *et al.*, 2011; Nakamura *et al.*, 2002). Intranuclear inclusions observed in necrotic pancreatic acinar cells. Even though proventriculitis with lymphocytic and epithelial hyperplasia in the mucosa noted along with gizzard erosions in an experimental study with adenovirus type1 (Lenz *et al*., 1998), the virus could not be detected in the proventriculus in EM examination. They opined the development of proventricular lesions as secondary to gizzard lesions, not because of virus.

Diagnosis of the FAdV is done by various techniques like EM examination, immunofluorescence and restriction endonuclease analysis. Raue and Hess (1998) developed hexon gene based PCR combined with restriction enzyme analysis for rapid detection and differentiation of FAdV from egg drop syndrome virus.

Fowl adenovirus group 1 has been detected in the intestine of chicken in healthy and disease condition, but their role in the disease production is not well-established (Mettifogo *et al.*, 2014).

Astrovirus infections

Astroviruses are small round, non-enveloped, single stranded +RNA virus 25-35 nm in diameter with a star-like morphology, associated with variety of gastro enteritis in mammals and birds including chicken (Schultz-Cherry, 2013; Smyth, 2017). The virus classified under the family *Astroviridae*. The family *Astroviridae* is divided into two genera, Mamastrovirus (MAstV) and Avastrovirus (AAstV) that consist of astroviruses of mammalian and avian species,

respectively. Based on genetic analysis of the complete capsid region avian astroviruses of the genus *Avastrovirus* have recently been reclassified into three species, Avastrovirus 1 (Turkey astrovirus type 1 and 2), Avastrovirus 2 (Chicken astrovirus and Avian nephritis virus) and Avastrovirus 3 (Duck astrovirus type 1 and 2) (Smyth, 2017; Virus Taxonomy: 2016 Release; https://talk.ictvonline.org/files/master-species-lists/m/msl/6776].

First report of *Avian astrovirus* came from UK in turkeys (Turkey astrovirus) suffering from diarrhoea and increased mortality (McNulty *et al.,* 1980). Gough *et al.* (1984) first detected the virus in electron microscope in ducklings affected with hepatitis.

In chicken two species of astrovirus are described, Avian nephritisvirus (ANV) and Chicken astrovirus (CAstV).

Prior to this genomic analysis CAstV was known as 'entero-virus like virus' duo to its similarity with *Enterovirus* of *Picornaviridae* family (Spackman, 1984). Using genomic analysis Chicken astrovirus was first discovered from broiler chicks of stunted growth in 2004 (Baxendale and Mebatsion, 2004). The virus genome has 3 open reading frames (ORFs), ORF-1a, ORF-1b and ORF-2. The hypervariable capsid gene ORF-2 is responsible for the antigenicity of the virus (Smyth,2012). Further the ORF region genetic analysis confirmed the existence of previously identified (McNeilly *et al*., 1994) two serogroups, A and B. Serogroup A comprises 3 subgroups and serogroups B comprises 2 subgoups (Smyth *et al.,* 2012).

CAstV being non-enveloped, highly resistant to chemicals and environmental conditions and retain in the farm premises for long period. The transmission of the virus occurs horizontally by faecal oral route in most of the time. Vertical transmission of the virus is not yet proved beyond doubt. Young chicks of age below one week are most susceptible to the infection. Furthermore viral load in the chicks infected in embryonic stage are found to be higher than that of chicks horizontally infected soon after hatch (Smyth, 2017). Pathogenicity study in SPF chickens detected CAstV in all the segments of the intestine as well as in liver, kidney and spleen (Smyth *et al.,* 2007). Developments of clinical signs depend upon the amount of the virus transmitted and the concurrent infection with other viruses like ANV, Orthoreovirus and fowl adenovirus. CAstV found to be associated with runting- stunting syndrome (RSS)

(McNeilly *et al*., 1994), kidney affection and visceral gout (Bulbule *et al*., 2013) and white chick hatchery disease (Sajewicz-Krukowska *et al*., 2016).

The study on RSS by McNeilly *et al.* (1994) with experimental inoculation by CAstV produced mild weight gain reduction not as typical as that of RSS. CAstV detected in gut and faecal samples from healthy broiler chickens and from flocks affected by enteritis and growth problems in the United States (Pantin-Jackwood *et al.*, 2006). Further, a study conducted by Smyth *et al.* (2009) detected CAstV in gut contents from 96% of growth retarded broiler flocks. Co-infection of CAstV with other enteric viruses observed by many workers (Koo *et al*., 2013a; Koo *et al*, 2013b; Smyth *et al.*, 2010; Day *et al*., 2007) and hence may not be the sole cause of the RSS.

ANV, previously regarded as picorna virus (Maeda *et al.*, 1979) and later classified under *Avastrovirus* after genome sequencing (Imada *et al.*, 2000) causes interstitial nephritis and stunting in chicken. Avian nephritisvirus 1, 2 and 3 are reported so far. Yamaguchi *et al.*, 1979, first isolated Avian nephritis virus 1 (ANV-1) from a 1- week-old healthy broiler chick. This virus associated with mild growth depression, interstitial nephritis and sometimes mortality. ANV-2 was reported by workers like Takase *et al.* (1989), Shirai *et al.* (1992). ANV 3 has been found in chicken and turkey flocks with enteric and locomotion problems and damages the kidney, pancreas, duodenum, thymus, bursa, and liver (de Wit *et al.*, 2011). The pathogenicity of ANV-1 is generally limited to the kidney of chickens. ANV mostly affect young chicks with clinical signs of dehydration and diarrhoea (Hewson *et al.*, 2010). In India Gouthaman *et al.* (2015) reported avian nephritis virus in broiler chicks with signs of diarrhoea, stunted growth with lesions like proventricular thickening and mortality up to 9.72%.

In an experimental study on runting- stunting syndrome, replication of CAstV and Avian nephritis virus (ANV1 and ANV2) were found in the duodenum of chicks on day 1 and 2 PI (CAstV) and for several days (ANV-1 and ANV- 2) by Kyung-Il Kang *et al.* (2012). Cystic dilatation of crypts of duodenum along with atrophy of villi was the main microscopic lesion in the study.

Avian rotavirus infection

Rotaviruses are non-enveloped icosahedral particles of approximately 70-75 nm size and its genome comprises 11 segments of double-stranded RNA, which encodes six structural proteins (VP1 to VP4, VP6 and VP7) and six non-structural proteins (NSP1 to NSP6). The NSP4 gene of non-structural protein, concerned with enterotoxin production has major difference in amino acids with that of mammalian rotavirus (Kusumakar *et al*., 2010). Replication of rotavirus occurs in cytoplasm and release by cytolysis. Based on antibody reactivity or genetic sequencing of structural protein VP6 of the internal capsid, rotaviruses have been classified into the groups A to H (Estes and Kapikian 2007; Matthijnssens *et al.,* 2011). Group A virus reported in both mammals and birds, the B, C and E groups only in mammals and the groups D, F and G only in birds. Incidence of AvRtV infection in chickens reported worldwide. A few incidences from India reported group A (Wani *et al*., 2003, Minakshiprasad *et al*., 2004) and recently group D (Niture *et al*., 2010; Kattoor *et al*., 2013b). The AvRtV-D infection rate found to have significantly higher in young age groups of 12 to 21 days by some workers (Dhama *et al*., 2015) and in the 16-day to 30-day age group (62.2%; 23/37) by other workers (Bezerra *et al*., 2014). However, infection in older birds also occur as reported by Jones *et al.* (1979) in 32 and 92 week old commercial layers and Niture *et al.* (2010) in 60-65 weeks layer chicken. In India study by Savita *et al.* (2008b) revealed 17.4% (8/446) incidence of rotavirus in the diarrhoea samples of chicken from Jabalpur using RNA polyacrylamide gel electrophoresis technique. Incidence studies in Indian states by Kattoor *et al.* (2013a) reported 11.6% occurrence of AvRtV-A in chicks with diarrhoea and 3 cases of concurrent occurrence of both AvRtV-A and AvRtV-D. The team reported occurrence of rotavirus in 7.4%, 2.8% and 1.4% of chicken faecal samples from Uttarakand, Haryana and Kerala respectively out of 215 samples screened. Transmission of the AvRtV occurs by faecal-oral route (Day, 2013). There is no conclusive evidence of vertical transmission in chicken. AvRtV infection in birds may be symptomatic or asymptomatic. Clinical signs are generally diarrhoea, dehydration, weakness, anorexia and general flock depression (Day, 2013). The most common finding at necropsy is the presence of abnormal amounts of fluid and gas in the

intestinal tract and caeca. The diarrhoea is caused by destruction of mature villi epithelium and its replacement by immature epithelium from the crypts that have reduced absorption ability, resulting in diarrhoea (Guy,1998). Role of non-structural protein NSP4, an enterotoxin produced by the rotavirus, considered to have a major role in the development of diarrhoea in rotavirus infection (Mori *et al*., 2002). Pallor of the intestinal tract accompanied by loss of tonicity may be evident. Within the small intestine, different rotavirus strains may show preference for specific areas. Group A rotavirus grew best in the duodenum of experimentally infected chickens and group D rotavirus favoured the jejunum and ileum (McNulty *et.al.*, 1983). Even though ballooning and degeneration of epithelial cells, reduced villi length may be seen in rotaviral enteritis (Mori *et al.*, 2002; Day, 2013), no gross or microscopic lesions are pathognomonic for rotavirus infection. Histologically lesions may even be absent in naturally occurring cases (Horrox, 1980). Concomitant infection with other common enteric viruses may exacerbate disease signs and lesions. Virus can be visualised from intestinal contents or faeces by direct electron microscopy. Migration pattern of 11 segment double stranded RNA in the RNA-Polyacrylamide Gel electrophoresis was the major technique used for the diagnosis of the rotavirus (Svensson *et al.,* 1986; Lozano *et al.,* 1992). Viral RNA identification by molecular methods like Rt-PCR and Real Time -PCR are now being popularised. Day *et al.,* 2007 developed Rt-PCR for simultaneous detection of different enteropathogenic virus mainly targeting NSP4 gene. Specific diagnosis of type D group by targeting VP6 and VP 7 were developed by a few workers (Bezerra *et al*., 2014; Kattoor *et al*., 2013b). Only type A could be isolated in cell cultures so far while isolation of other serogroups is extremely difficult (Day, 2013).

Avian reovirus infections

Avian reovirus (AvRV) are reported to be associated with runting-stunting syndrome, enteric diseases (Pantin-Jackwood *et al*., 2008) and other non-intestinal conditions like tenosynovitis (De Gussem *et al*., 2010) and myocarditis (Davis *et al*., 2012). The association between the virus and the disease is clearly established in tenosynovitis while less clear with other conditions (Jones, 2000).

The AvRV has 10 double-stranded RNA (dsRNA) segments enclosed in a non-enveloped icosahedral double capsid. The 10 genomic segments are separated into three size classes-large (L1, L2, L3), medium (M1, M2, M3), and small (S1, S2, S3, S4) based on their electrophoretic mobility in agarose gel (Spandidos and Graham, 1976). The AvRV genome expresses at least eight structural proteins (λA, λB, λC, μA, μB, σA, σB, and σC) and four non-structural proteins (μNS, P10, P17, and σNS) (Martinez-Costas *et al.*, 1997).

Intestinal lesions by reo viral infections are not specific. In an experimental study by Lenz *et al*. (1998) dark green-brown, foamy caecal contents (caecal gas) at day 5 PI that persisted during the experimental period and randomly scattered raised 1–2-mm-diameter pale yellow foci in the superficial mucosa of the proventriculus at day 15 PI were observed .

AvRV infection potentiates the coccidia infection. Concurrent infection of coccidia and reovirus cause more reduction in weight gain in broilers than when either virus or coccidia were present alone (Ruff and Rosenberger, 1985).

Infectious bronchitis virus- enteric strain

Even though infectious bronchitis virus (IBV) is primarily affecting respiratory system, the virus can replicate in intestinal tract and may cause pathological changes (Ambali and Jones, 1990). Villarreal *et al.* (2007) isolated enterotropic strains of IBV and pointed out that they could be a possible pathogenic agent of enteritis in chickens. There are reports of IBV or IBV like viruses associated with RSS (Montgomery *et al*., 1997; Hauck *et al.*, 2016). In recent studies (Mettifogo *et al*., 2014; Chacon *et al*., 2014), though IBV detected frequently the intestine, they could not produce any enteritis in the experimental inoculation and opined that IBV may not a primary pathogen for enteritis.

Mixed enteric infections

Mixed viral enteritis were studied by various workers. Early reports on mixed enteric infection of poultry by rotavirus, reovirus, adenovirus and pseudopicorna virus started in 1980s (Andral *et al*., 1985; Saif *et al*., 1985). Roussan *et al.* (2012b) reported mixed enteric infection of AvRtV, CAstV, AvRV and adenovirus group I in broiler flocks of Jordan. The study revealed

AvRtV in 18.75%, AvRV in 21.4%, Adenovirus in 14.3%, CAstV in 38.6% and ANV in 44.4% of tested flocks. Another study made by Koo *et al*. (2013a) identified enteric viruses in 85.3% of tested flocks. The viruses identified in the study is in order ANV (44.1%), CAstV (38.2%), ChPV (26.5%), IBV (20.6%), AvRtV (8.8%), AvRV (5.9%), and FAdV (2.9%).

Runting- Stunting Syndrome (RSS)

Runted chicks hatches small while stunted bird show failure to grow and delayed development (Smyth, 2017). The bird will be active and normally feeding and exhibit pale and ruffled feathers (helicopter disease), small comb and beak along with the poor weight gain. Leg weakness and diarrhoea may also accompany the condition. The stunting signs are evident in second week onwards in broilers. Histologically cystic crypts in the intestine are seen in majority of the instances that may be resulting in malabsorption of the nutrients. Even though many workers were able to reproduce RSS experimentally with viral inoculation (Kisary, 1985; McNeilly *et al.*, 1994) no single virus can be attributed as a sole cause of the condition. Interestingly a latest metagenomic study on RSS affected and normal chicks (Devaney, 2016) detected all the suspected viruses in both groups but a little bit higher amount in the RSS affected group. Metagenomic studies do have its own limitations with bias formation during the steps (Pinard *et al*., 2006).

Viruses- in healthy chicken

The above said viruses are detected from the birds with clinical signs of enteritis. The same family of viruses are detected in healthy birds (Pantin-Jackwood*et al.*, 2008). Rotavirus in healthy birds were identified by Bazerra *et al.* (2014). Study utilizing the next generation sequencing Shah *et al.*, 2016 characterized the RNA viral community in the healthy broiler chicken. They identified 333 genera belonging to 80 RNA viral families. The most abundant RNA viral family detected at 2, 4 and 6 weeks of age was Astroviridae, which decreased in abundance with age while the abundance of Picornaviridae increased with age.

Avian Intestinal spirochaetosis (AIS)

Avian intestinal Spirochaetosis is generally a diarrheal condition/ reduction in egg production in birds caused by lower intestinal tract colonisation of *Brachyspira* sp. The major

species affecting poultry are *B. pilosicoli*, *B. alvinipulli* and *B. intermedia.* Even though, *B.hyosdysenteriae* cause swine dysentery and necrotising typhlocolitis in ducks (Glavitis *et al.,* 2011) and rheas (Sagartz *et al.,* 1992), considered non-pathogenic to poultry. They do not colonize small intestine. Unlike *B. hyosdysenteriae,* species affecting chicken such as *B. intermedia* and *B. pilosicoli* are short lived and do not persists in the poultry houses. The species *B. innocens*is associated with reduction in egg production in free range flocks (Burch *et al.,* 2009). The estimated impact of AIS on the UK laying industry was £18 million (Mappley *et al.,* 2014).

Brachyspira are Gram-negative, spiral organism with single circular genome (Hampson, 2013; LeRoy *et al.,* 2015). There are about Sequences of 16S rRNA are highly conserved (Stanton, 2006). The major clinical signs caused by AIS are diarrhoea, increased feed intake and mortality (up to 5-10%). Economic significance is mainly by reduced egg production, wetting of litters, increased fly problem, and resultant increased labour cost. Reduction of egg weight and faecal staining of eggs may also be seen. In broilers, retarded growth is seen in chicks of infected parent hens (Smit *et al.,*1998). Major infection in poultry is reported to be by *B. intermedia* followed by *B. pilosicoli* (Stephens *et al.,* 1999; Bano *et al.,* 2008; Myers *et al.*2009). Even though infrequent, detection of B. alvinipulli and B. hyodysenteriae were also reported (Feberwee *et al.,* 2008; Jansson *et al.,* 2008).

Some of the species are seen as commensals such as *B. murdochii, B. innocens* and *B. pulli* and may cause subclinical infection in chickens while *B. alvinipulli, B intermedia*, and *B. pilosicoli* cause mild to moderate signs in broiler breeder and layer hens (Hampson, 2013).

Natural infections usually do not develop any gross pathological lesions in poultry. Histologically spirochetes are seen apical surface of caecal epithelial cells. Histological lesions such as mild lymphocytic infiltration in the caecum and mild hyperplasia of epithelial cells may be developed. The species, *B. pilosicoli* characteristically attach to luminal surface of enterocytes with its one of the pointed ends, lying right angle to the epithelial surface and gives a "false brush border" on the surface (Hampson, 2013). Caecalcolonisation of spirochete produces

severe lesions in turkeys and rheas (Buckles *et al.,* 1994; Hampson, 2013). Dark field microscopy, electron microscopy and indirect florescent antibody tests and PCR studies are employed for the diagnosis of spirochaetosis (Hampson, 2013; LeRoy *et al.,* 2015).

Coccidiosis

Eimeria sp, one of the major protozoan parasite affecting the intestine of the poultry affect nutrient absortion, blood loss, intestinal mucosal damage, dehydration and mortality. Nine species of *Eimeria* have been described in chickens such as *E. acervulina, E. brunette*, *E. maxima*, *E. mitis*, *E. mivati*, *E. necatrix*, *E. praecox*, *E. tenella* and *E. hagani*.

A multiplex PCR was developed for simultaneous detection of seven species of *Eimeria* by Fernandez *et al.* (2003). Lew *et al.* (2003) developed nested PCR for identification of *Eimeria* species. Kumar *et al.* (2014) optimized the protocol for identification of *Eimeria* species. Raman *et al.* (2011) made a lesion scoring technique for assessing the pathogenicity of Indian isolate of *Eimeria* species.

Dysbacteriosis

Dysbacteriosis has been defined as the presence of a qualitatively and/or quantitatively abnormal microbiota in the proximal parts of the small intestine, inducing a cascade of reactions in the gastro-intestinal tract including reduced nutrient digestibility and impaired intestinal barrier function, increasing the risk of bacterial translocation and inflammatory responses (Fabri, 2000; Panneman, 2000). The condition observed mostly in young ages (Pattison, 2002; Wilson *et al.,* 2005) with signs of undigested food particle in the dropping coloured pale orange. The birds may show signs like reduced activity, increased water intake and reduced food intake with or without reduction in weight gain (Fabri, 2000; Wilson *et al.,* 2005; De Gussem, 2007). The major gross lesions reported were intestinal inflammation, thin often translucent wall, orange mucus and undigested feed in the lumen(Pattison, 2002) while the villous atrophy and reduction in thickness of tunica muscularis and increased infiltration of T lymphocytes observed histologically (Teirlynck *et al.,* 2011).

Dysbacteriosis can be seen associated with infectious and non-infectious causes (Mortimer, 2002; De Gussem, 2007). Stressors like dietary changes nutritional imbalances, enzymatic dysfunction, genetic factors and mycotoxin were believed to be the major non-infectious players (Langhout *et al.*,1999; Teirlynck *et al.*, 2009). Bacteria like *Clostridium perfringens* and other toxic metabolite producing bacteria and protozoans like coccidia were attributed for infectious causes of dysbacteriosis (Morrow, 2001; De Gussem, 2007).

Helminthiasis

The major helminths reported from the gut of the chicken are following. In proventriculus- *Tetramerus* sp., in gizzard- *Cheilospirua* sp., in intestine *Ascaridia sp., Capillaria sp., Heterakis sp., Strongyloides sp., Trichostrongylus, sp.* and cestodes *like Davainea sp.,* and *Raillietina sp*. (McDougald, 2013).

Miscellaneous conditions

Tumors

In chickens, proventricular adenomas (Campbell and Appleby, 1966) and proventiricular, gizzardal and intestinal adenocarcinomas (Campbell, 1969; Reece, 1996) have been described. Primary adenocarcinomas affecting the intestinal tract must be differentiated from metastatic adenocarcinomas of the reproductive tract, which readily and rapidly metastasize to involve the intestine (Campbell, 1969).

Leiomyomas and leiomyosarcomas have been described affecting the proventriculus, gizzard and duodinum (Campbell, 1969; Reece, 1996).

Malformation and derangement of gut

Intussusception is telescoping of one segment of into its adjacent segment. Intestinal volvulus is a twisting across the long axis of the gut characterized by compression of the thin-walled veins and obstruction of the influx of arterial blood, which eventually progresses to dilation, devitalization and venous infarction of the affected segment (Brown *et al*., 2007). Intussusception is more commonly seen in intestine. Sharma (1972) reported intussusception

of proventriculus. Coccidiosis, ulcerative enteritis and helminthiasis are considered to be the possible cause for intestinal intussusception and volvulus in chicken (Williams, 1986).

Materials and Methods

3 MATERIALS AND METHODS

3.1 MATERIALS

3.1.1.Sample collection

Samples for the present study include tissues collected from routine post-mortem examination at ICAR-Directorate of Poultry Research, Hyderabad during the period from December 2015 to November 2016. All the chicks were hatched in the institute hatchery unit and reared in the floor or cage. The breeds in the farm and their grouping for the present study are given in table 3.1.

Table 3.1. Details of chicken necropsied from the farms of ICAR-DPR, Hyderabad

Line type	Breed/line name	Chick	Grower	Adult	Total
Layers	White leghorn	405	464	1226	**2095**
Broilers	PB1	517	165	150	**832**
	PB2	312	207	183	**702**
	Krishibro	101	7	0	**108**
	Control Broiler	375	462	195	**1032**
	Crosses	47	42	20	**109**
Synthetic breeds	PD1	885	359	468	**1712**
	PD2	969	751	2160	**3880**
	PD3	1051	1220	1952	**4223**
	GML	1025	480	127	**1632**
Native breeds	Aseel	124	93	124	**341**
	Nicobari	233	135	58	**426**
	Ghagus	277	61	54	**392**
Single gene lines	Naked neck	63	143	51	**257**
	Dwarf	55	58	124	**237**
	Total	**6439**	**4647**	**6892**	**17978**

All standard hygienic measures were followed in the farm with regular vaccination against Marek's Disease, Newcastle disease, Infectious coryza, Fowl pox, Infectious Bursal disease and Infectious bronchitis. Anticoccidial program and starter antibiotic supplementation were practiced in all the flocks.

A total of 17978 chicken carcasses were screened for gut lesions. The tissue sections were collected from proventriculus, gizzard, different parts of intestine along with its contents from chickens having gross lesions in the gut.

Other than the ICAR-DPR farms, a total of 120 chicken carcasses from the commercial farms located in the Hyderabad area were necropsied during the period. Samples collected from chickens died with proventricular lesions from three farms located at Hyderabad. The farms included one broiler breeder farm and two layer farms.

3.1.2 Sample data

The study was carried out for a period of one year from December, 2015 to November, 2016 and the data regarding age, sex, breed/ line, vaccination history, clinical signs, mortality pattern were recorded.

3.1.3. Chick embryo

Chick embryos for virus isolation studies were procured from the hatchery unit, ICAR-Directorate of Poultry Research, Rajendranagar, Hyderabad.

3.1.4. Day old chicks

Commercial day old chicks for experimental reproduction of proventricular thickening was procured from the hatchery unit, ICAR-Directorate of Poultry Research, Rajendranagar, Hyderabad.

3.1.5. Glass wares and plastic wares.

The glass wares used in the present study were procured from Borosil (India). The glasswares were cleaned as per standard protocol and autoclaved after overnight treatment with 0.01% diethylpyrocarbonate (DEPC) treated water to render them DNase and RNase free.

The plastic wares used in the present study were procured from Tarsons (India). Disposable syringes used in this study was from Dispovan. The plastic wares were either certified to be free from DNase and RNase activity or were DEPC treated and autoclaved to render them nuclease free.

3.1.7. Equipment and instruments

Standard equipment and instruments available in the Avian Health Laboratory and Central Instrumentation Facility , ICAR-Directorate of Poultry Research, Hyderabad were utilized for requisite purpose. Specifications of the instrument/equipment were given at appropriate places in the text wherever necessary.

3.1.8. Buffers, media and reagents

Double distilled water was used for preparation of all aqueous solutions and autoclaved at 121^{0}C for 15 minutes. Common reagents like PBS (pH 7.2) were prepared as per standard procedures.

3.1.9 Chemicals, molecular biology reagents, enzymes and kits

All the chemicals, molecular biology reagents, enzymes and kits used in this study were obtained from Merck, Invitrogen, ThermoScientific, Applied Biosystems and Himedia. Wherever necessary, molecular biology grade chemicals and biochemical were used. Specifications of the chemicals, molecular biology reagents, enzymes and kits are given at appropriate places in the text wherever necessary.

3.1.10 Oligonucleotide primers

Oligonucleotide primers used in the present study were obtained commercially synthesized from IDT. The nucleotide sequence and other relevant details are given in Table 3.2 and 3.3.

3.1.11 Software for phylogenetic analysis

'EditSeq' programme of Lasergene version.6 (DNASTAR Inc, USA) was used for editing the raw sequences obtained after sequencing. 'Megalign' programme of Lasergene version 6 was used for aligning and joining the truncated sequences to obtain genome sequences of ALV, ChPV, CAstV, CAV, AvRtV and FAdV and phylogenetic analysis in the present study.

3.2 METHODS

3.2.1 Pathological studies

3.2.1.1 Post mortem examination and recording of gross lesions

Carcasses of varying age and breeds/ lines of chicken were subjected to detailed post mortem examination and major gut lesions were recorded. All the data regarding age, sex, breed/ lines, clinical signs, vaccination status and mortality pattern of flocks were recorded.

The birds were grouped age wise into chick, grower and adult. Birds with 8 weeks and below were grouped under chicks, 9-17 weeks in layers, 9-20 weeks in broiler, single gene and native breeds grouped under growers and above that under adult group.

Proventricular thickening was classified grossly as mild, moderate and severe thickening qualitatively. Proventricular haemorrhage were classified as scattered and diffuse. Scattered haemorrhage were those with haemorrhage seen at a few glandular tips. In diffuse haemorrhage, all the glandular tips or whole of mucosa were affected. Proventricular ulceration was grouped as focal and diffuse where, one or two ulcerations can be seen in focal and whole mucosa was ulcerated in diffuse type. Intestinal haemorrhages were grouped as per the affection of intestinal segments such as duodenum, jejunum, ileum, caecum, colon/rectum. The large intestine of chicken, colon/ rectum will be termed as rectum hereafter. The neoplastic lesions in the intestine were classified as focal and diffuse. Focal lesions were those with tumours affecting a few areas of intestine while diffuse for affections affecting whole length of the intestine.

3.2.1.2 Collection of tissue samples

Representative tissues from proventriculus, gizzard, duodenum, jejunum/ileum, caecum and rectum were collected in 10% buffered neutral formalin. Representative proventriculus

Table 3.2: Gene specific primers used for viruses screening in the proventriculus

Pathogen	Primer name	Primers (5' – 3')	Region	Product Size (bp)	Reference
CPNV	B2F	F- CGTAGACCTCGTCCTTCTGC	VP1 gene	171	Guy *et al.*, 2011
	B2R	R- GGGCGGTAACCATTCAGATA			
Marek's	M1	M1- TAC TTC CTA TAT AGA TTGAGA CGT	132bp	300	Becker *et al.*, 1992
disease	M2	M2- GAG ATC CTC GTAAGG TGTAAT ATA	repeats		
IBV	XCE2+	XCE2+ CAC TGG TTT TTC AGA	S	466	Adzhar *et al.*, 1997
	XCE2-	TGGXCE2-CCT TAA ACA CCC TTG CA			
Avian	H1F	H1F- TGGACATGGGGGCGACCTAH2	Hexon	1219	Raue and Hess, 1998
adenovirus	H2R	R- AAGGG ATTGACGTTGTCCA			
group I					
Chicken	CAVF	CAVF- ACG CTC TCC AAG AAG ATA	ORF3	298	Vilmaz *et al.*, 2001
infectious	CAVR	CTC CAC CC CAVR- TTT AGC TCG			
anaemia		CTT ACC CTG TAC TCG GAG G 3'			
virus					
Avian	NF	5'CATGAGGCTATWGATTGGATTA	N	389	Weissenböck
bornavirus	NR	5'TAGCCNGCCMKTGTWGGRTTYT	gene		*et.al.* (2009)
Avian	H5F	GGA TGA GGT GAC TAA GAA A	Env	544bp	Smith *et al.*, 1998
Leucosis	H7/Br	GCGA ACC AAA GGT AAC ACA		2.4kb	
virus	All F	CGCGA GAG TGG CTC GCG AGA			
	All R	TGGAC A CTA CAT TTC CCC CTC CCT AT			
Reticulo	ENV1	TCG ATT GCG GTA GCT CCA CCCA	Env	642bp	
Endothelial	ENV2	TCG AGA GTG ACA TTG C			
Virus					
New Castle	HN-NDV	TTT TTC TTA ATC AAG TGA	HN	535bp	Peroulis-Kourtis
Disease virus	304HN-NDV314	CTATA TCC CGC AGT CGC ATA AC			*et.al.*, 2002

Table 3.3. Gene specific primers used for viruses screening in the intestine

Pathogen	Primer name	Primers (5' – 3')	Region	Product Size (bp)	Reference
Chicken	PVF1	5'TTCTAATAACGATATCACTCAAGTTTC 3'	NS	561	Zsak*et al*., 2009
parvo virus	PVR1	5'TTT GCGCTTGCG GTGAAGTCTGGC TCG 3'			
Chicken	CASpol1F	CASpol1F-GAYCARCGAATGCGRAGRTTG	ORF	362	Day *et al*., 2007
astrovirus	CASpol1R	CASpol1R- TCAGTGGAAGTGGGKARTCTA			
Rota virus	RD6F	RD6F- GGAGGCGCTGTCTTCAATTG	VP6	742	Bazerra *et al*., 2012
D	RD6R	CGRD6R-TGGCCA ATAGTGTGTGGCAGC			
Rota virus	F30 and	TF30- GTG CGG AAA GAT GGA GAA C	NSP4	630	Day *et al*., 2007
	R660	R660- GTT GGG GTA CCA GGG ATT AA			
Clostridium	CPAF	CPAF-TGCATGAGCTTCAATTAGGTCPA	*C. perfringens*	400	Roussan *et al*., 2012
perfringens	CPAR	R-TTA GTT TTG CAA CCT GCT GT	alpha toxin		
Clostridium	CCF	CCF- GTC GAG CGG AGT TTT ATG GGCC			
colinum	CCR	R- CAT TAC ACA GAT TGT CAT CGG G	*C.colinum*- 16s RNA	936	
Avian nephritis	ANV F	5'-ACG GCG AGT ACC ATC GAG-3'	3'UTR	182	Todd *et al*., 2010
virus	ANV R	5'-AAT GAA AAG CCC ACT TTC GG-3'			
Avian reovirus	S4-F13	5' GTG CGT GTT GGA GTT TCC CG3'	S4	1120	Pantin-Jackwood *et al*. 2008
	S4-R1133	5' TAC GCC ATC CTA GCT GGA 3'			
Eimeriatenella	K04-F	CCG CCC AAA CCA GGT GTC ACGCCG	SCAR	539	Fernandez *et al*., 2003
	K04-R	CCCAAA CAT GCA AGA TGG C			
Eimerianecatrix	A18-F	TTC ATT TCG CTT AAC AAT ATT TGG CCT	SCAR	200	Fernandez *et al*., 2003
	ENec-R	CAACA ACG CCT CAT AAC CCC AAG AAA TTT TG			
Eimeriaacer	A03F	AGT CAG CCA CAC AAT AAT GGC AAA CAT GAGT	SCAR	811	Fernandez *et al*., 2003
vulina	A03R	CAG CCA CAG CGA AAG ACG TAT GTG			
Eimeria maxima	A09F	GGG TAA CGC CAA CTG CCG GGT ATGAGC	SCAR	272	Fernandez *et al*., 2003
	A09R	AAA CCG TAA AGG CCG AAG TCC TAG A			

and intestinal parts with gross lesions were collected on screw capped sterile polypropylene containers using sterile forceps and scissors, transported in ice and stored in -20°C until use. Intestinal contents from various anatomical parts of intestine were collected after scraping the mucosa for identification of coccidia and stored in a vial containing 2.5% potassium dichromate until use.

3.2.1.3 Histopathological examination

Tissues collected for histopathological examination were processed for paraffin embedding, sectioned at 4 micron thickness and routinely stained with haematoxylin and eosin (Bancroft and Gamble, 2008). A few selected proventricular sections were stained with Gomori's one step trichrome staining for deomonstrating connective tissue. Microscopic changes were recorded and correlated with the gross and clinical findings.

Histological lesions of proventricular thickening were scored. For scoring, pointes were given as 0- normal, 1- for mild, 2- moderate or locally extensive and 3- severe or extensive. Scoring were done separately for lesions in mucosa, lamina propria, muscularis mucosa, submucosa, muscularis externa and serosa with conditions like plicae fusion, mononuclear cell infiltration, oedema, thickening by fibroplasia, necrosis, duct cell hyperplasia and sinus dilatation of the submucosal glands. Mean of the scored values were taken after multiplying number of samples with the histological score.

3.2.2. Diagnostic assays

3.2.2.1. Preparation of sample for PCR and virus isolation studies

3.2.2.1.1. Preparation of proventriculus samples

Proventriculi were washed twice in sterile PBS for removing feed materials. Samples of thickened proventriculus from same flock were pooled together for homogenisation. The proventriculi were homogenised in the sterile porcelain mortar and pestle with sterile PBS (1:10 w/v) and sand. A portion of the homogenate was kept at -20^{0}C. Rest of the homogenate was clarified by centrifuging (Remi R-24)at 3000 rpm for 10 minutes, supernatant was collected and kept at -20^{0}C till use.

3.2.2.1.2.Preparation of intestinal samples

Intestinal contents were diluted 10 % w/v ratio with sterile PBS (pH: 7.2- 7.4) and homogenised in sterile porcelain mortar and pestle. Clarified the homogenate by centrifuging at 3000 rpm for 10 minutes, supernatant collected and kept at -20^{0}C till used for DNA and RNA isolation.

3.2.2.2. Polymerase chain reaction (PCR)/Reverse transcription-polymerase chain reaction (RT-PCR) amplification and sequencing of viral genome

3.2.2.2.1. Total RNA extraction

Commercial TRIzolLS® Reagent (Life technologies) was used for RNA isolation. 250µl of clarified tissue homogenate was taken in 2ml micro centrifuge tube (MCT) in to which750 ml of cold TRIzol LS® Reagent added, mixed and incubated at room temperature for 5 minutes to permit complete dissociation of the nucleoprotein complex. After incubation 200µl of chilled chloroform was added, mixed vigorously with hand for 15 seconds and incubated for 3 minutes at room temperature. Centrifuged (eppendorf centrifuge 5417R) the sample for 5 minutes at 12000x g at -4^{0}C, collected and transferred the upper aqueous phase into a 1.5 ml MCT. Added 500µl of chilled isopropylalcohol to the supernatant collected and incubated for 10 minutes at room temperature. After incubation, RNA was pelleted by centrifuging at12000xg at -4^{0}C for 10 minutes. Supernatant discarded and the pellet was washed in 1000µl of 0.75% ethanol by briefly vortexing and centrifuging at 7500xg at -4^{0}C for 5 minutes. Discarded the supernatant and allowed to dry the pellet at room temperature for 10 minutes. Partially dried pellet was suspended in 10µl of nuclease free water, incubated in water bath for 10 minutes at 60^{0}C and kept at -70^{0}C until further use.

3.2.2.2.2. First strand complementary DNA (cDNA) synthesis (Reverse Transcription)

The cDNA was prepared usingHigh Capacity cDNA Reverse Transcription Kit (Applied Biosystems) as per the manufacturers instruction. Briefly 10µl2x RT master mix was prepared in ice by adding the following components- 10' RT Buffer 2µl, 25' dNTP Mix (100 mM) -0.8µl, 10' RT Random Primers- 2 µl, MultiScribe™ Reverse Transcriptase- 1µl, RNase Inhibitor- 1µl and Nuclease-free water- 3µl. Prepared 2x RT master mix were mixed

with 10µl sample RNA in 0.2ml MCT kept on ice. The prepared 20µl mix was kept in the thermocycler and run on the thermal conditions of 25^{0}C for 10 minutes, 37^{0}C for 120 minutes and 85^{0}C for 5 minutes. The synthesized cDNA was stored at -20^{0}Ctill further use.

3.2.2.2.3. DNA extraction

3.2.2.2.3.1. Tissue lysis

To about 250µl of tissue homogenate in 2ml MCT, 600µl of lysis buffer and 10µl proteinase K(10mg/ ml) were added,mixed by vortex at high speed and incubated at 37^{0}C for overnight.

3.2.2.2.3.2. DNA extraction

DNA from lysed samples was isolated using phenol-chloroform method. Briefly, lysed sample was mixed with equal volume of phenol-chloroform-isoamyl alcohol (25:24:1 v/v), by vortex mixing for 10 sec, and centrifuged at 14000 rpm for 5 minutes at room temperature. The aqueous phase was collected by pipetting and placed in 1.5ml MCT. The DNA was precipitated in 1volume of 100% isopropyl alcohol at room temperature for 15 minutes. The precipitated mixture was centrifuged at 14000 rpm for 15 minutes at room temperature. The DNA pellet was washed in 70% isopropyl alcohol, dried at 37^{0}C, suspended in nuclease free water and kept at -20^{o}C.

3.2.2.2.3.3. DNA extraction from *Eimeria sp.*

The oocyst separation and DNA isolation of *Eimeria* sp. was done as per the procedure adopted by Moraes *et al*. (2015) with minor variations. Intestinal samples collected in the 2.5% potassium dichromate solution mixed thoroughly using sterile mortar and pestle, filtered through the nylone mesh and the suspension was centrifuged for 5 minutes at 1000 x g. The supernatant was discarded and the pellet was suspended in double distilled water and homogenized. The remains of potassium dichromate were removed by repeating the procedure until getting clear supernatant. The pellet was resuspended in saturated sodium chloride solution and centrifuged at 200xg for 10 minutes. Oocysts from the top layer were collected using a Pasteur pipette in to a 15ml centrifuge tube and washed with double distilled water. The pellet was homogenised by inversion with 20% Sodium Dodecyl Sulfate (SDS). The sample then centrifuged at 2500xg for 10 minutes and pellet suspended in nuclease free water. This again pelletized and suspended

in 500 µl of lysis buffer (10 mMTris- HCl pH 8.0; 50 m Methylene diamine tetraacetic acid pH 8.0; and 200 mMNaCl) and 0.5 g of glass beads 300-500 micrometer in diameter added, vortexed at maximum speed for 10 minutes and incubated for 30 minutes at 37°C. Then the homogenate was centrifuged at 1000xg for 5 minutes and the supernatant collected in 1.5 ml MCT. Added 2.5µl of proteinase K (20 microgram / microliter) and 12.5 µl of 20% SDS solution and incubated at 50°C for 30 minutes.

3.2.2.2.3.4. DNA extraction from *Clostridium* species

Deep swab was taken from the intestinal lesion areas and cultured in the cooked meat broth (Himedia) and incubated in anaerobic jar at 37°C for 3 days. After incubation the broth kept at -70°C till use. About 500µl of cultured broth was taken in screw capped 5ml glass tube and boiled for 5 minutes. The boiled solution was used as DNA template for the bacteria.

3.2.2.2.4. Polymerase chain reaction amplification

Polymerase chain reaction (PCR) conditions for various genomic regions were performed in a thermal cycler (Esco/eppendorf). The reaction mix and thermal cycling conditions were as described below:

Chicken Proventricular Necrosis Virus

B2F and B2R (Guy *et al.*, 2011; Expected product size-171bp) PCR reaction set up (Standard Taq DNA Polymerase, Thermo Scientific)

PCR reaction contents	Quantity
10X Standard Taq Reaction Buffer	2.5 µl
10 mMdNTP	0.5 µl
B2F (20pM)	0.5µl
B2R (20pM)	0.5µl
Taq DNA Polymerase (5U/ µl)	0.2µl
Template DNA	2.0 µl
Nuclease-free water	18.8µl

THERMAL PROFILE

Initial Denaturation	95^{0}C, 5 min.
Denaturation	95^{0}C, 1 min
Annealing	55^{0}C, 1 min
Extension	72^{0}C, 1min.
Cycles	30
Final Extension	72^{0}C, 5 min.

Marek's Disease

M1 and M2 (Becker *et al.*, 1992; Expected product size-300bp) PCR reaction set up (Standard Taq DNA Polymerase, Thermo Scientific)

PCR reaction contents	**Quantity**
10X Standard Taq Reaction Buffer	2.5µl
10 mMdNTP	0.5µl
M1(20 pM)	0.5µl
M2 (20 pM)	0.5µl
Taq DNA Polymerase (5U/ µl)	0.2µl
Template DNA	2.0µl
Nuclease-free water	18.8µl

THERMAL PROFILE

Initial Denaturation	94^{0}C, 4 min.
Denaturation	94^{0}C, 1 min
Annealing	55^{0}C, 1 min
Extension	72^{0}C, 1min.
Cycles	35
Final Extension	72^{0}C, 10 min.

Avian Leukosis virus

All F and all R Expected product size-2.4kb PCR reaction set up (Standard Taq DNA Polymerase, Thermo Scientific)

PCR reaction contents	Quantity
10X Standard Taq Reaction Buffer	2.5 µl
10 mMdNTP	1.0µl
ALL F (20 pM)	1.0µl
ALL R (20 pM)	1.0µl
Taq DNA Polymerase (5U/ µl)	0.5µl
Template DNA	2.0 µl
Nuclease-free water	17.0µl

THERMAL PROFILE

Initial Denaturation	95^{0}C, 3 min.
Denaturation	95^{0}C, 1 min
Annealing	57^{0}C, 1 min
Extension	72^{0}C, 2 min.
Cycles	30
Final extension	72^{0}C, 5 min.

Avian Leukosis virus subgroup J

H5f and H7bR (Smith *et al*., 1998); Expected product size- 544 kb) PCR reaction set up (Standard Taq DNA Polymerase, Thermo Scientific)

PCR reaction contents	Quantity
10X Standard Taq Reaction Buffer	2.5 µl
10 mMdNTP	0.5 µl
B2F (20 pM)	0.5 µl
B2R (20 pM)	0.5 µl
Taq DNA Polymerase (5U/ µl)	0.2 µl
Template DNA	2.0 µl
Nuclease-free water	18.8 µl

THERMAL PROFILE

Initial Denaturation	94^0C, 4 min.
Denaturation	94^0C, 1 min
Annealing	55^0C, 1 min
Extension	72^0C, 1 min.
Cycles	30
Final extension	72^0C, 10 min.

Avian Rotavirus

F30 and R660 (Day *et al*., 2007; Expected product size- 630 bp) PCR reaction set up (Standard Taq DNA Polymerase, Thermo Scientific)

PCR reaction contents	**Quantity**
10X Standard Taq Reaction Buffer	2.5 µl
10 mMdNTP	0.5 µl
PVF1 (20pM)	0.5 µl
PVR1 (20pM)	0.5 µl
Taq DNA Polymerase (5U/ µl)	0.2 µl
Template DNA	2.0 µl
Nuclease-free water	18.8 µl

THERMAL PROFILE

Initial denaturation	94^0C, 2 min.
Denaturation	94^0C, 30 sec
Annealing	52^0C, 30 sec
Extension	72^0C, 80 sec.
Cycles	40
Final Extension	72^0C, 5 min.

RD6F and RD6R (Bazerra *et al*., 2012; Expected product size- 742 bp) PCR reaction set up (Standard Taq DNA Polymerase, Thermo Scientific)

PCR reaction contents	Quantity
10X Standard Taq Reaction Buffer	2.5 µl
10 mMdNTP	0.5 µl
RD6F (20pM)	0.5 µl
RD6R (20pM)	0.5 µl
Taq DNA Polymerase (5U/ µl)	0.2 µl
Template cDNA	2.0 µl
Nuclease-free water	18.8 µl

THERMAL PROFILE

Initial Denaturation	93^0C, 3 min.
Denaturation	93^0C, 1 min
Annealing	55^0C, 1 min
Extension	72^0C, 1min.
Cycles	35
Final Extension	68^0C, 7 min.

Fowl Adeno Virus1

H1 and H2 (Raue and Hess, 1998 ; Expected product size-1219 bp) PCR reaction set up (Standard Taq DNA Polymerase, Thermo Scientific)

PCR reaction contents	Quantity
10X Standard Taq Reaction Buffer	2.5 µl
10 mMdNTP	0.5 µl
H1 (20pM)	0.5 µl
H2 (20pM)	0.5 µl
Taq DNA Polymerase (5U/ µl)	0.25µl
Template DNA	2.0 µl
Nuclease-free water	18.75µl

THERMAL PROFILE

Initial Denaturation	95^{0}C, 5 min.
Denaturation	95^{0}C, 45 sec
Annealing	55^{0}C, 45 sec
Extension	72^{0}C, 1.5min.
Cycles	35
Final extension	72^{0}C, 10 min.

Chicken parvovirus

PVF1 and PVR1 (Zsak *et al.*, 2009; Expected product size- 561 bp) PCR reaction set up (Standard Taq DNA Polymerase, Thermo Scientific)

PCR reaction contents	**Quantity**
10X Standard Taq Reaction Buffer	2.5 µl
10 mMdNTP	0.5 µl
PVF1 (20pM)	0.5 µl
PVR1 (20pM)	0.5 µl
Taq DNA Polymerase (5U/ µl)	0.2 µl
Template DNA	2.0 µl
Nuclease-free water	18.8 µl

THERMAL PROFILE

Initial Denaturation	94^{0}C, 3 min.
Denaturation	94^{0}C, 30 sec
Annealing	55^{0}C, 30 sec
Extension	72^{0}C, 1min.
Cycles	30
Final Extension	72^{0}C, 5 min.

Chicken Astrovirus

CASpolIF and CASpolIR (Day *et al.,* 2007; Expected product size- 362bp) PCR reaction set up (Standard Taq DNA Polymerase, Thermo Scientific)

PCR reaction contents	Quantity
10X Standard Taq Reaction Buffer	2.5 µl
10 mMdNTP	0.5 µl
CASpol1F (20pM)	0.5 µl
CASpol1R (20pM)	0.5 µl
Taq DNA Polymerase (5U/ µl)	0.2 µl
Template DNA	2.0 µl
Nuclease-free water	18.8 µl

THERMAL PROFILE

Initial Denaturation	94^0C, 15 min.
Denaturation	94^0C, 30 sec
Annealing	55^0C, 30 sec
Extension	72^0C, 1min.
Cycles	35
Final Extension	72^0C, 5 min.

Avian nephritisvirus

ANV F and ANV R (Todd *et al.,* 2010; Expected product size- 182bp) PCR reaction set up (Standard Taq DNA Polymerase, Thermo Scientific)

PCR reaction contents	Quantity
10X Standard Taq Reaction Buffer	2.5 µl
10 mMdNTP	0.5 µl
ANVF (20pM)	0.5 µl
ANVR (20pM)	0.5 µl
Taq DNA Polymerase (5U/ µl)	0.2 µl
Template DNA	2.0 µl
Nuclease-free water	18.8 µl

THERMAL PROFILE

Initial Denaturation	94^{0}C, 2min.
Denaturation	94^{0}C, 30 sec
Annealing	57^{0}C, 30 sec
Extension	68^{0}C, 1min.
Cycles	30
Final Extension	68^{0}C, 7 min.

Infectious Bronchitis Virus

XCE 2+ and XCE 2- (Adzhar *et al.*, 1997; Expected product size- 466bp) PCR reaction set up (Standard Taq DNA Polymerase, Thermo Scientific)

PCR reaction contents	**Quantity**
10X Standard Taq Reaction Buffer	2.5 µl
10 mMdNTP	0.5 µl
XCE2+ (20pM)	0.5 µl
XCE 2- (20pM)	0.5 µl
Taq DNA Polymerase (5U/ µl)	0.2 µl
Template DNA	2.0 µl
Nuclease-free water	18.8 µl

THERMAL PROFILE

Initial Denaturation	94^{0}C, 5 min.
Denaturation	94^{0}C, 40 sec
Annealing	56^{0}C, 40 sec
Extension	72^{0}C, 50 sec.
Cycles	40
Final extension	72^{0}C 7 minutes

Eimeria sp

K04-F and K04-R; A18-F and ENec-R; A03F and A03R; A09F and A09R (Fernandez *et al.,* 2003; Expected product size- 539bp, 200bp, 811bp, 272bp) PCR reaction set up (Standard Taq DNA Polymerase, Thermo Scientific)

PCR reaction contents	Quantity
10X Standard Taq Reaction Buffer	2.5 µl
10 mMdNTP	0.5 µl
F (20pM)	0.6µl
R (20pM)	0.6µl
Taq DNA Polymerase (5U/ µl)	0.25µl
Template DNA	3.0 µl
Nuclease-free water	17.55µl

THERMAL PROFILE

Initial Denaturation	96^0C, 5 min.
Denaturation	94^0C, 1 min
Annealing	65^0C, 1 min
Extension	72^0C, 1 min
Cycles	35
Final extension	72^0C 10 minutes

Clostridium perfringens and Colstridium colinum

CCF&CCR; CPAF&CPAR- (Roussan *et al.,* 2012 ; Expected product size- 936 and 400 bp) PCR reaction set up (Standard Taq DNA Polymerase, Thermo Scientific)

PCR reaction contents	Quantity
10X Standard Taq Reaction Buffer	2.5 µl
10 mMdNTP	0.5 µl
XCE2+ (20pM)	0.5 µl
XCE 2- (20pM)	0.5 µl
Taq DNA Polymerase (5U/ µl)	0.25 µl
Template DNA	3.0 µl
Nuclease-free water	17.75 µl

THERMAL PROFILE

Initial Denaturation	94^0C, 4 min.
Denaturation	94^0C, 1 min
Annealing	56^0C, 1 min
Extension	72^0C, 1 min
Cycles	25
Final extension	72^0C 4 min

3.2.2.2.5 Analysis of PCR/Rt-PCR amplicons

The PCR/RT-PCR amplicons (15µl) were electrophoresed along with 100 bp plus molecular weight marker (Thermo Scientific) on a 1.5% agarose gel in 1XTris borate EDTA (TBE) buffer (90 mM Tris borate, 2 mM DTA, pH- 8.0) containing 0.5 ug/ml ethidium bromide for 50 min at 120 V. Following electrophoresis, the gel was visualized under UV light and photographed in a gel documentation system (GelDoc- UVITEC, Cambridge).

3.2.2.2.6. Sequencing of PCR/ RT-PCR amplicons

The PCR amplicon was subjected to DNA sequencing using respective forward and reverse primers used for PCR amplification at DNA sequencing facility of Regene, Hyderabad. The sequence data generated was received as colored electropherograms and text files.

3.2.3. Isolation and identification of viruses.

Isolation of the virus was attempted by chick embryo inoculation through appropriate routes.

3.2.3.1. Preparation of samples for inoculation

Clarified proventriculus was treated with antibiotic at 10% rate (1000 IU penicllin-1000µg streptomycin per ml of clarified sample) and incubated at 4^0C overnight.

3.2.3.2.Chicken Proventricular Necrosis Virus (CPNV)

Fifteen day old embryonated eggs were inoculated with the clarified and antibiotic treated tissue homogenate @0.1ml/ egg and incubated at 37°C till hatch. Proventriculus were collected on second day post hatch, homogenised and repeated the passages. From the third passage onwards RNA were isolated from proventricular sample and subjected for RT-PCR specific for CPNV.

In cases of higher occurrence of proventricular thickening in the same flock attempt was made for isolation of other possible viruses.

3.2.3.3. Infectious bronchitis virus (IBV), Avian Nephritis Virus (ANV) and haemagglutinating virus isolation

Inoculums prepared as mentioned above were inoculated through intrallantoic route in 9 day old chick embryos and harvested allantoic fliud from the embryos died after 24 hours post inoculation and from other live chicks after 72 hours and repeat the passages. RNA isolated from the allantoic fluid and screened for the IBV, NDV and ANV by Rt-PCR using virus species specific primers.Haemagglutinating viruses were screened by performing haemagglutination (HA) test of the allantoic fluid.

3.2.3.3.1. Haemagglutination test

The HA test was performed according to the standard procedure (OIE Terrestrial Manual 2009). Briefly, 0.025 ml of PBS was dispensed into each well of a plastic 96 well V bottomed microtitre plate and 0.025 ml of the allantoic fluid was added to the first well and

two fold dilutions of the allantoic fluid was made across the plate. Then 0.025 ml of 1% chicken RBCs was dispensed to each well. The solution was mixed by tapping the plate gently and the RBCs were allowed to settle for about 40 minutes at room temperature. HA was determined by tilting the plate and observing the presence or absence of tear-shaped streaming of RBCs. The end point titration was considered to the highest dilution giving complete HA (no streaming).

3.2.3.4. Infectious Bursal Disease Virus (IBDV)

The inoculum prepared as mentioned above were inoculated through Chorio- Allantoic Membrane in11 day old chicken embryo (Hitchner, 1970) and done 3 passages for checking IBDV specific lesions.

3.2.3.5 Chicken Parvo virus (ChPV) isolation

The 200 µl intestinal samples found positive only for ChPVby PCR method were inoculated to 7day old chicken embryo through intra yolk route. Negative controls were inoculated with 200 µl of sterile PBS (pH 7.2). Embryos died within 24 days were discarded. The inoculated eggs were checked for embryo mortality two time daily. Embryos were harvested on 7^{th}, 9^{th} and 13^{th} day of post inoculation, chilled and screened for embryonic changes.Embryo visceral organs were collected, homogenated after diluting with PBS (1:1 ratio) and supernatant collected after centrifuging at 6000 rpm for 15 minutes were treated with antibiotic and utilized for subsequent passage. Part of embryonic visceral homogenate collected were utilized DNA and RNA extraction and PCR/ Rt-PCR screening for viruses using species specific primers.

3.2.3.6 Experimental inoculation in day old chick

Clarified and antibiotic treated proventriculus homogenate, prepared from thickned and proventriculi of PD3 chicks were administered oro-nasally to day old chicks. 1ml of the inoculum was administered to 10 chicks and reared for 7 weeks. Body weights were taken on weekly basis. Two birds each were sacrificed from 3rd week onwards up to 7 weeks and checked for proventricular thickening and compared with controls.

3.2.3.7. Molecular detection of pathogens in proventriculus and intestine

Molecular detection of Chicken Proventricular Necrosis Virus, Marek's disease, Avian leukosis virus, Infectious bronchitis virus, adenovirus group 1, Chicken parvovirus, Chicken infectious anaemia virus, Avian nephritis virus were carried out from the proventricular samples showing lesions of proventriculitis/proventricular dilatation/mucosal ulceration by PCR technique using gene specific primers. In case of proventricular dilatation with thinning of the wall, detection of borna virus were attempted by Rt-PCR using specific primers. Tissues from brain also utilized for the same.Selected intestinal samples collected with intestinal lesions were screened for avian Fowl adenovirus1 (FAdV1), Avian nephritisvirus (ANV), Chicken astrovirus (ChAstV), chicken parvovirus (ChPV), Avian rotavirus (AvRtV) and Avian reovirus (AvRV). Intestinal samples with suspected neoplastic lesions were screened for Marek's Disease Virus (MDV), Avian Leucosis virus (ALV) and Reticulo endothelial Virus (REV). Intestinal samples with gross lesions of necrotic enteritis were screened for Clostridium species by PCR using species specific primers for *Clostridium perfringens* and *Clostridium colinum*. A few of the coccidial outbreak samples were randomly screened for *Eimeria tenella, E necatrix* , *E maxima* and *E acervulina* by PCR technique using species specific primers.

Genomic RNA and DNA were extracted from the collected samples of proventriculus and intestinal contents following the standard protocol. RNA extraction was carried out byTrizol method. The cDNA was synthesised from RNA samples using commercial kit (High-Capacity cDNA Reverse Transcription Kits, Applied Biosystems). DNA was extracted the samples using phenol-chloroform method. The extracted DNA and cDNA were stored at -20°C till further use. The cDNA and DNA samples were screened for presence of suspected etiological agent by polymerised chain reaction (PCR) using specific primers. The PCR products were analysed by agarose gel electrophoresis.

3.2.3.8. Molecular characterization

PCR amplicons of variable gene segments of viral pathogens detected were sequenced commercially. The gene segments were aligned by using sequence analysis software like DNA star. The sequence data were compared with the published database and the results interpreted accordingly for molecular characterisation of the pathogens.

3.2.3.9. Statistical analysis

The frequency of gut pathogenic conditions with respect to age and breed were calculated by dividing condition identified with the number of birds necropsied of respective age group or breed/line and multiplied with hundred. The risk factor was assessed by Pearson's Chi-square test and the association considered significant if computed p-value is less than 0.05.

Results

4 RESULTS

4.1 OVERALL OCCURRENCE OF GUT LESIONS

A total of 17,978 dead birds from the ICAR-DPR Hyderabad farms and 120 birds from commercial farms, were necropsied for gut lesions. The chickens necropsied from ICAR-DPR were belonging to 307 different flocks of 15 different breeds and multiple age groups. The breeds/lines of chicken were grouped into 5 categories such as layer, broiler, synthetic, single gene and native breeds. The layer lines include White leghorns of lines IWA, IWD, IWF, IWH, IWI and IWK. Broiler breeds include Punjab Broiler 1 and 2, control broiler, crosses and Krishibro. Synthetic lines include PD1, PD2, PD3 and Gramapriya Male line (GML). The single gene breeds are Naked Neck and Dwarf while the native breeds include Aseel, Ghagus and Nicobari. Total occurrence of gut lesions were 9.22% (1658/17978) (Table 4.1). The details of total number of birds examined in different breeds from ICAR-DPR and overall occurrence of proventricular and intestinal lesions among different breeds are presented in Table 4.2a. A total of 329 (1.83%) birds were exhibited proventricular lesions with highest (4.99%) occurrence in Aseel and the lowest (0.42%) occurrence in Dwarf line. While, 1405 (7.82%) out of 17,978 birds examined were showed intestinal lesions with highest (15.96%) occurrence in Nicobari and lowest (5.17%) in PB1 breed.

The overall age-wise occurrence of gut lesions is presented in Table 4.2b. The occurrence of proventricular lesions was 2.30% (148/6439), 2.26% (105/4647) and 1.10% (76/6892) in chicks, grower and adult, respectively. The intestinal lesions were observed in 12.44% (801/6439), 5.85% (272/4647) and 4.82% (332/6892) in chicks, grower and adult

respectively. In 120 commercial birds examined, proventricular lesions were observed in 17 out of 80 layers and 8 out of 40 broiler breeders.

4.2 PROVENTRICULUS LESIONS

Proventricular lesions were grossly divided into 5 major types such as proventricular thickening, proventricular dilatation, proventricular haemorrhage, proventricular ulcers and miscellaneous. The occurrence of different proventricular lesions among different breeds is depicted in Table 4.3. The occurrence of proventricular thickening, proventricular dilatation, proventricular haemorrhage, proventricular ulcers and miscellaneous proventricular lesions was 0.94% (168/17,978), 1.07% (191/17,978), 0.09% (16/17978), 0.07% (13/17,978) and 0.06% (10/17,978) respectively.

The occurrence of various proventricular lesions in different age groups is presented in Table 4.4. The occurrence of proventricular haemorrhage was higher in most age groups as compared to those of other proventricular conditions. In a few birds, more than one proventricular lesion was observed as listed in the Table 4.5. A total of 44 chicks and 13 growers had both proventricular thickening and haemorrhage and 1 grower and 8 adults exhibited proventricular ulceration along with the proventricular thickening. Occurrence of proventricular haemorrhage alone was higher (40.43%) than the other single or combined lesions. Haemorrhage alone (55.26%) was the major proventricular lesion in adults.

4.2.1 Proventricular thickening

4.2.1.1 Occurrence of proventricular thickening

The proventricular thickening was observed in 168 (0.94%) out of 17,978 birds necropsied from ICAR-DPR farms (Table 4.4) and in 17 chicks from commercial farms. On bird basis, the breed wise occurrence of proventricular thickening was highest in Aseel (2.05%) and zero in Dwarf and crosses (Table 4.4). In breed and age wise occurrence, highest occurrence was noticed in PD3 chicks (3.24%, 34/1051), Ghagus grower (8.20%, 5/81) and adult Naked neck (1.96%, 1/51) in the concerned age group (Table 4.6). On month wise calculation of occurrence from December 2015 to November 2016, highest occurrence was observed on the month July (3.17%) followed by November (3.14%) and December (2.15%) (Fig.4.1).

Table 4.1 Total lesions of gut (proventriculus and intestine)

Total birds	Birds with gut lesions	Both proventricular & intestinal lesions	Proventricular lesion only	Intestinal lesions only
17978	1658(9.22%)	76	253	1329

Table 4.2a. The overall breed wise occurrence of gut lesions (proventriculus and intestine)

Line type	Line name	Birds necropsied	Carcasses with proventricular lesions	Percentage	No. of carcasses with intestinal lesions	Percentage
Layers	White leghorn	2095	35	1.67	193.00	9.21
Broilers	PB1	832	15	1.80	43.00	5.17
	PB2	702	9	1.28	75.00	10.68
	Krishibro	108	2	1.85	11.00	10.19
	Control broiler	1032	17	1.65	71.00	6.88
	Crosses	109	1	0.92	6.00	5.50
Synthetic breeds	PD1	1712	19	1.98	137.00	8.00
	PD2	3880	77	2.23	279.00	7.19
	PD3	4223	94	2.32	246.00	5.83
	GML	1632	9	0.55	129.00	7.90
Native breeds	Aseel	341	17	4.99	47.00	13.78
	Nicobari	426	15	3.52	68.00	15.96
	Ghagus	392	13	3.32	43.00	10.97
Single gene lines	Naked Neck	257	5	1.95	38.00	14.79
	Dwarf	237	1	0.42	19.00	8.02
Total		**17978**	**329**	1.83	1405.00	7.82

Table 4.2b. Overall age wise occurrence of proventricular and intestinal lesions

Age group	Chick	Grower	Adult	Total
Proventricular lesion	148 (2.30)	105 (2.26)	76 (1.10)	329
Intestinal lesion	801 (12.44)	272 (5.85)	332 (4.82)	1405 (7.82)
Total necropsied	**6439**	**4647**	**6892**	**17978**

(Figures in the parenthesis are percentage)

Table 4.3. Occurrence of different types of proventricular lesions among different breeds/lines of chicken

Type of chicken	Breed/Line name	Total Birds necropsied	Proventricular thickening	Mucosal haemorrhage	Mucosal ulcer	Proventricular dilatation	Miscellaneous
Layers	White leghorn	2095	16(0.76)	22(1.05)	3(0.14)	0(0.00)	2(0.10)
Broilers	PB1	832	9(1.08)	6(0.72)	1(0.12)	3(0.36)	0(0.00)
	PB2	702	4(0.57)	2(0.28)	2(0.28)	1(0.14)	0(0.00)
	Krishibro	108	2(1.85)	1(0.93)	0(0.00)	0(0.00)	0(0.00)
	Control broiler	1032	6(0.58)	10(0.97)	2(0.19)	1(0.10)	0(0.00)
	Crosses	109	0(0.00)	0(0.00)	1(0.92)	0(0.00)	0(0.00)
Synthetic	PD1	1712	7(0.41)	13(0.76)	0(0.00)	1(0.06)	0(0.00)
breeds	PD2	3880	34(0.88)	52(1.34)	1(0.03)	1(0.03)	2(0.05)
	PD3	4223	64(1.52)	52(1.23)	2(0.05)	2(0.05)	4(0.09)
	GML	1632	1(0.06)	8(0.49)	1(0.06)	0(0.00)	(0.00)
Native	Aseel	341	7(2.05)	5(1.47)	2(0.59)	3(0.88)	1(0.29)
breeds	Nicobari	426	8(1.88)	12(2.82)	0(0.00)	0(0.00)	1(0.23)
	Ghagus	392	9(2.30)	5(1.28)	0(0.00)	0(0.00)	(0.00)
Single gene	Naked Neck	257	1(0.39)	2(0.78)	1(0.39)	1(0.39)	(0.00)
lines	Dwarf	237	0(0.00)	1(0.42)	0(0.00)	0(0.00)	0(0.00)
Total		**17929**	**168 (0.94)**	**191(1.07)**	**16(0.09)**	**13(0.07)**	**10(0.06)**

Table 4.4. Age wise occurrence of proventricular lesions

Age(total necropsied)	Proventricular thickening	Proventricular dilatation	Proventricular haemorrhage	Proventricular ulcers	Miscellaneous
Chick (n=6439)	85(1.32)	6(0.09)	97(1.51)	1(0.02)	3(0.05)
Grower (n=4647)	62(1.33)	4(0.09)	52(1.12)	1(0.02)	2(0.04%)
Adult (n=6892)	21(0.30)	3(0.04)	42(0.61)	14(0.20)	5(0.07%)
Total	168(0.94%)	13(0.07%)	191(1.07%)	16(0.09%)	10(0.06%)

(Figures in the parenthesis are percentage)

Table 4.5. Concomitant occurrence of proventricular lesions

Proventricular lesions	Chick (N=148)	Grower (N=105)	Adult (N=76)	Total (N=329)
Proventricular thickening alone	41 (27.7)	47 (44.76)	12 (15.79)	100 (30.39)
Proventricular thickening+ haemorrhage	44 (29.7)	13 (12.38)	0	57 (17.33)
Proventricular thickening+ ulcer	0	1 (0.95)	8 (10.52)	9 (2.74)
Proventricular thickening+ miscellaneous	0	1 (0.95)	1 (1.32)	2 (0.61)
Proventicular haemorrhage alone	53 (35.8)	38 (36.19)	42 (55.26)	133 (40.43)
Proventicular haemorrhage + miscellaneous	0	1 (0.95)	0	1 (0.30)
Proventricular ulcer alone	1 (0.67)	0	6 (7.89)	7 (2.13)
Proventricular dilatation	6 (4.05)	4 (3.81)	3 (3.95)	13 (3.95)
Miscellaneous	3 (2.02)	0	4 (5.26)	7 (2.13)
Total	148	105	76	329

(Figures in the parenthesis are percentage)

Table 4.6 Breed and age wise occurrence of proventricular thickening

Breed	Total birds examined				Proventricular thickening			
	Chick	Grower	Adult	Total	Chick	Grower	Adult	Total
White leghorn	405	464	1226	**2095**	7(1.73)	4(0.86)	5(0.41)	16(0.76)
PB1	517	165	150	**832**	8(1.55)	1(0.61)	0(0.00)	9(1.08)
PB2	312	207	183	**702**	1(0.32)	2(0.97)	1(0.55)	4(0.57)
Krishibro	101	7	0	**108**	2(1.98)	0	0	2(1.85)
Control Broiler	375	462	195	**1032**	4(1.07)	0	2(1.03)	6(0.58)
Crosses	47	42	20	**109**	0	0	0	0
PD1	885	359	468	**1712**	2(0.23)	4(1.11)	1(0.21)	7(0.41)
PD2	969	751	2160	**3880**	18(1.86)	13(1.73)	3(0.14)	34(0.88)
PD3	1051	1220	1952	**4223**	34(3.24)	25(2.05)	5(0.26)	64(1.52)
GML	1025	480	127	**1632**	0(0.00)	0(0.00)	1(0.79)	1(0.06)
Aseel	124	93	124	**341**	0(0.00)	6(6.45)	1(0.81)	7(2.05)
Nicobari	233	135	58	**426**	5(2.15)	2(1.48)	1(1.72)	8(1.88)
Ghagus	277	61	54	**392**	4(1.44)	5(8.20)	0(0.00)	9(2.30)
Naked neck	63	143	51	**257**	0	0	1(1.96)	1(0.39)
Dwarf	55	58	124	**237**	0	0	0	0
total	**6439**	**4647**	**6892**	**17978**	85(1.32)	62(1.33)	21(0.30)	168(0.93)

(Figures in the parenthesis indicate percentage)

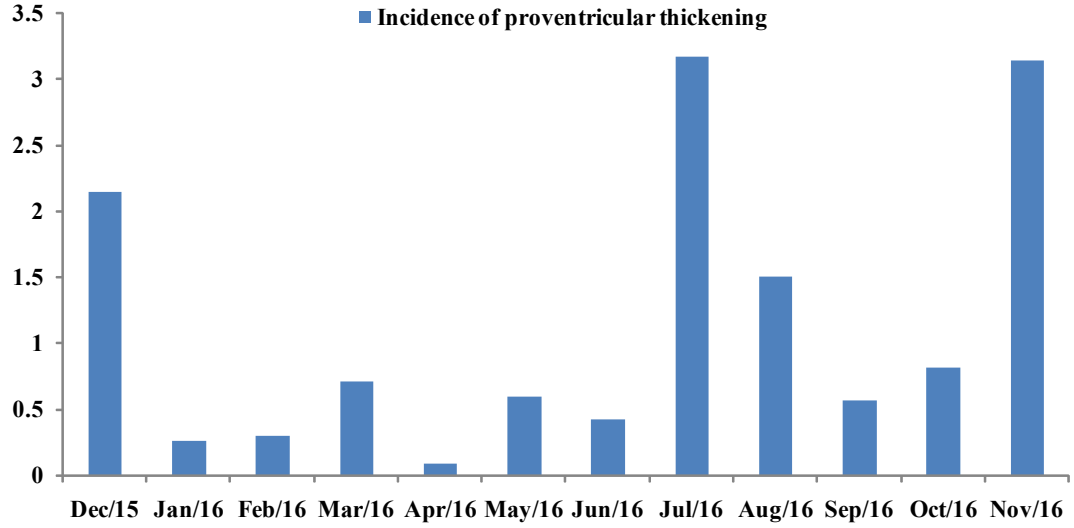


Fig. 4.1: Month wise occurrence of proventricular thickening

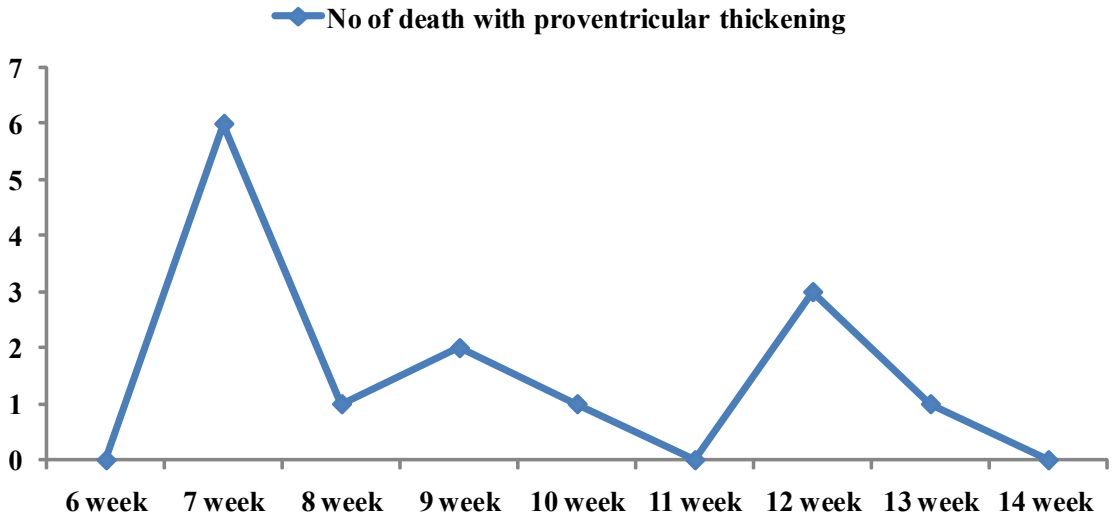


Fig. 4.2: Age wise occurrence of proventricular thickening cases in a PD2 flock

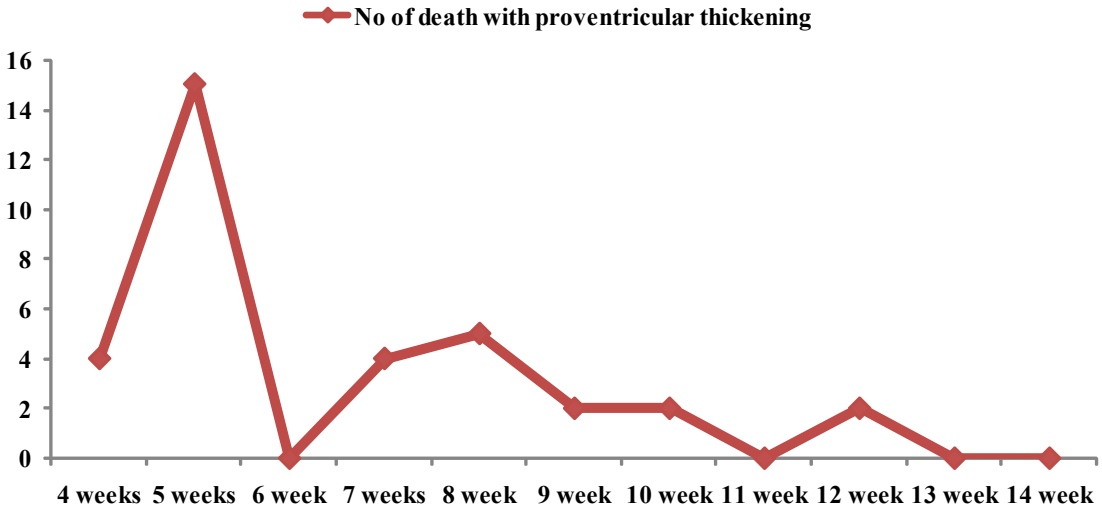


Fig. 4.3: Age in week wise occurrence of proventricular thickening cases in a PD3 flock

Fig. 4.4: Higher mortality with proventricular thickening in a PD3 flock

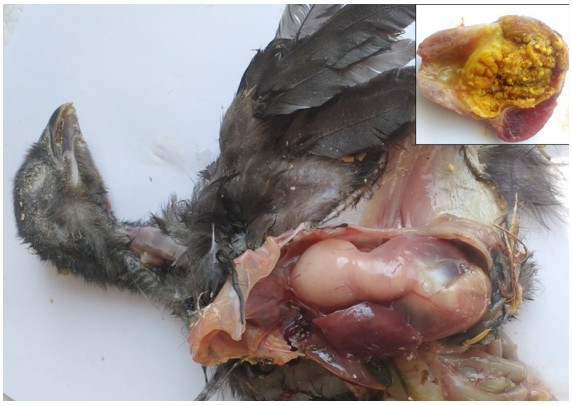


Fig. 4.5: Moderate type of proventricular thickening in an Aseel chick

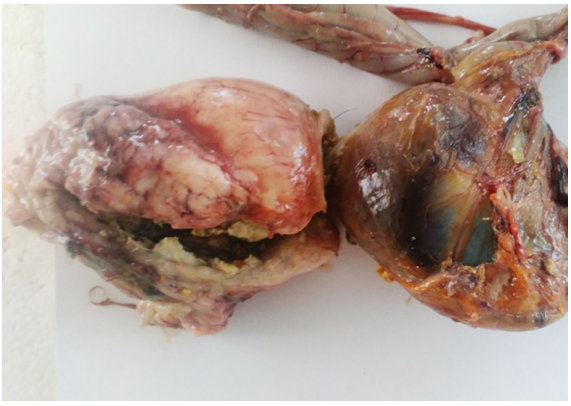


Fig. 4.6: Severe type of proventricular enlargement in an adult bird

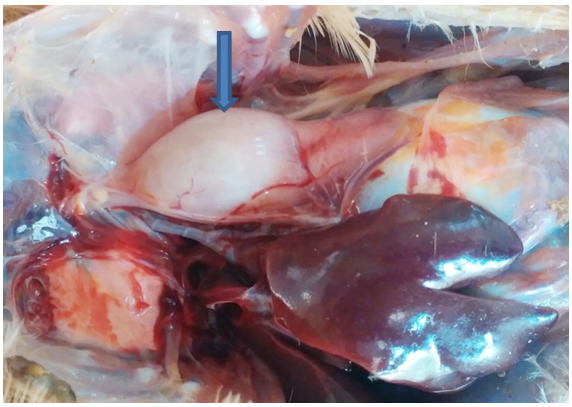


Fig. 4.7: Pale white serosa in proventricular thickening cases

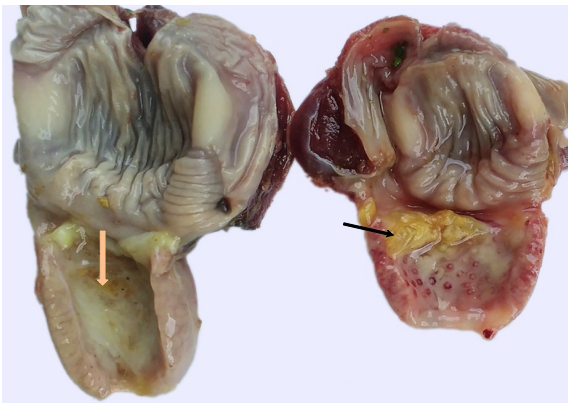


Fig. 4.8: Proventricular thickening. Excess mucus production in the mucosa (thick arrow), haemorrhage (quad arrow) and thickened soft koilin at isthmus junction (thin arrow)

Fig. 4.9: Proventricular thickening in a white leghorn chick. The lumen contain moderate quantity of feed

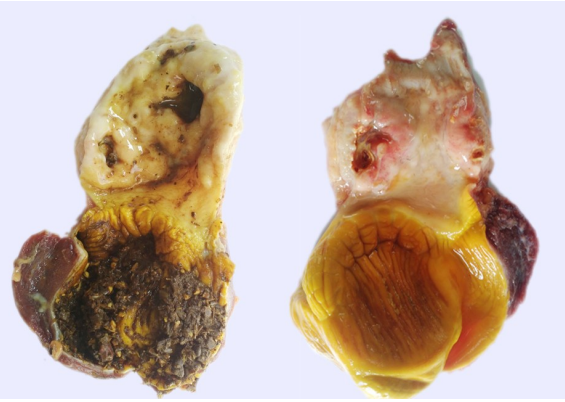


Fig. 4.10: Proventricular thickening with localised mucosal ulcerations

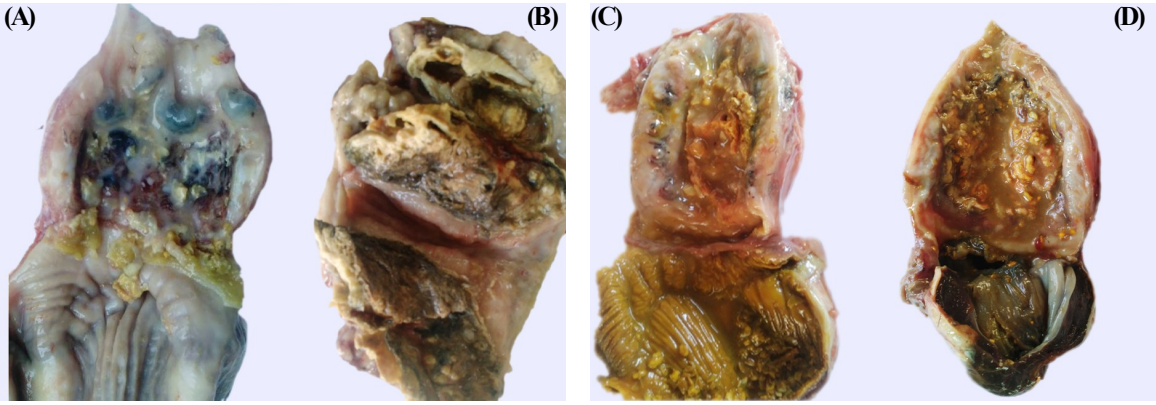


Fig. 4.11: Proventricular thickening- diffuse ulceration(A) and diphtheretic membrane formation (B,C &D)

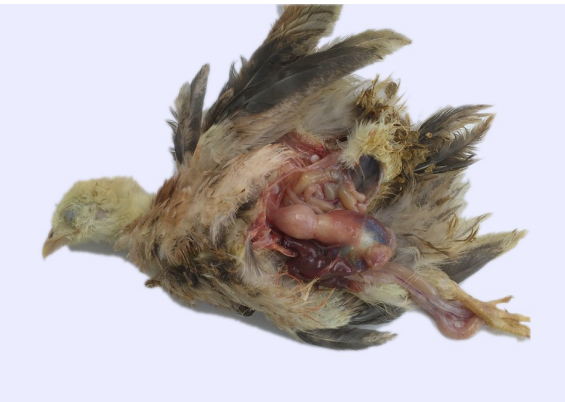


Fig. 4.12: Six week old chick with runting-stunting syndrome, catarrhal enteritis and faecal smearing of feathers around vent region

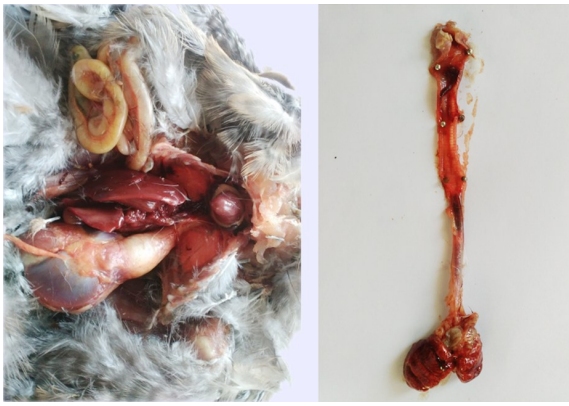


Fig. 4.13: Proventricular thickening with laryngotracheitis in a grower chicken

Lowest occurrence found in the month of April. Moreover, the proventricular thickening was not observed in all flocks of the same breed. Out of 307 flocks of 15 different breeds, proventricular thickening was noticed in 62 (20.19%) flocks belonging to 13 breeds (Table 4.7).The occurrence of flocks affected with proventricular thickening was highest (33.33%) in Ghagus and Krishibro, while no occurrences found in Dwarf and Crosses. Again, the occurrence varied widely in affected flocks within each breed (Table 4.8) and highest occurrence was observed in Nicobari (NIC5- 33.33%, 4/60). Even though proventricular thickening observed as sporadic occurrences, a few flocks exhibited higher number of birds died with proventricular thickening in shorter period or on same day. In a few flocks like DR1, DR10, VR3 etc, the occurrence were extending from its chick to grower age (Table 4.8). Occurrence of proventriculus thickening with age in weeks of a PD2 flock (flock no. VR3) and a PD3 flock in an outbreak is depicted in the Fig. 4.2, 4.3 and 4.4. The proventricular thickening started from 4 to 6 weeks in the PD2 and PD3, and in some white leghorns as young as 1 week. In addition to the birds from the farms of ICAR-DPR, the proventricular thickening was recorded in 17 layer chicks out of 120 commercial birds examined during the study period.

4.2.1.2 Macroscopic lesions

The affected proventriculus were grossly enlarged with firm and thickened walls. Depending on severity, proventricular thickening was grouped into mild, moderate (Fig 4.5) and severe (Fig 4.6). Serosa of the affected proventriculus was mostly pale white in colour (Fig. 4.7). On applying digital pressure, thick white mucus materials oozes out through the glandular opening of the proventriculus. In most of the cases, proventricular mucosa was covered with excess mucus. The mucosa of isthmus region was covered with thick yellowish jelly like or semi hardened koilin material in most of the thickening cases (Fig 4.8). In a few birds, proventricular thickening was associated with mucosal haemorrhage and ulceration (Table 4.5). The mucosal haemorrhage were observed either as scattered, affecting only small number of glandular tips or diffuse covering all glandular tips or entire mucosa (Fig.4.8). Lumen of the proventriculus contained small or moderate quantity of feed in most of the cases (Fig. 4.9). Ulceration of proventricular mucosa was observed in a few older birds either as localised ulcers or diffuse with diphtheritic membrane formation (Fig. 4.10 and 4.11).Mild atrophy of gizzard musculature was observed in a very few cases.

Most common concurrent lesion with the proventricular thickening was catarrhal enteritis (45/168) especially in chicks (Fig.4.12) and growers (Table 4.9). Runting-stunting syndrome was observed in 40% (34/85) of birds with thickened proventriculus in the chick age group. Other concurrent conditions observed along with proventricular thickening were laryngotracheitis or air-sacculitis (4/168) (Fig. 4.13, 4.14), fibrinous pericarditis (3/168), gout (1/168) (Fig 4.15), splenomegaly (8/168), hepatic neoplasia (9/168) (Fig. 4.16), tumors in flat bones and other parts of the body (2/168) (Fig. 4.17).

4.2.1.3 Microscopic lesions of proventricular thickening

Microscopic changes in proventricular thickening were observed in all layers of proventricular wall in varying degree and nature. Major histological lesions in the mucosa were proliferation of mononuclear cells, fusion of plicae, congestion and haemorrhage (Fig. 4.18). Oedema, proliferation of mononuclear cells and fibrous tissue were noticed in lamina propria and muscularis mucosa (Fig. 4.19, 4.20). Glandular epithelial cell degeneration and necrosis were evident in most of the samples. Metaplasia of glandular epithelium to cuboidal or pseudo columnar epithelium (duct cell hyperplasia) were noticed in many proventriculus samples (Fig. 4.21). Other lesions in the submucosa include, dilatation of lobular sinus (Fig. 4.22), mononuclear cell proliferation (Fig. 4.23) and septal thickening (Fig. 4.24, 4.25). Degeneration and cystic gland formation (Fig.4.26) was observed in a grower bird with mild proventricular thickening. Lesions with in a proventriculus are not uniformily distributed. Normal glands can be observed adjacent to the severely affected gland (Fig. 4.23). In the muscularis externa, lesions such as oedema, mild necrosis and mononuclear cell proliferations were observed in a few cases. The histological lesions were scored mild(1), moderate (2) and severe(3) and mean score was calculated (Fig. 4.27). The lesion scoring in 185 samples (including commercial chicks) revealed higher scoring for duct cell hyperplasia, glandular cell degeneration and necrosis and mononuclear cell proliferation in glands. Severe proliferation of the mononuclear cells was observed in severely thickened proventriculus. In case of moderately and mildly thickened proventriculus, chief changes were thickening of lamina propria and muscularis mucsoa by oedema or fibroplasia, mild haemorrhage, glandular ductal cell proliferation and septal thickening. A higher occurrence of glandular degeneration and necrosis was observed commonly in all types of proventricular thickening.

Table 4.7. Flock occurrence of proventricular thickening

Line name	No of flocks necropsied	No of flocks with proventricular thickening
White leghorn	50	10(20.00)
PB1	10	2(20.00)
PB2	13	2(15.38)
Krishibro	3	1(33.33)
Control broiler	22	4(18.18)
Crosses	7	0(0.00)
PD1	35	6(17.14)
PD2	46	13(28.26)
PD3	40	10(25.00)
GML	16	1(6.25)
Aseel	16	2(12.50)
Nicobari	18	5(27.78)
Ghagus	15	5(33.33)
Naked Neck	9	1(11.11)
Dwarf	7	0(0.00)

Table 4.8. Details of the flocks affected with proventricular thickening

Sl. No	Breed	Flock No.	Necropsied	No. of birds with prov. thickening			Total	Incidence/ flock
				Chick	Grower	adult		
1	Aseel	AS1	48			1	1	2.08
		AS2	60		6		6	10
2	Control Broiler	CB1	9	1			1	11.11
		CB2	60	1			1	1.67
		CB3	36			2	2	5.56
		CB4	215	2			2	0.93
3	PD1	CR1	42		1		1	2.38
		CR2	24	1			1	4.17
		CR3	36			1	1	2.78
		CR4	30		1		1	3.33
		CR5	35	1			1	2.86
		CR6	38		2		2	5.26
4	PD3	DR1	298	29	3		32	10.74
		DR2	40		3		3	7.5
		DR3	175		1	2	3	1.71
		DR4	85		1		1	1.18
		DR5	90		1		1	1.11
		DR6	10		2		2	20
		DR7	884			3	3	0.34
		DR8	35		2		2	5.71
		DR9	420		5		5	1.19
		DR10	114	5	7		12	10.53
5	Ghagus	GH1	21		1		1	4.76
		GH2	40	2	3		5	12.5
		GH3	7		1		1	14.29
		GH4	13	1			1	7.69
		GH5	49	1			1	2.04
6	GML	GM1	73			1	1	1.37
7	White Leghorn	WL1	45		1		1	2.22
		WL2	61	2	2		4	6.56
		WL3	751			1	1	0.13
		WL4	5			1	1	20
		WL5	97	1			1	1.03
		WL6	8		1		1	12.5
		WL7	15	1			1	6.67
		WL8	273	3			3	6.67
		WL9	86			2	2	1.1
		WL10	72			1	1	1.39
8	Naked neck	NN1	20			1	1	2.33
9	Nicobari	NIC1	156	1			1	1.39
		NIC2	28		1		1	5
		NIC3	3			1	1	0.64
		NIC4	33		1		1	3.57
		NIC5	66	4			4	33.33
10	PB1	PB1 1	57		1		1	1.75
		PB1 2	435	8			8	1.83
11	PB2	PB2 1	144	1	2		3	2.08
		PB2 2	139			1	1	0.72

Table 4.8. Condt...

Sl. No	Breed	Flock No.	Necropsied	No. of birds with prov. thickening			Total	Incidence/ flock
				Chick	Grower	adult		
12	Krishbro	PX1	31	2			2	6.45
13	PD2	VR1	46		1		1	2.17
		VR2	181	2	1		3	1.66
		VR3	609	6	8		14	2.3
		VR4	52	3			3	5.77
		VR5	73	1			1	1.37
		VR6	241			2	2	0.83
		VR7	153			1	1	0.65
		VR8	129		1		1	0.78
		VR9	149	4			4	2.68
		VR10	30	1			1	3.36
		VR11	35	1			1	2.86
		VR12	38		1		1	2.63
		VR13	42		1		1	2.38
White leghorn from commercial farm		WLO 1		17			17	

Table 4.9a Viruses identified by PCR in proventricular thickening cases

Age group	No. of proventricular thickening	MD	MD+ CAV	CAV	ALV	ALV+ CAV	ChPV	ChPV+ CAV
CHICK	85	0	0	12 (14.12)	0	0	0	2 (2.35)
GROWER	62	7 (11.29)	4 (6.45)	3 (4.84)	2 (3.23)	0	0	1 (1.61)
ADULT	21	11 (52.38)	0	2 (9.52)	0	1 (4.76)	0	0

Fig. 4.14: Proventricular thickening with caseous air sacculitis (arrow)

Fig. 4.15: One week old chick with visceral gout and proventricular thickening

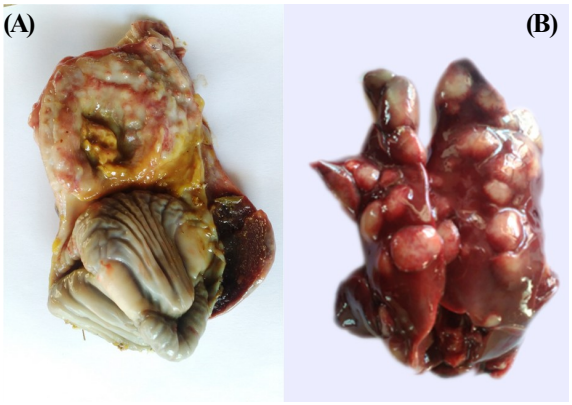


Fig. 4.16: Severe proventricular thickening with a large mucosal ulcer and diphtheritic membrane formation (A) concurrently with tumour nodules in the liver (B) in an adult chicken.(MDV infection)

Fig. 4.17: Proventricular thickening. Concurrently with tumour masses in the kidney and adjacent areas (A). Enlarged proventricular glands seen from mucosa (B). ALV infection

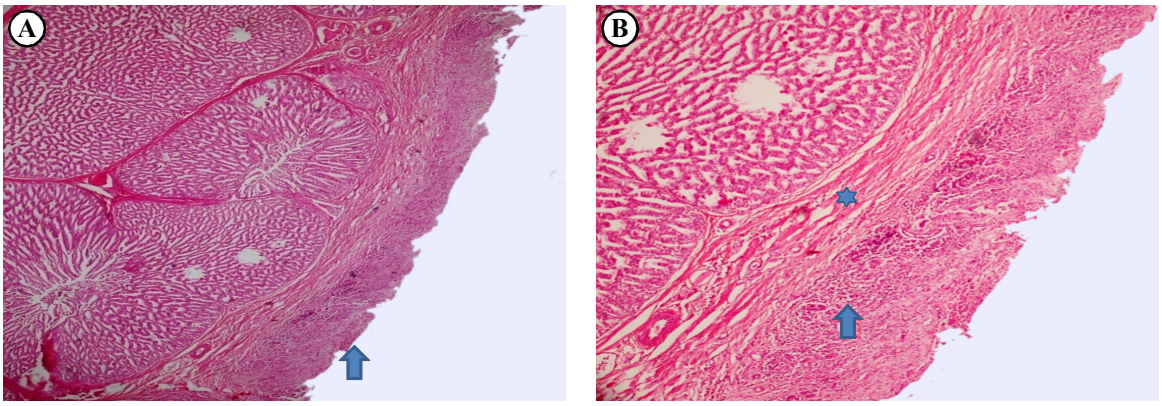


Fig. 4.18: Proventricular plicae showing shortening and fusion (arrow). Mild MNC proliferation in the mucosa and oedema of muscularis mucosa (star). H&E, (A) x40 and (B) x100

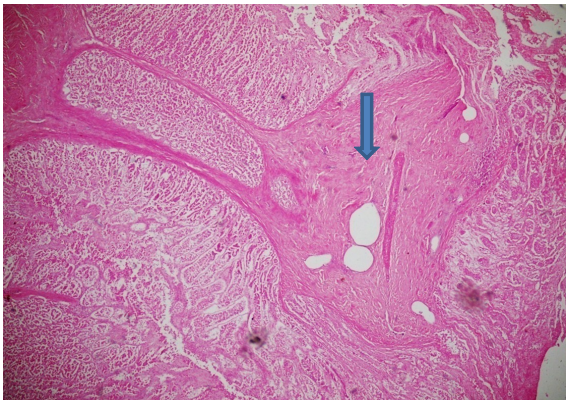


Fig. 4.19: Proventriculus - severe thickening of muscularis mucosa (arrow) H&E, x100

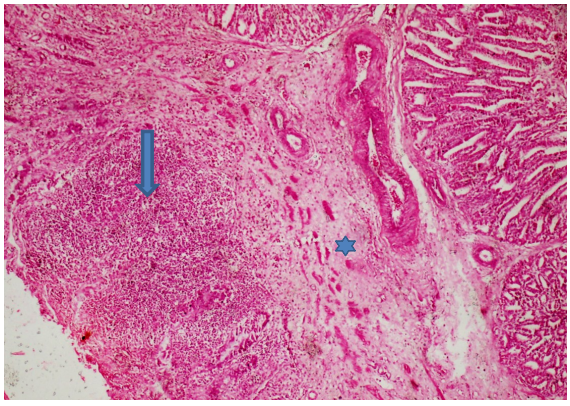


Fig. 4.20: Proventriculus – moderate infiltration of lymphocytes in the mucosa (arrow), edema and fibroplasia of muscularis mucosa (star) H&E, x400

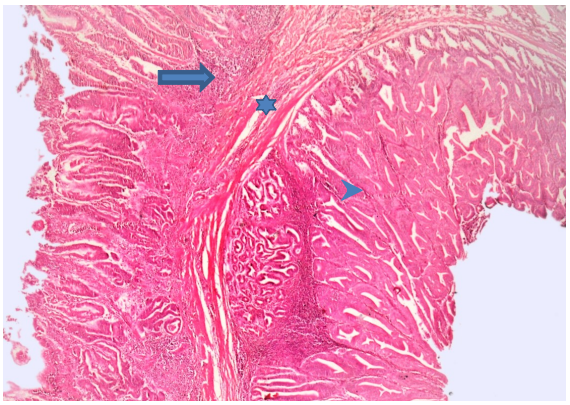


Fig. 4.21: Proventriculus showing moderate MNC proliferation in the mucosa and lamina propria (arrow), oedema of muscularis mucosa (star) and duct cell hyperplasia of submucoal glands (arrow head) H&E, x100

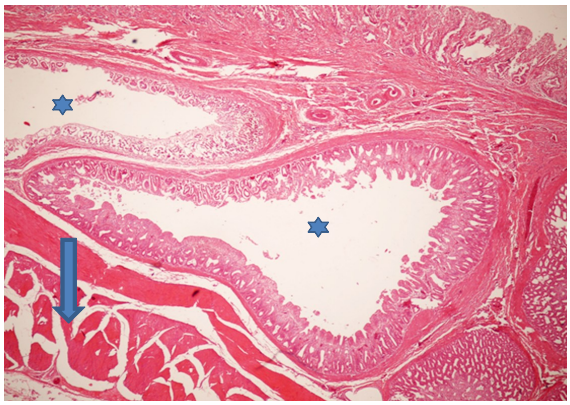


Fig. 4.22: Proventriculus showing moderately dilated lobular sinuses of the submucosal glands (star) and oedema in the muscularis externa (arrow) H&E, x40

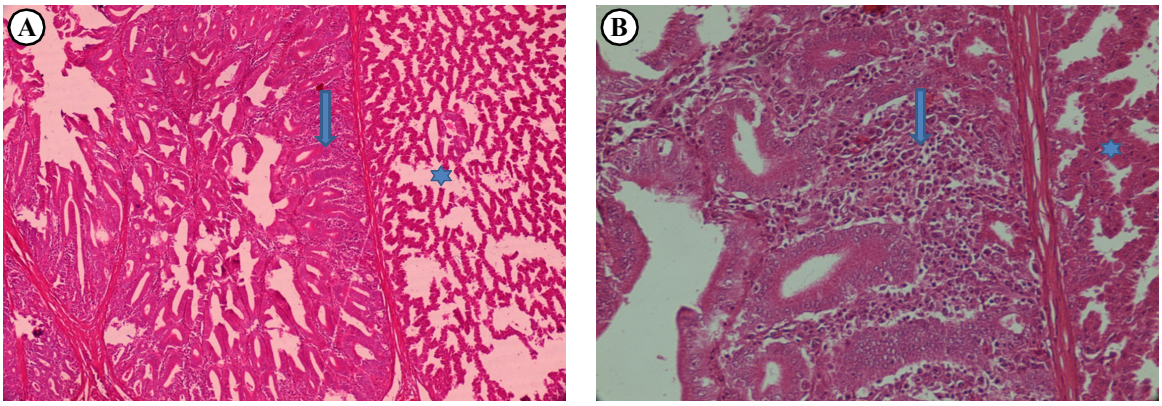


Fig. 4.23: Proventriculus submucus glands. One lobe affected with ductal cell hyperplasia with moderate infiltration of mononuclear cells (arrow). The adjacent lobe is free of infiltration and hyperplasia (star) H&E, (A) x100 and (B) x400

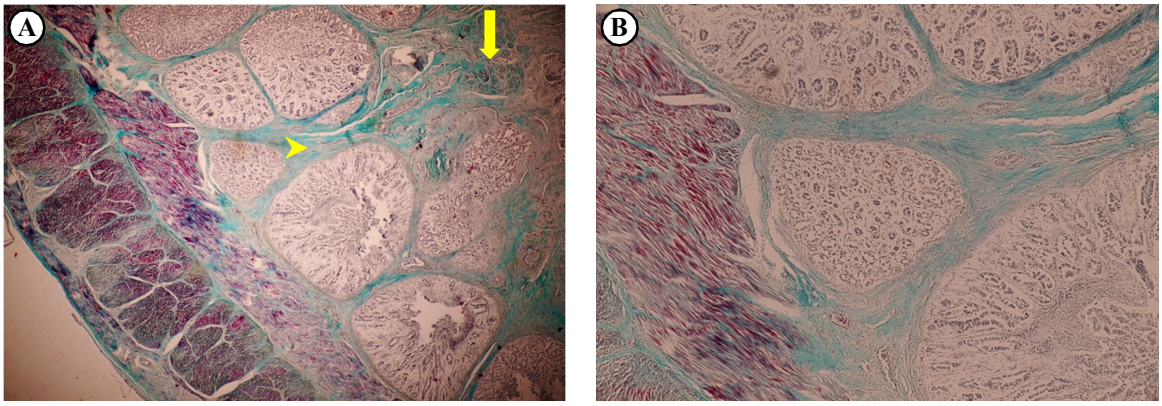


Fig. 4.24: Proventriculus- mild thickening of muscularis mucosa (arrow) and glandular septum (arrow head) by fibroplasia. Gomori's trichrome. H&E, (A) x40 and (B) x100

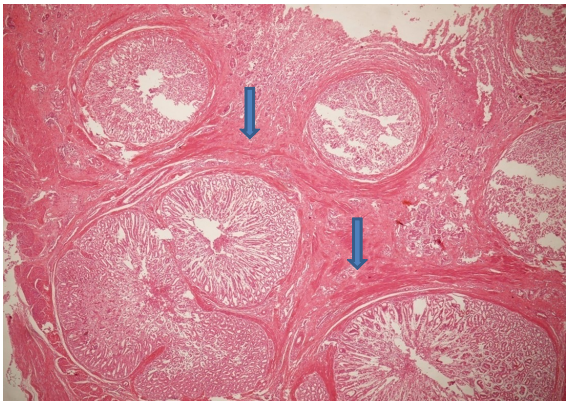


Fig. 4.25: Proventriculus submucosa-severe glandular septal thickening. H&E, x40

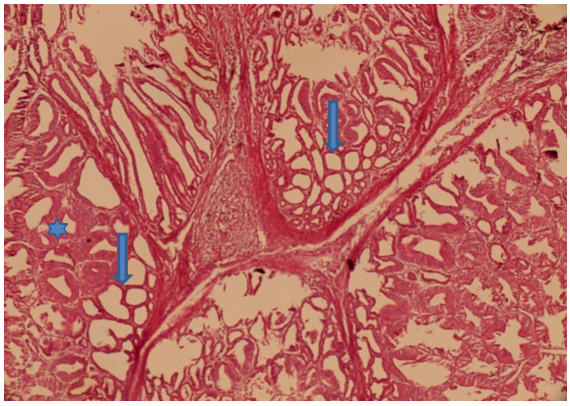


Fig. 4.26: Proventriculus submucosal glands- degeneration of glandular epithelium and cystic gland formation(arrow) duct cell hyperplasia (star) H&E, x40

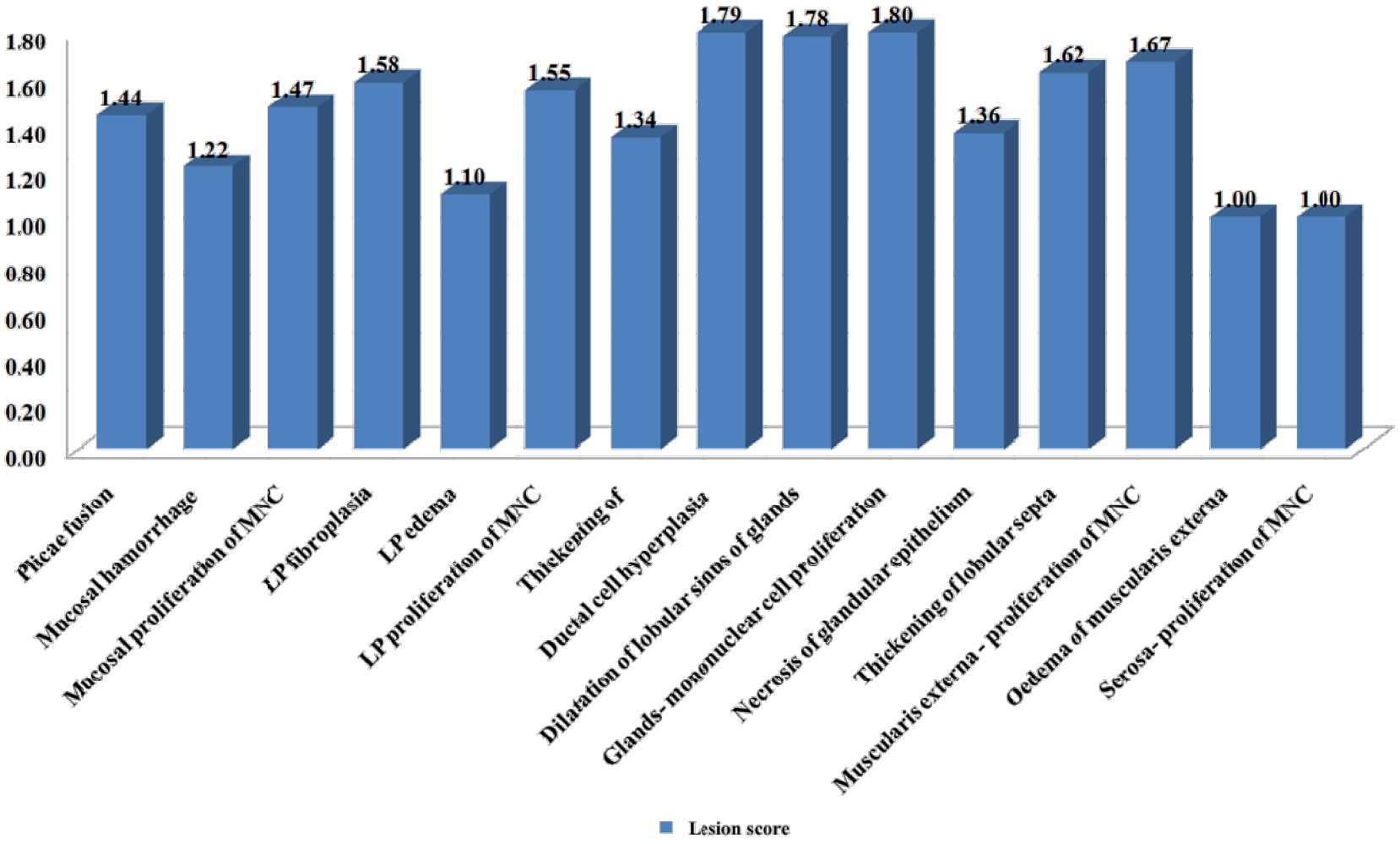


Fig. 4.27: Proventricular thickening- Histological lesion scoring

In case of severe proliferation of mononuclear cells, two cellular types were observed, pleomorphic lymphocytic proliferation and myeloid cell proliferation. Such types of cellular proliferation were identified mostly in cases of severe proventricular thickening found primarily in adult birds. Pleomorphic lymphocyte proliferation included cells like plasma cells, lymphoblasts with vesicular nucleus and lymphocytes. These cells were proliferating in all the layers of proventriculus. In the submucosal glandular region, proliferation of cells and necrosis of glandular epithelium were evident (Fig. 4.28). Pleomorphic lymphoid proliferation was observed in 17.7% (11/62) growers and 52.38 % (11/21) adults with proventricular thickening. Myeloid cells were observed in 3.23% (2/62) growers and 9.52% (2/21) adults. The proliferating myeloid cells were characterized by large vesicular and eccentrically placed nucleus with eosinophilic cytoplasm filled with eosinophilic granules (Fig. 4.29, Fig. 4.30). Histologically, in the Aseel growers (AS2), pleomorphic lymphocytic proliferation found only in one bird. In other birds of the same flock, enlargement of proventriculus was not directly due to lymphocytic proliferation but due to ductal cell hyperplasia, sinus dilatation and thickening of muscularis mucosa.

4.2.1.4 Electron microscopic study

Electron microscopic examination was performed in a proventriculus from PD3 chick, flocks in which had higher occurrence of proventricular thickening observed. Examination of the glandular area revealed 100-120nm sized virus like particles (VLP) in the intercellular space (Fig. 4.31).

4.2.1.5. Molecular diagnosis

Tissues from selected proventricular thickening were screened for presence of pathogens such as MDV, ALV, REV, CAV, FAdV1, ChPV, CPNV, NDV and IBV by PCR or Rt PCR using specific primers. The viruses identified in proventricular thickening were MDV (Fig.4.32), ALV (Fig 4.33), ChPV (Fig.4.34) and CAV (Fig. 4.35). In a chick with proventricular thickening and gout, Chicken Astrovirus and Avian Nephritis virus was detected in kidney but not in proventriculus. Age group wise details of virus identified from proventriculus in proventricular thickening cases were shown in the Table 4.8. Details of microscopic lesions,

breeds and age of the birds from which viruses detected are presented in Table (4.9a). Proliferation of myeloid cells was evident in the ALV positive proventriculus. The histological findings were correlated with results of molecular detection. The microscopic lesions in MD positive cases include proliferation of pleomorphic lymphocytes, plasma cells and lymphoblasts affecting all the layers of proventriculus in varying degree. Proventricular haemorrhage was a common finding (11/17) in proventriculus from which CAV alone was detected (table. 4.9b). At the same time proventriculus with both CAV and MD or ALV did not show haemorrhage.

4.2.1.6. Isolation and identification of virus from proventricular thickening

4.2.1.6.1- Chicken embryo inoculation

Isolation of CPNV, IBV, NDV from tissues collected from proventricular thickening cases were attempted and results indicate that all the samples were negative for above mentioned viruses. Mild proventricular thickening was observed in an intra-amniotic inoculated sample (Fig. 4.36). Histological examination showed thickening of muscularis mucosa, enlargement of submucosal gland and thickening of a part of muscularis externa (Fig.4.37). No virus could be identified from the same using PCR and Rt-PCR using virus specific primers. Proventricular thickening could not be reproduced by any of the inoculation routes attempted in chicken embryos even after 5-8 passages. Haemagglutination test performed to detect haemagglutinating viruses, from the allantoic fluids collected from embryos inoculated through intra-allantoic route revealed all sample as non-haemagglutinating.

4.2.1.6.2 Inoculation in day old chicks

The chicks were oro-nasally administered the clarified proventriculus homogenate after treatment with antibiotic. None of the birds showed proventricular thickening during the 7 weeks experimental period. No significant weight difference was observed between the test and control groups.

4.2.2 Proventricular dilatation

Proventriculus of the birds with dilated lumen and thinning of the wall were categorized as having proventricular diatation. Age and breed wise occurrence of the proventricular dilatation is shown in the Table 4.3 and 4.4.

Table 4.9b. Lesion scoring proventricular thickening in which viruses were identified by PCR

Sl. No	Sample detail				Histopathology scoring											
	Breed	Flock No.	Age group (No. positive)	PCR diagnosis	Mucosa plicae fusion	Congestion and hemorrhage in the villi	Mucosa proliferation of cells	LP oedema	LP proliferation of MNCs	Muscularis mucosa thickening by fibroplasia	Glands – duct cell hyper plasia	Glands – proliferation of MNCs	Necrosis of glandular epithelium	Thickening of lobular septa	Dilatation of the sinus of lobueles	Muscular layer proliferation of MNCs
1	PD4	AS1	A	MD	2	0	3	0	3	0	0	0	2	0	0	2
		AS2	G	MD, CAV	1	0	1	0	2	0	0	2	0	0	0	0
		AS2	G	MD, CAV	0	0	1	0	1	0	0	0	0	2	2	0
		AS2	G	MD, CAV	0	0	0	0	3	0	0	2	0	0	0	0
		AS2	G	MD, CAV	0	0	0	0	0	0	0	1	1	0	0	0
2	CB	CB3	A	MD	0	0	2	0	2	0	0	2	2	0	0	2
			A	MD	0	0	3	0	2	0	0	3	3	0	0	2
3	PD3	DR2	G	MD	1	0	3	0	3	0	0	3	2	0	0	2
			G	MD	0	0	1	0	0	0	0	0	1	0	0	1
			G	MD	0	0	0	0	2	2	0	0	0	2	0	1
		DR6	G (2)	ALV-J	0	0	1	0	1	0	0	0	0	0	1	0
		DR7	A	MD	0	0	3	0	3	0	0	0	0	0	0	0
			A (2)	ALV-J, CAV	0	0	0	0	2	0	0	3	1	0	0	2
		DR9	G	CAV(1/5)	0	0	1	0	1	0	2	0	1	0	0	0
	GHAGUS	GH1	G	CAV	2	0	0	1	1	2	0	0	1	2	0	0
		GH2	C(2)	1 CAV	0	2	1	1	0	0	0	0	2	0	2	0
5	White	WL1	G	MD	2	1	3	0	3	0	0	2	2	0	0	2
	leghorn	WL2	C(2)	CAV	0	0	3	1	2	1	2	2	1	1	0	0
		WL3	A	MD	1	0	2	0	2	0	0	2	2	0	0	0
		WL6	G	CAVChParvo	0	0	1	0	2	1	0	1	0	0	0	0
		WL8	C	CAV	0	2	1	0	1	2	3	1	3	0	1	0
			C	CAV	0	2	1	1	0	1	3	0	3	0	2	0
		WL9	A	MD	0	0	3	0	3	0	0	3	0	0	0	2
6	Naked neck	NN1	A	MD	0	0	2	0	0	0	0	3	2	0	0	0

Table 4.9b. Contd...

Sl. No	Sample detail				Histopathology scoring											
	Breed	Flock No.	Age group (No. positive)	PCR diagnosis	Mucosa plicae fusion	Congestion and hemorrhage in the villi	Mucosa proliferation of cells	LP oedema	LP proliferation of MNCs	Muscularis mucosa thickening by fibroplasia	Glands – duct cell hyper plasia	Glands – proliferation of MNCs	Necrosis of glandular epithelium	Thickening of lobular septa	Dilatation of the sinus of lobueles	Muscular layer proliferation of MNCs
7	Nicobari	NIC3	A	MD	0	0	3	0	2	0	0	0	1	0	0	0
		NIC5	C	CAV	0	2	0	1	0	0	2	0	0	0	1	0
			C	CAV	0	2	0	0	0	0	2	0	0	0	0	0
			C	CAV	0	1	0	1	0	0	2	0	0	2	1	0
			C	CAV	0	2	0	1	0	1	0	1	1	0	1	0
8	PB2	PB2 1	G(2)	MD	0	2	3	0	3	0	0	2	2	0	0	2
		PB2 2	A	MD	0	0	1	2	1	2	0	0	0	0	0	0
9	Krishibro		C	ChPV	0	0	1	0	0	1	1	1	0	0	0	0
			C	ChPV	0	2	0	0	0	2	0	1	1	0	0	0
10	PD2	VR2	C(2)	CAV	0	0	1	1	2	1	2	0	1	1	0	0
			G	MD	2	1	3	0	3	0	0	3	2	0	0	1
		VR3	C	CAV	0	1	0	0	0	0	0	0	1	1	0	0
			G	CAV	0	1	0	0	0	2	1	0	0	2	0	0
		VR6	A	CAV	0	1	1	0	1	1	0	0	0	0	0	0
			A	CAV	0	0	3	0	1	2	2	3	2	2	0	0
		VR12	G	MD	2	2	0	0	0	0	2	0	0	0	1	0
11	Layer commercial farm	IBL 1	C	CAV	0	1	0	0	0	0	0	0	0	0	0	0

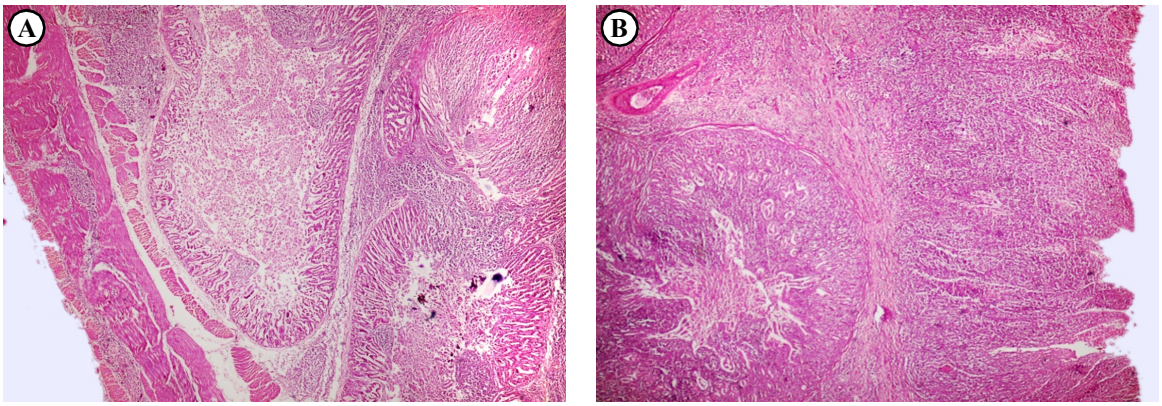


Fig. 4.28: Proliferation of lymphocytes in all the layers of proventriculus with diffuse glandular epithelial cell necrosis H&E 100x

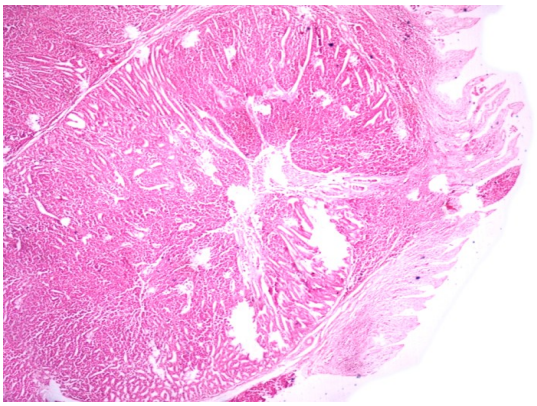


Fig. 4.29: Severe proliferation of myeloid cells in the submucosal glands of proventriculus H&E, x40

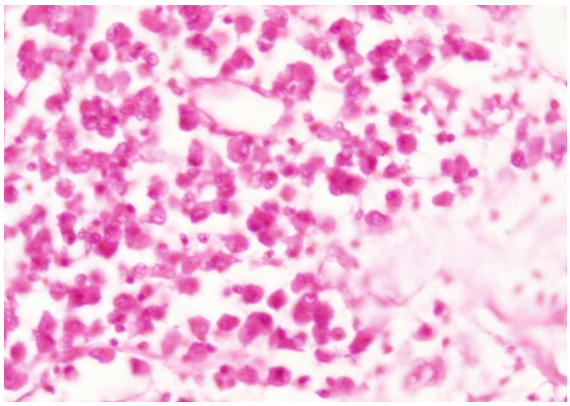


Fig. 4.30: Severe proliferation of myeloid cells in the submucosal glands of proventriculus H&E, x1000

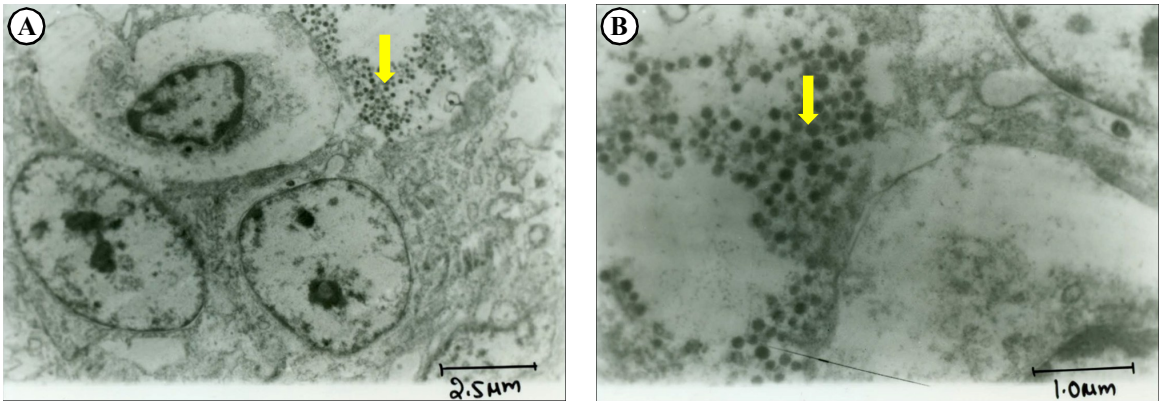


Fig. 4.31: Electron micrograph of proventriculus glandular area- virus like particles (arrow) of size 100-120nm seen in the intercellular space

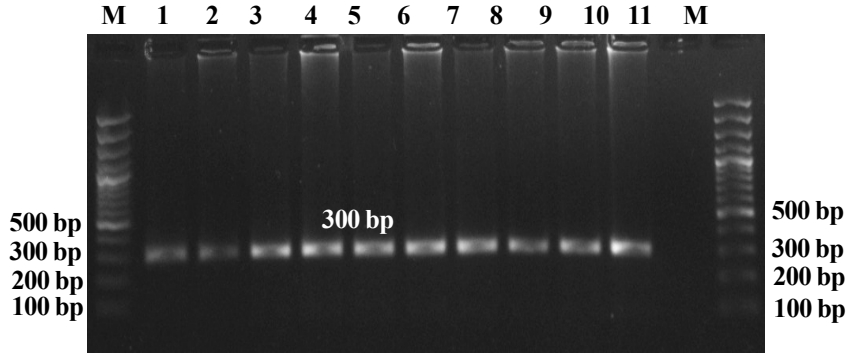


Fig. 4.32: Ethidium bromide stained 1.5% agarose gel showing amplification of 300 bp fragment of MDV

Lane M : 100 bp DNA ladder
Lanes 1-10 : Positive samples (300 bp)
Lane 11 : Negative control

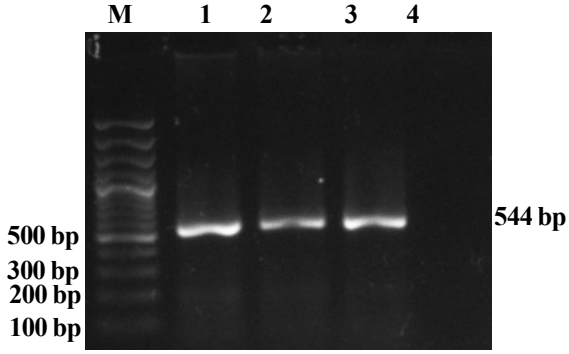


Fig. 4.33: Ethidium bromide stained 1.5% agarose gel showing amplification of 544 bp fragment of ALV

Lane M : 100 bp DNA ladder
Lanes 1-3 : Positive samples (544 bp)
Lane 4 : Negative control

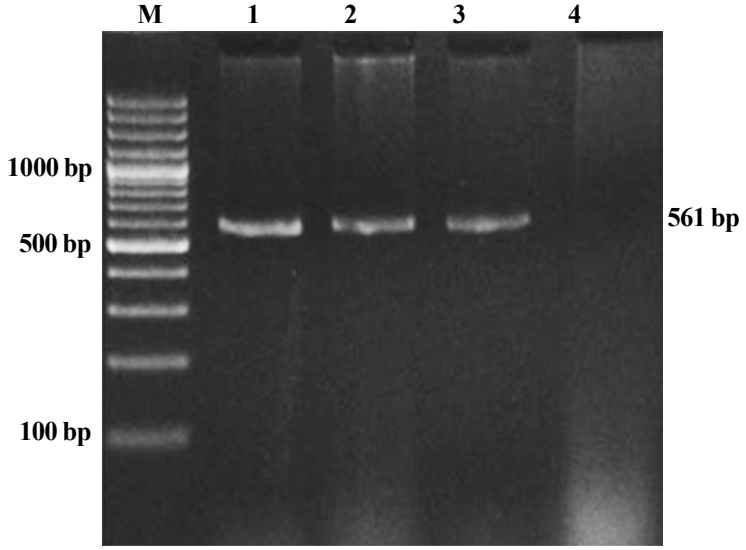


Fig. 4.34: Ethidium bromide stained 1.5% agarose gel showing amplification of 561 bp fragment of Chicken parvovirus
Lane M : 100 bp DNA ladder
Lanes 1-3 : Positive samples (561bp)
Lane 4 : Negative control

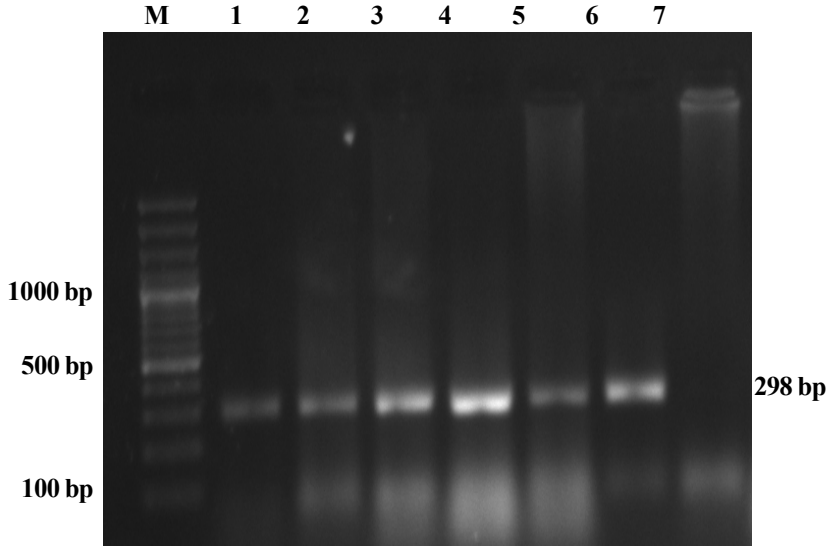


Fig. 4.35: Ethidium bromide stained 1.5% agarose gel showing amplification of 298 bp fragment of chicken infectious anaemia virus
Lane M : 100 bp DNA ladder
Lanes 1-6 : Positive samples (298bp)
Lane 7 : Negative control

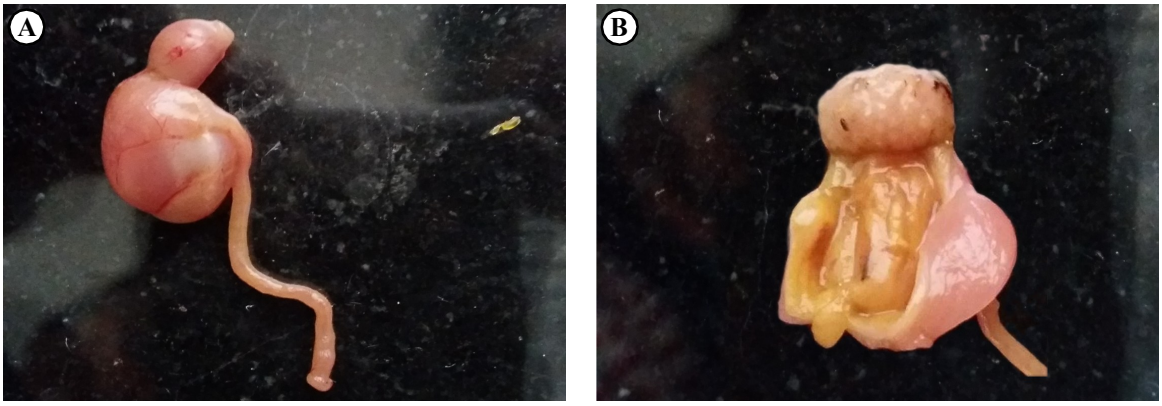


Fig. 4.36: Proventricular thickening in P1 passage of gut 209

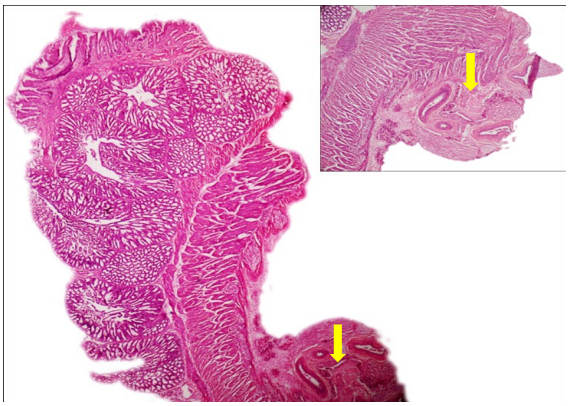


Fig. 4.37: Gut 209 P1 embryo with proventricular thickening. Enlargement of submucosal glands and abnormal thickening of outer muscular layer H&E, x40 and insert x100

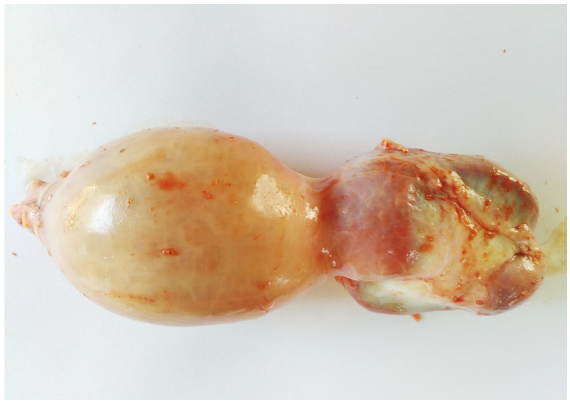


Fig. 4.38: Proventricular dilatation- severely engorged proventriculus

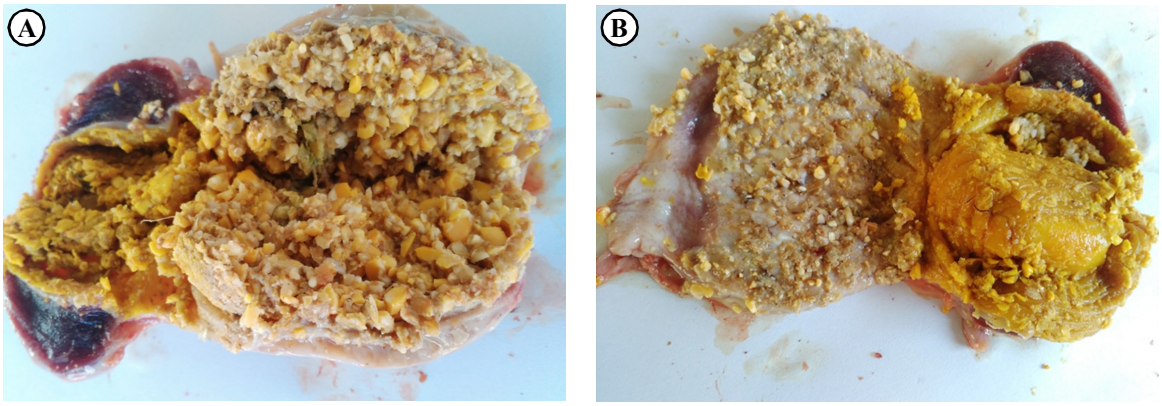


Fig. 4.39: Proventricular dilatation. Opened proventriculus (A) proventricular lumen engorged with feed. (B) flaccid and thin proventricular wall

The proventricular dilatation was observed in 13 (0.07%) out of 17,978 birds necropsied from ICAR-DPR farms (Table 4.3). On bird basis, the breed wise occurrence of proventricular dilatation was highest in Aseel (0.88%) and zero in Krishibro, WLH, GML, Nicobari, Gaghus, and Dwarf (Table 4.3). Age wise occurrence of proventricular dilatation was 0.09% in chicks and growers and 0.04% in adults (Table 4.4).

4.2.2.1 Gross lesion

The proventricular lumen was engorged with feed material in 46.15% (6/13) cases of proventricular dilatation (Fig 4.38, 4.39, 4.40 and 4.41). Colour of the proventriculus serosa varied from pale pink to pale white. The mucosa was flat without any glandular prominence in 5(38.46%) cases while others had only mild prominence of gland tips. Dilatation of gizzard (Fig. 4.42) was also observed in 30.77% (4/13) of proventricular dilatation. Undigested feed materials in the intestine (Fig. 4.43.) were observed in 2 cases (15.38%).

4.2.2.2 Microscopic lesions in the proventricular dilatation

Other than the overall thinning of all layers of proventriculus, histological lesions were minimal in all cases of proventricular dilatation. Mild to moderate degeneration and necrosis of the glandular epithelium was observed in 7 (53.84%) cases. One case (7.69%) showed moderate denudation of primary glandular epithelium with cystic dilatation (Fig. 4.44).

4.2.2.3 Molecular diagnosis

Tissues from dilated proventriculus and brain were screened for presence of MDV and Borna virus by PCR or Rt PCR using specific primers. Results indicate that all the samples tested were negative for both MDV and Borna virus.

4.2.3 Proventricular haemorrhage

Proventricular haemorrhage was observed in 191 (1.08%) out of 17,978 birds necropsied from ICAR-DPR farms (Table 4.3). The breed wise occurrence of proventricular haemorrhage was highest in Nicobari (2.82%) and nil in crosses (Table 4.3). Age-wise occurrence of proventricular haemorrhage was 1.51% in chicks, 1.12% in growers and 0.61% in adults (Table 4.4). Breed and age wise occurrence shows highest occurrence in PD3 chick

(2.85%, 30/1051), Aseel grower (4.30%, 4/93) and GML adult (3.15%, 4/127) in the respective age groups (Table 4.10). The condition was associated with concurrent lesions like proventricular thickening (57/191), laryngotracheitis (27/191), fibrinous pericarditis and perihepatitis (32/191), air sacculitis (35/191), intestinal coccidiosis (15/191) and dehydration (25/191). The condition was observed as sporadic in most of the cases.

4.2.3.1. Gross lesions

Grossly proventricular haemorrhage was observed as scattered or diffuse. In scattered haemorrhage, a few of glandular tips were affected while in diffuse cases most of the glandular tips (Fig. 4.45) or entire mucosa was affected. Scattered haemorrhage was observed in 92.14% (176/191) birds with proventricular haemorrhage. Among the scattered glandular haemorrhage, haemorrhage appeared horizontally near the proventriculo-oesophageal junction (Fig 4.46) in one PD3 adult and a PD2 chicks . Haemorrhages, horizontally at isthmus junction were observed in one case (Fig. 4.47).

4.2.3.2. Microscopic lesions

Grossly, scattered type of haemorrhage was observed on the mucosa adjacent to the opening of the glands (Fig. 4.48) whereas haemorrhages extended to lamina propria and muscularis mucosa and even up to submucosal glands in diffuse type. Glandular epithelial cell degeneration and necrosis was observed in varying degrees in all the cases.

4.2.4 Proventricular mucosal ulceration

The proventricular mucosal ulceration was observed in 16 (0.09%) out of 17,978 birds necropsied from ICAR-DPR farms (Table 4.3). The breed wise occurrence of proventricular mucosal ulceration was highest in Crosses (0.92%) and zero in Krishibro, PD1, Nicobari, Gaghus and Dwarf (Table 4.3). Age-wise occurrence of proventricular mucosal ulceration was 0.02% in chicks and growers and 0.20% in adults (Table 4.4). In breed and age wise occurrence, proventricular ulceration were highest among the adults of Naked neck 1.9% (1/51) (Table 4.11).

Table 4.10. Breed and age wise occurrence of proventricular haemorrhage

	Chick	Grower	Adult	Total
White leghorn	102.47	20.43	100.82	251.19
PB1	00.00	53.03	10.67	91.09
PB2	10.32	00.00	10.55	20.33
Krishibro	10.99	00.00	00.00	21.84
Control Broiler	61.60	40.87	00.00	121.21
Crosses	00.00	00.00	00.00	00.00
PD1	70.79	10.28	51.07	140.82
PD2	252.58	152.00	120.56	571.46
PD3	302.85	141.15	80.41	561.33
GML	40.39	00.00	43.15	80.51
Aseel	10.81	44.30	00.00	102.96
Nicobari	73.00	53.70	00.00	194.39
Ghagus	51.81	00.00	00.00	71.74
Naked neck	00.00	10.70	11.96	31.05
Dwarf	00.00	11.72	00.00	31.15
Total	971.51	521.12	420.61	1941.08

Table 4.11. Breed and age wise occurrence of proventricular ulcers

	Chick	Grower	Adult	Total	Chick	Grower	Adult	Total
White leghorn	405	464	1226	2095	0	0	30.24	30.14
PB1	517	165	150	832	0	0	10.67	10.12
PB2	312	207	183	702	0	0	21.09	20.28
Krishibro	101	7	0	108	0	0	0.00	00.00
Control Broiler	375	462	195	1032	0	0	21.03	20.19
Crosses	47	42	20	109	12.13	0	0	10.92
PD1	885	359	468	1712	0	0	0	00.00
PD2	969	751	2160	3880	0	10.13	0	10.03
PD3	1051	1220	1952	4223	0	0	20.10	20.05
GML	1025	480	127	1632	0	0	10.79	10.06
Aseel	124	93	124	341	0	11.08	10.81	20.59
Nicobari	233	135	58	426	0	0	0	0
Ghagus	277	61	54	392	0	0	0	0
Naked neck	63	143	51	257	0	0	11.96	10.39
Dwarf	55	58	124	237	0	0	0	00.00
total	6439	4647	6892	17978	1	2	13	16

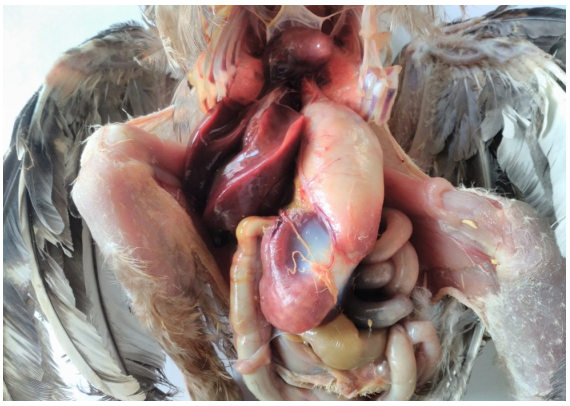


Fig. 4.40: Dilatation of proventriculus and isthmus in an Aseel grower

Fig. 4.41: Proventricular dilatation in a naked neck grower

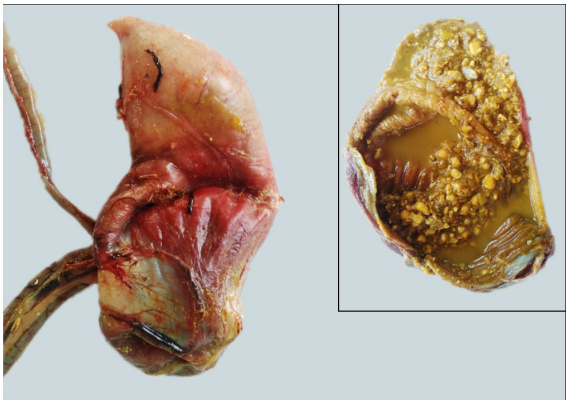


Fig. 4.42: Proventricular dilatation in a PD1 adult bird. Both gizzard and proventriculus were dilated with thinning of wall (insert- opened proventricular and gizzard)

Fig. 4.43: Undigested feed materials in the intestine in a case of proventricular dilatation

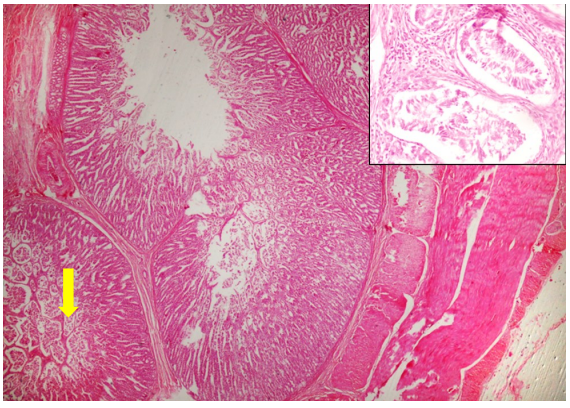


Fig. 4.44: Proventriculus - degeneration and denudation of glandular epithelium with cystic dilatation of primary glands (arrow) H&E, x100 (insert-denudation of glandular epithelium H&E, x400)

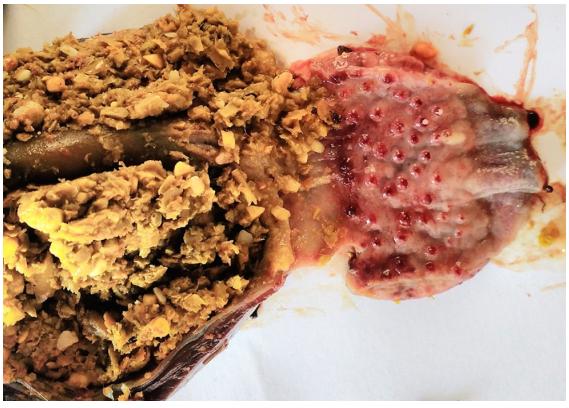


Fig. 4.45: Diffuse glandular haemorrhage in a dehydrated carcass

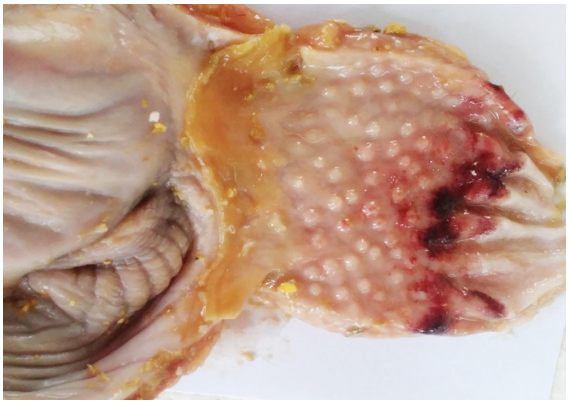


Fig. 4.46: Haemorrhage at the oesophageal junction

Fig. 4.47: Proventricular haemorrhage in the isthmus junction

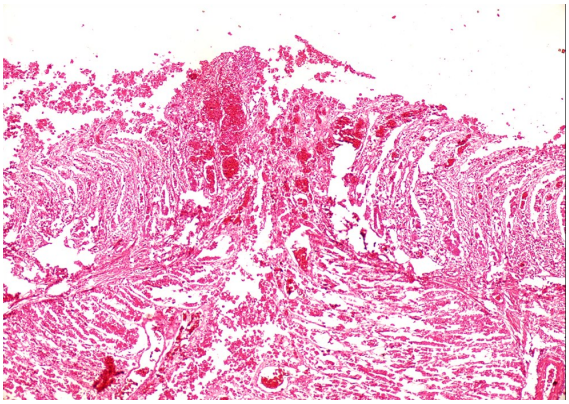


Fig. 4.48: Congestion and haemorrhage in the mucosa of proventriculus H&E, x100

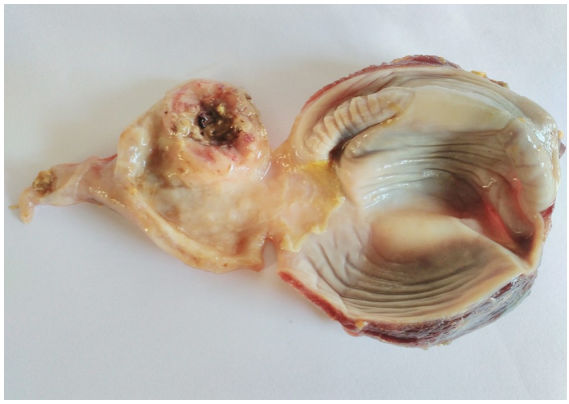


Fig. 4.49: Proventricular ulcer with localised wall thickening

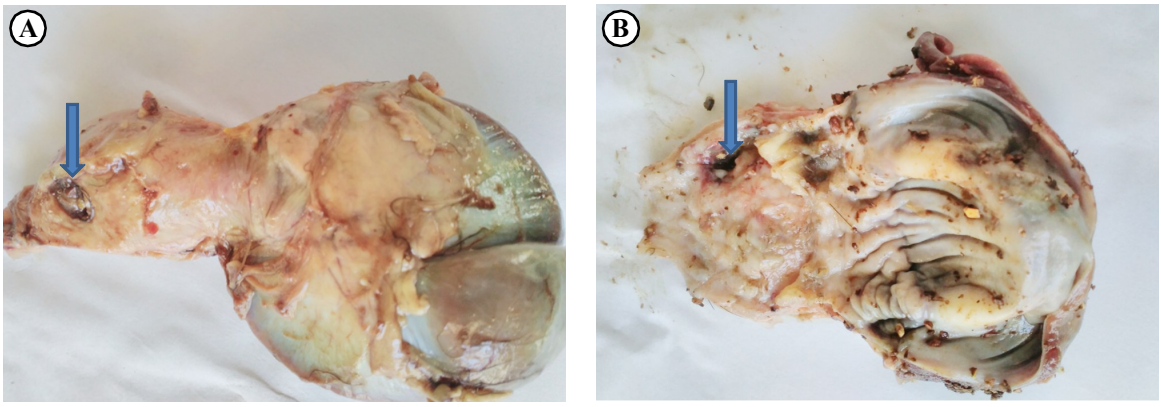


Fig. 4.50: Proventricular ulcer penetrating whole layer of proventriculus in a crosses chick (A) Serosal surface (B) Mucosal surface

4.2.4.1 Gross lesion

Proventricular mucosal ulcerations were observed along with proventricular thickening in 9 (56.25%) cases and with intestinal wall nodular thickening in 3 (18.75%). Size and extent of ulceration varied from localised to diffuse, affecting whole proventricular mucosa and resultant diphtheritic membrane formation (Fig. 4.10 and 4.11) and uniform proventricular thickening. Ulceration observed in one Aseel grower and one White Leghorn adult bird (Fig. 4.49) was focal type with localised proventricular thickening. A hole was observed on the wall of the proventriculus in a Crosses chick without any wall thickness or inflammatory signs (Fig. 4.50).

4.2.4.2 Microscopic lesions

Severe lymphocytic proliferation was observed in all the layers of proventriculus in 93.75% (15/16) cases of mucosal ulceration. Necrosis of the mucosa and glandular tissues were observed in affected areas. Ulceration in a Crosses chick elicited minimal tissue reaction with mild proliferation of heterophils and lymphocytes in the area surrounding the ulcer.

4.2.4.3 Molecular diagnosis

MDV and ALV were detected in the proventriculus tissue by PCR using specific primers. Results revealed presence of Marek's disease virus in one grower and 12 adult chickens and ALV in one adult PD3 chicken.

4.2.5 Miscellaneous conditions

4.2.5.1 Flaccid proventriculus and gizzard

Flaccid proventriculus and gizzard was observed as an outbreak in commercial broiler breeders at 32 weeks of age. The farm suffered death of about 20 birds per day for a week period. Necropsy examination of the birds revealed severe flaccidity of both proventriculus and gizzard (Fig. 4.51). The wall of the proventriculus and gizzard was very thin and lumen contained small amount of soft feed material. Intestine contained undigested feed material in some birds. No other gross lesions were detected in any of the visceral organs. Histological examination of the proventriculus showed thinning of proventricular wall, denudation of mucosal and glandular epithelium, degeneration of plicae epithelium, mild infiltration of mononuclear

cells in lamina propria and mild necrosis of muscle of muscularis mucosa and muscularis externa (Fig. 4.52).

The molecular screening of proventriculus and gizzard homogenates for MDV, ALV, CAV by PCR using specific primers and embriyonic inoculation of proventricular suspension in the allontoic rout and subsiquent HA test and Rt-PCR for NDV revealed that all the samples tested were negative for the viruses. Withdrawal of old feed and feeding new feed with vitamin supplements containing vitamin E and selenium resulted in complete recovery from the problem within few days.

4.2.5.2 Proventriculo-ventricular intussusception

Proventricular intussusception was observed in 3 (0.017%) out of 17,978 birds necropsied from ICAR-DPR farms. Two cases were observed in PD2 chicks (aged 4 days and 3 weeks) and one Nicobari grower aged 9 weeks. (Fig. 4.53, 4.54, 4.55)

Grossly, the proventriculus was telescoped into the lumen of anterior thin walled part of the ventriculus in all the cases. The telescoping was found to be originated posterior to the proventriculo- oesophageal junction without the eversion of the oesophagus. The intact oesophagus was drawn posteriorly along with the telescoped proventriculus. In both PD2 chicks (Fig. 4.53), small part of the inverted proventriculus was protruded into the lumen of ventriculus while moderately protruded in Nicobari grower (Fig.4.54). Though the muscular wall of the proventriculus was mildly thin, it was not flaccid. While gross examination, mild cranial pulling of proventriculus holding the oesophagus relieved the intussusception in the 3 weeks old Vanaraja chick. On the other hand, the intussusception was not relieved with mild pulling of the oesophagus in case of Nicobari grower. Isthmus had a thick layer of jelly like material in the mucosa. The koilin layer of the ventriculus of Nicobari grower was pealed from the area just posterior to the isthmus and pushed into the lumen by the protruding proventriculus (Fig. 4.54). Even though the proventricular serosa of the outer layer appeared normal coloured, the intussusceptumwas affected with intense congestion and haemorrhage (Fig. 4.55). No fibrinous adhesions found in any of the cases. Crop and gizzard contained only a small amount of feed particles. Histologically, degeneration and compression of the submucosal glands were found at affected areas.

Table 4.12a. Age wise occurrence of different types of intestinal lesions

Intestinal lesions	Chick (N=6439)	Grower (N=4647)	Adult (N=6892)	Total (N=17978)
Haemorrhagic enteritis	96	75	92	263
	(1.49)	(1.61)	(1.33)	(1.46)
Necrotic enteritis	4	13	7	24
	(0.06)	(0.28)	(0.10)	(0.13)
Catarrhal enteritis	698	181	192	1071
	(10.84)	(3.89)	(2.79)	(5.96)
Neoplastic lesions	0	0	36	36
	(0.00)	(0.00)	(0.52)	(0.20)
Miscellaneous	3	1	4	8
	(0.05)	(0.02)	(0.06)	(0.04)
Total	**8.1**	**272**	**332**	**1405**
	(12.44)	(5.85)	(4.82)	(7.82)

Figures in the parenthesis are percentage

Table 4.12b. Breedwise occurrence of intestinal lesions

Line name	Haermorrhagic enteritis	Catarrhal enteritis	Necrotic enteritis	Neoplastic lesions	Miscellaneous	Total intestinal lesions
White leghorn	61	113	2	14	3	193
(N=2095)	(2.91)	(5.39)	(0.10)	(0.67)	(0.14)	(9.21)
PB1	3	36	1	3	0	43
(N=832)	(0.36)	(4.33)	(0.12)	(0.36)	(0.00)	(5.17)
PB2	6	62	2	4	1	75
(N=702)	(0.85)	(8.83)	(0.28)	(0.57)	(0.14)	(10.68)
Krishibro	0	11	0	0	0	11
(N=108)	(0.00)	(10.19)	(0.00)	(0.00	(0.00)	(10.19)
Control Broiler	20	49	2	0	0	71
(N=1032)	(1.94)	(4.75)	(0.19)	(0.00)	(0.00)	(6.88)
Crosses	0	6	0	0	0	6
(N=109)	(0.00)	(5.50)	(0.00)	(0.00)	(0.00)	(5.50)
PD1	8	126	2	1	0	137
(N=1712)	(0.47)	(7.36)	(0.12)	(0.06)	(0.00)	(8.00)
PD2	71	200	2	2	4	79
(N=3880)	(1.83)	(5.15)	(0.05)	(0.05)	(0.10)	(7.19)
PD3	28	202	5	10	1	246
(N=4223)	(0.66)	(4.78)	(0.12)	(0.24)	(0.02)	(5.83)
GML	13	112	4	0	0	129
(N=1632)	(0.80)	(6.86)	(0.25)	(0.00)	(0.00)	(7.90)
ASEEL	4	38	2	1	2	47
(N=341)	(1.17)	(11.14)	(0.59)	(0.29)	(0.59)	(13.78)
Nicobari	19	48	1	0	0	68
(N=426)	(4.46)	(11.27)	(0.23)	(0.00)	(0.00)	(15.96)
Ghagus	15	26	1	1	0	43
(N=392)	(3.83)	(6.63)	(0.26)	(0.26)	(0.00)	(10.97)
Naked neck	12	26	0	0	0	38
(N=257)	(4.67)	(10.12)	(0.00)	(0.00)	(0.00)	(14.79)
Dwarf	3	15	0	0	1	19
(N=237)	(1.27)	(6.33)	(0.00)	(0.00)	(0.42)	(8.02)
Total	263	1070	24	36	12	1405
(N=17978)	(1.46)	(5.95)	(0.13)	(0.20)	(0.07)	(7.82)

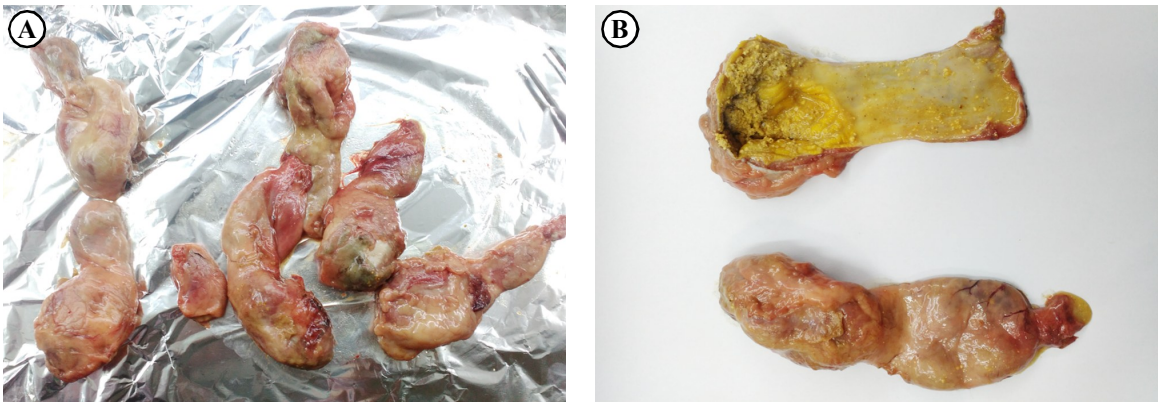


Fig. 4.51: Flaccid proventriculus and gizzard in broiler breeders(A). Very thin wall of both proventriculus and gizzard (B)

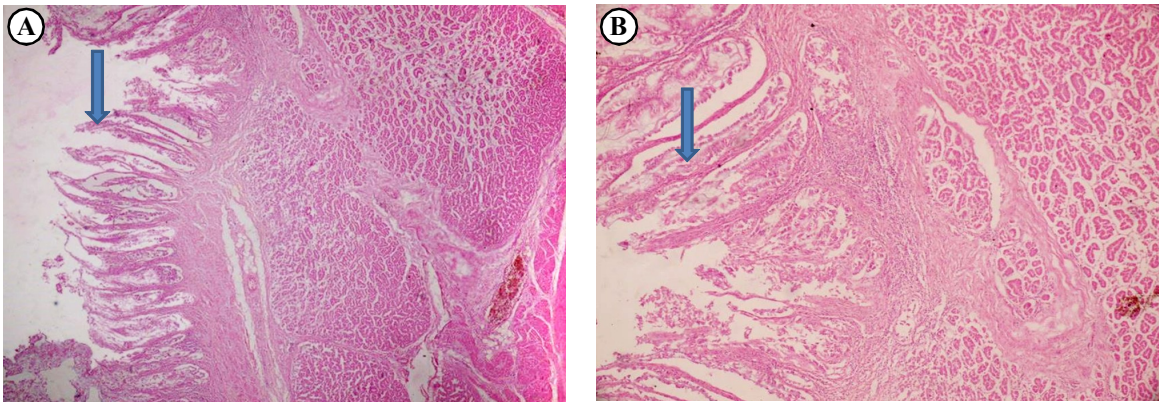


Fig. 4.52: Section of flaccid proventriculus. Severe denudation of mucosal epithelium, thinning of plicae and degeneration of glands H&E, (A) x40 and (B) x100

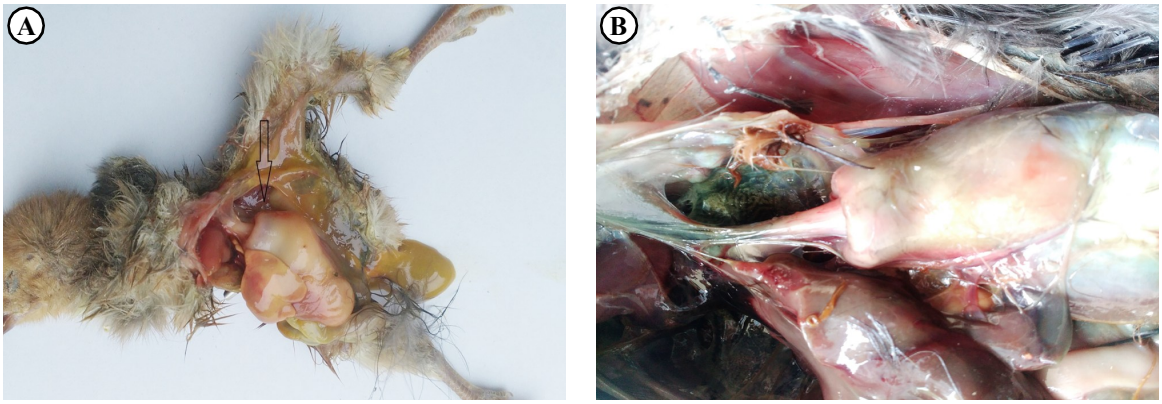


Fig. 4.53: Proventriculo-ventricular intussusception in 4 day (A) and 3 week (B) chicks of PD2

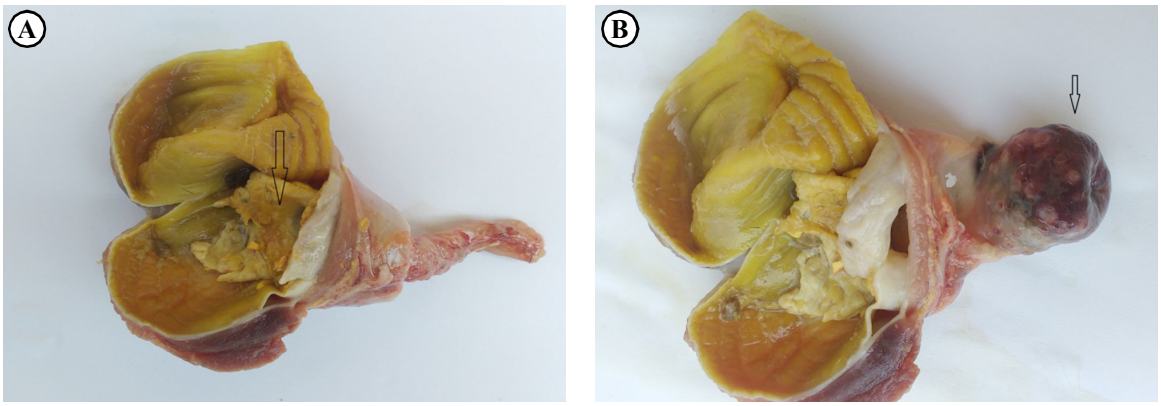


Fig. 4.54: Proventriculo-ventricular intussusception in Nicobari (A) koilin layer pushed into the ventricular lumen (arrow) (B) haemorrhagic proventriculus (arrow)

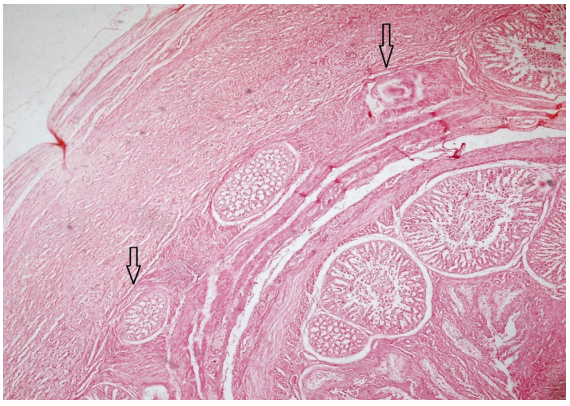


Fig. 4.55: Histopathology- Intususception in PD2. Atrophy of glands of outer layer (arrow) H&E, x40

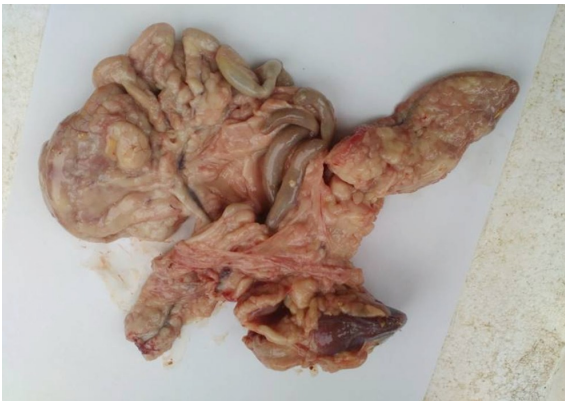


Fig. 4.56a: Miliary nodules in the serosa of proventriculus and intestine with thickened mesentary

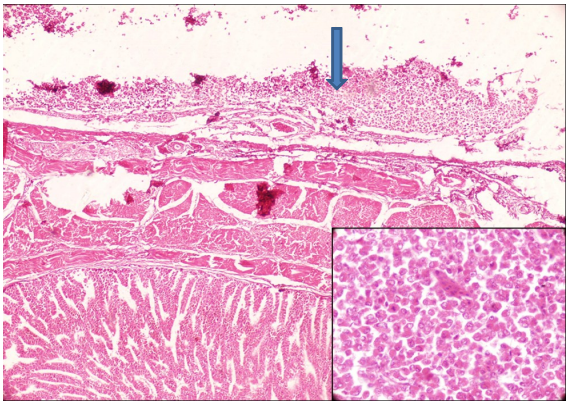


Fig. 4.56b: Histology of proventriculus with infiltration of myeloid cells in the serosa (arrow) H&E, x100 (insert-x1000)

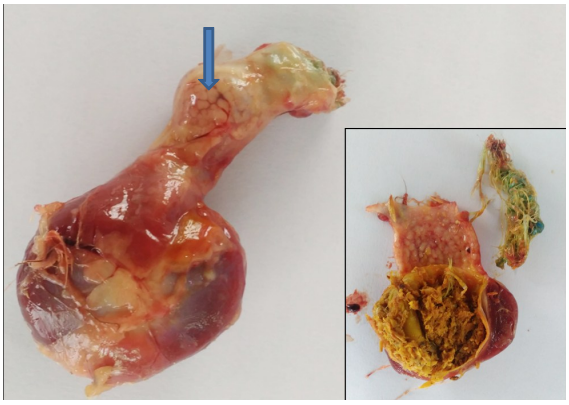


Fig. 4.57: Proventricular impaction by nylon thread in a PD3 chick

4.2.5.3 Serosal affections

Miliary nodular growth along the serosa of the proventriculus was noticed in three PD3 and in one White leghorn adult birds (Fig 4.56A). These nodules were observed extending from the intestinal serosa and mesentery. Tumors were detected in the ovary/ flat bones in all the four birds. Histologically myeloid cells were found proliferating on the serosa (Fig.4.56B).

4.2.5.4. Proventricular impaction by foreign bodies

Proventriculus was found impacted by nylon rope in a PD3 chick (Fig. 4.57). Proventricular mucosa was congested and gizzard contained small quantity of feed material. Microscopic examination revealed congestion in the mucosa and muscularis mucosa area and degeneration and necrosis of the glandular epithelium.

4.3 INTESTINAL LESIONS

A total of 1,405 (7.83%) out of 17,798 chickens were identified with gross intestinal lesions. The intestinal lesions were classified grossly as catarrhal enteritis, haemorrhagic enteritis, necrotic enteritis, neoplastic lesions, malformations and miscellaneous conditions. Age and breed wise occurrence of different types of intestinal lesions is shown in the Table 4.12a and. 4.12b

4.3.1 Haemorrhagic enteritis

Haemorrhagic enteritis was noticed in 263 (1.46%) out of 17,978 birds examined (Table 4.13) either as sporadic incident or as outbreak. The extent of haemorrhage varied from affection of single intestinal segment to extensive mucosal haemorrhage affecting entire length of intestine. Age group and breed wise occurrence of haemorrhagic enteritis in each breed/line is presented in Table 4.13. The occurrence of haemorrhagic enteritis in chicks, growers and adults was 1.49%, 1.61% and 1.33%, respectively (Table 4.11). The highest occurrence of haemorrhagic enteritis observed in Naked neck breed (4.67%) and zero in Krishibro. The breed wise and age wise data revealed that the haemorrhagic enteritis was highest in White Leghorn growers (8.41%), Naked neck chicks (7.94%), and Naked neck adults (7.84%). Segment wise occurrence of haemorrhagic enteritis in each breed is shown in

Table 4.14. Occurrence of haemorrhagic enteritis among different segments was found to be high (31.94%, 84/263) in rectal region, followed by caecal region (26.24%, 69/263), and lowest in duodenal region (0.38%, 5/263). Breed wise occurrence of haemorrhagic enteritis affecting the jejunum and ileum was highest in naked neck (77.78%, 7/9). On segment wise haemorrhage, affection of jejunum, ileum and caecum in Ghagus (60.0%, 9/15) and Control broiler (60%, 12/20) were high. Examination of intestinal scrapings revealed coccidiosis in 166 birds. Age and segment wise occurrence of haemorrhagic enteritis due to coccidiosis is presented in the Table 4.15. In coccidiosis cases, caecal haemorrhagic enteritis had the highest occurrence in chick (37.23%, 35/94) and growers (46.48%, 33/71). The rectal haemorrhages observed were non-coccidial cause, and observed mostly in adults (29.27%, 77/263) and a few in growers (2.66%, 7/ 263)

4.3.1.1. Gross lesions

Haemorrhagic enterits affecting the mid intestinal portion was characterised by ballooning of intestine and red or black coloured petechiae (salt and pepper appearance) visible from serosa (Fig. 4.58A). The characteristic ballooning was starts from middle of jejunum and ending towards middle of ileum (Fig. 4.58B). In the affected segments, lumen was filled with blood, mucus, tissue debris and gas. In haemorrhagic typhlitis, the caecum was bulged with blood mixed contents. Petechiae were observed from the serosa itself (Fig. 4.59). In a few cases haemorrhage was observed in cecum along with other segments (Fig 4.60).

In a white leghorn bird, diffuse congestion and haemorrhage on entire length of the intestinal mucosa and large amount of viscid mucus in the intestinal lumen along with severe congestion of the mesenteric blood vessels, mesenteric and pericardial petechiae and cardiac dilatation were observed. Liver of the bird had white foci distributed all over the lobes. A few birds were ailing chronically in the flock. No coccidia found in the intestine.

The rectal haemorrhage observed in adults and growers were patchy and linear (Fig. 4.61) sparing other intestinal segments. Various concurrent conditions observed with rectal haemorrhage were congestion of mesenteric blood vessels (20/84), nephritis (15/84), polyserositis (19/84) and proventricular mucosal haemorrhage(4/84).

Table 4.13. Breed and age wise occurrence of haemorrhagic enteritis

Breed	Birds examined				Birds with haemorrhagic enteritis			
	Chick	Grower	Adult	Total	Chick	Grower	Adult	Total
White leghorn	405	464	1226	**2095**	1 (0.25)	39 (8.41)	21 (1.71)	61 (2.91)
PB1	517	165	150	**832**	1 (0.19)	0 (0.00)	2 (1.33)	3 (0.36)
PB2	312	207	183	**702**	2 (0.64)	2 (0.97)	2 (1.09)	6 (0.85)
Krishibro	101	7	0	**108**	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)
Control	375	462	195	**1032**	18 (4.80)	1 (0.22)	1 (0.51)	20 (1.94)
Broiler Crosses	47	42	20	**109**	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)
PD1	885	359	468	**1712**	0 (0.00)	0 (0.00)	8 (1.71)	8 (0.47)
PD2	969	751	2160	**3880**	30 (3.10)	8 (1.07)	33 (1.53)	71 (1.83)
PD3	1051	1220	1952	**4223**	2 (0.19)	10 (0.82)	16 (0.82)	28 (0.66)
GML	1025	480	127	**1632**	2 (0.20)	9 (1.88)	2 (1.57)	13 (0.80)
ASEEL	124	93	124	**341**	0 (0.00)	3 (3.23)	1 (0.81)	4 (1.17)
Nicobari	233	135	58	**426**	18 (7.73)	0 (0.00)	1 (1.72)	19 (4.46)
Ghagus	277	61	54	**392**	15 (5.42)	0 (0.00)	0 (0.00)	15 (3.83)
Naked neck	63	143	51	**257**	5 (7.94)	3 (2.10)	4 (7.84)	12 (4.67)
Dwarf	55	58	124	**237**	2 (3.64)	0 (0.00)	1 (0.81)	3 (1.27)
Total	**6439**	**4647**	**6892**	**17978**	**96** (1.49)	**75** (1.61)	**92** (1.33)	**263** (1.46)

(Figures in the parenthesis are percentage)

Table 4.14. Breed and Segment wise occurrence of haemorrhagic enteritis

	Jejunum, ileum, caecum & rectum	Duodinum	Dudodinum to caecum	Duodinum to ileum	Jejunum and ileum	Jejunum to caecum	Caecum	Ileum and rectum	Jejunum, ileum and rectum	Rectum	Total
White leghorn	1 (1.64)	0.00	1 (1.64)	1 (1.64)	11 (18.03)	11 (18.03)	18 (29.51)	0	0	18 (29.51)	61
PB1	0	0	0	0	0	0	1 (33.33)	0	0	2 (66.67)	**3**
PB2	0	0	0	0	1 (16.67)	0 0	3 (50.00)	0	0	2 (33.33)	6
Krishibro	0	0	0	0	0	0	0	0	0	0.00	**0**
Control Broiler	0	1 (5.00)	0	0	4 (20.00)	12 (60.00)	2 (10.00)	0 0	0 0	1 (5.00)	20
Crosses	0	0	0	0	0	0	0	0	0	0.00	**0**
PD1	0	0	0	0	0	0	0	0	0	8 (100)	8
PD2	0	0	0	2 (2.82)	11 (15.49)	5 (7.04)	18 (25.35)	8 (11.27)	1 (1.41)	26 (36.62)	71
PD3	1 (3.13)	0	0	0	2 (6.25)	0	7 (21.88)	0	0	22 (68.75)	32
GML	0	0	2 (15.38)	0	1 (7.69)	0	8 (61.54)	0	0	2 (15.38)	13
ASEEL	0	0	0	0	1 (25.00)	0	2 (50.00)	0	0	1 (25.00)	4
Nicobari	0	0	0	0	0.00	9 (47.37)	9 (47.37)	0	0	1 (5.26)	19
Ghagus	0	0	0	0	5 (33.33)	9 (60.00)	1 (6.67)	0	0	0	15
Naked neck	0	0	0	0	7 (77.78)	1 (11.11)	0	0	0	1 (11.11)	9
Dwarf	0	0	0	0	0	2 (100)	0 0	0 0	0 0	0 0	2
total	**2** (0.76)	**1** (0.38)	**3** (1.14)	**3** (1.14)	**43** (16.35)	**49** (18.63)	**69** (26.24)	**8** (3.04)	**1** (0.38)	**84** (31.94)	**263**

(Figures in the parenthesis are percentage)

Table 4.15. Age and segment wise occurrence of coccidiosis

Segments affected with haemorrhage	Chick (N=94)	Grower (N=71)	Adult (N=1)	Total (n=166)
Jejunum, Ileum,caecum	35 (37.23)	12 (16.90)	0	**47 (28.31)**
Jejunum, ileum	23 (24.47)	19 (26.76)	1 (100)	43 **(25.90)**
Caeum	35 (37.23)	33 (46.48)	0 0	68 **(40.96)**
Duodinum, jejunum, ileum, caecum	0	3 (4.23)	0	3 **(1.81)**
Ileum to rectum	0	2 (1.41)	0 0	2 **(0.60)**
Jejunum, ileum, caecum, rectum	0	2 (2.81)	0 0	2 **(1.21)**
jejunum , ileum rectum	1 (1.06)	0 0	0 0	1 **(0.60)**

(Figures in the parenthesis are percentage)

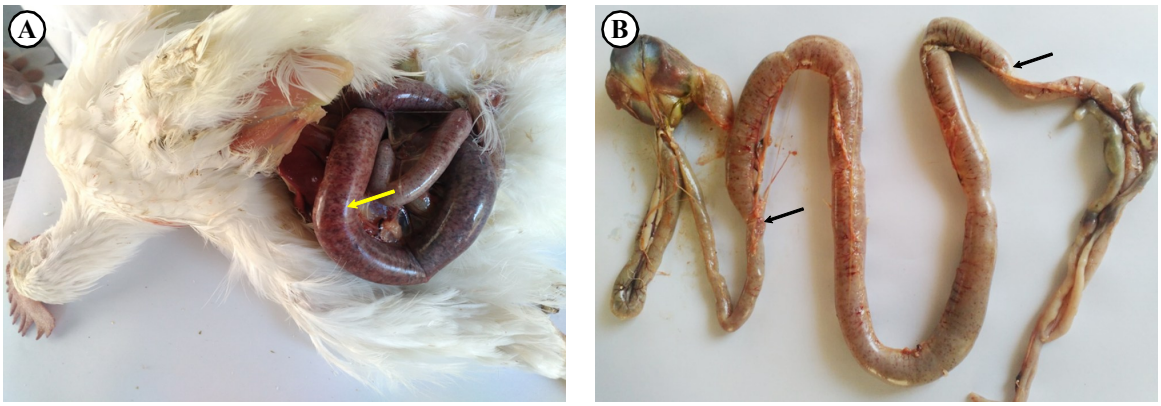


Fig. 4.58: Haemorrhagic enteritis and ballooning of mid intestine. (A) Red coloured petechae mixed with white plaques (Salt and pepper appearance) is visible from serosa (arrow) (B) Haemorrhagic enteritis-Ballooning of intestine from mid of jejunum to mid of ileum (between arrows)

Fig. 4.59: Haemorrhagic typhlitis. Petchae visible from serosa (arrow)

Fig. 4.60: Haemorrhagic enteritis affecting both caecum and small intestine

Fig. 4.61: Patchy and linear haemorrhage in the rectal mucosa

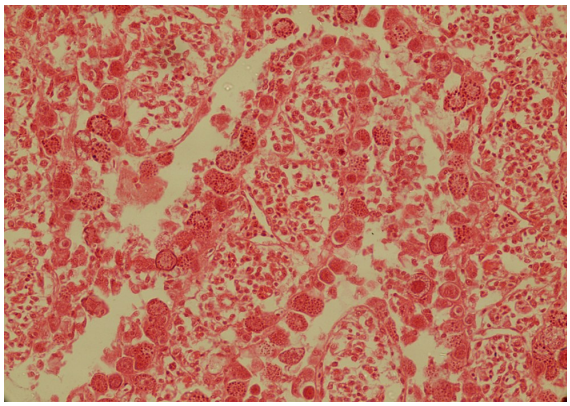


Fig. 4.62: Sexual life stages of coccidia in the intestinal villi epithelium H&E, x100

4.3.1.2 Microscopic lesions

The haemorrhagic portions of small intestine showed moderate to severe mucosal haemorrhage, degeneration and denudation of villi epithelium, mild to moderate proliferation of mononuclear cells and heterophils in the lamina propria. In the coccidiosis cases, different life cycle stages of coccidial organisms were found in the luminal contents, epithelium of villi (Fig. 4.62), crypts (Fig. 4.63) and in some cases in submucosa (Fig. 4.64). Small intestinal sections were mostly affected with asexual stages of coccidia like groups of large schizonts (Fig. 4.65). The coccidial organisms were found in various intestinal segments including rectum.

In the White Leghorn adult chicken without coccidial infestation, diffuse haemorrhage and severe congestion in the mucosa was noticed with mild infiltration of the inflammatory cells. No protozoans could be identified in the intestinal sections. Liver revealed coagulative necrosis of hepatocytes. Heart blood smear of the birds revealed moderate number of bipolar organisms. (No bipolar organisms found in the birds of same flock which were chronically ill).

Rectal haemorrhage in the adult and growers were mostly restricted to the tip of the villi (Fig. 4.66) with mild degenerative changes of villi epithelium. No other major histological lesions were noticed.

4.3.1.3 Molecular diagnosis

Single and mixed infestaton of *E tenella* and *E necatrix* were confimed by PCR (Fig. 4.67). Two cases of linear haemorrhage in the rectum along with nephritis in adults birds were positive for ChPV and ANV by PCR. The intestine of white leghorn birds were found negative for FAdV 1.

4.3.2 Necrotic enteritis

A total of 24 (0.13%) out of 17,978 cases of necrotic enteritis involving jejunum, ileum, cecum and rectum, was observed during the study. Age and breed wise occurrence of necrotic enteritis is shown in Table 4.16. The total occurrence was highest in the growers (0.28%, 13/4647) compared to other age groups and in Aseel (0.59%, 2/341) than the other breeds. Details of age and intestinal segments affected is shown on the Table 4.17. Chicks

exhibited 50% (2/4) of necrotic enteritis as necrotic typhlitis while that of growers were 84.62% (11/13) in the caecum. In adults, affection of caecum alone and affection of distal ileum, caecum and rectum together were 42.86% (3/5) each.

4.3.2.1. Gross lesions

Necrotic enteritis were observed either as diffuse lesions, affecting a large area of mucosa of intestinal segment or as focal ulcerartion with or without diphteritic membrane formation. In the diffuse type diphtheritic membrane formation in the mucosa were observed along with formation of necrotic cores in the lumen (Fig 4.68). This type of lesions often found in the caecum concurrently with catarrhal enteritis of small intestine or infrequently with haemorrhagic enteritis of small intestine (Fig. 4.69). Bulging of caecum with friable necrotic cores and diffuse mucosal necrosis was evident in 14/24 cases. In a PB2 adult bird, necrotic typhlitis with blood clot and necrotic cores were observed along with severe proventricular thickening and mucosal ulceration (MDV identified) (Fig.4.70). Distal ileum engorged with necrotic core and diffuse mucosal ulceration and diphtheritic membrane formation was observed in a PB1 chick (Fig. 4.71). The caecum of the chick showed necrotic changes. Bulging of the intestine with necrotic core and content mixed fluid was observed in the mid intestinal region in a white leghorn adult bird (Fig. 4.72). The necrotic core has about 5cm length and 3cm diameter occluding the intestinal lumen and the proximal part of intestine was bulged out by content mixed fluid up to a length of 15cm in the mid intestine. The lumen of the intestine distal to the necrotic core was empty. In a layer chick, necrotic enteritis was detected in the proximal part of ileum, with necrotic contents.

In the focal type, very small ulcers or button shaped ulcers, often covered with thin diphtheritic membrane were found distributed in the mucosa of caecum, distal ileum and rectum. Nicobari grower and control broiler adult birds exhibited small (0.5-1mm diameter) button ulcers in the caecal mucosa with semisolid necrotic contents (Fig.4.73). Similar button ulcers with diphtheritic membrane formation and granular eruptions in the mucosa affecting distal ileum, caecum and rectum was observed in an Aseel grower, two PD2 and one PD3 adutls. When small focal necrosis was observed in the rectum, ileum and caeca of PD3 adult

Table 4.16. Breed and age wise occurrence of necrotic enteritis

Breed	Chick	Grower	Adult	Total
White leghorn	1(0.25)	0	1(0.08)	**2**(0.10)
PB1	1(0.19)	0	0	**1**0.12)
PB2	0	1(0.48)	1(0.55)	**2**(0.28
Krishibro	0	0	0	**0**(0.00)
Control Broiler	0	1(0.22)	1(0.51)	**2**(0.19)
Crosses	0	0	0	**0**(0.00)
PD1	1(0.11)	0	1(0.21)	**2**(0.12)
PD2	0	0	2 (0.09)	**2**(0.05)
PD3	0	4(0.33)	1(0.05)	**5**(0.12)
GML	0	4(0.83)	0	**4**(0.25)
ASEEL	0	2(2.15)	0	**2**(0.59)
Nicobari	0	1(0.74)	0	**1**(0.23)
Ghagus	1(0.36)	0	0	**1**(0.26)
Naked neck	0	0	0	0
Dwarf	0	0	0	0
total	**4**(0.06)	**13**(0.28)	**7**(0.10)	**24**(0.13)

(Figures in the parenthesis are percentage; number of birds in an age group of the concerned line is used for calculating percentage)

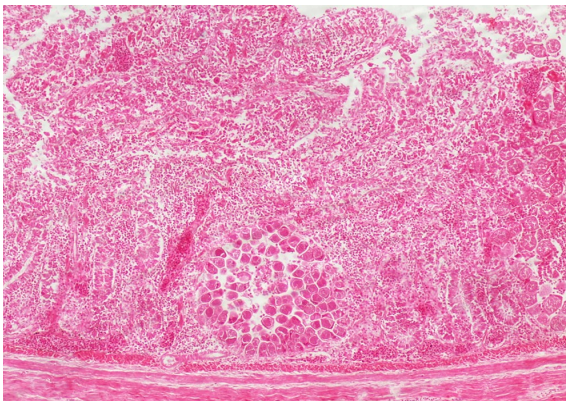


Fig. 4.63: Cluster of coccidial life stages in the caecum H&E, x100

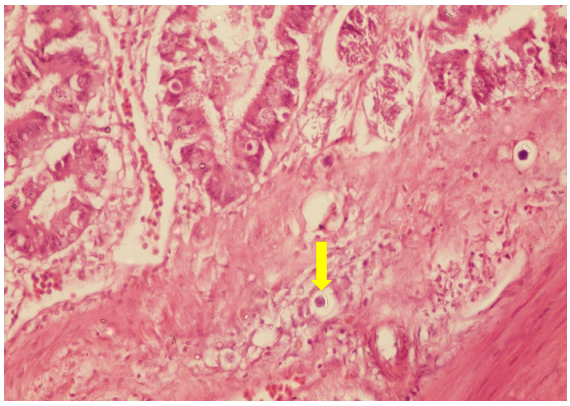


Fig. 4.64: Haemorrhagic enteritis: Oocysts in the crypts and submucosa (arrow) of caecum H&E, x400

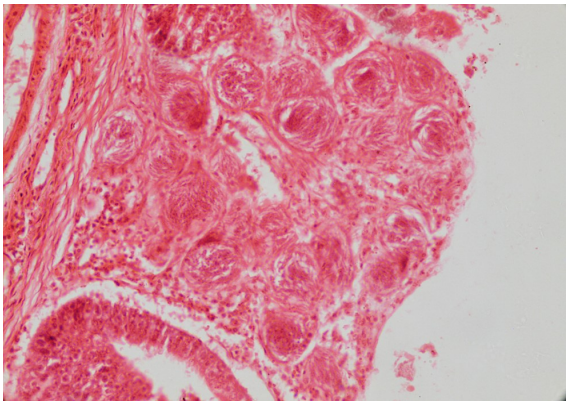


Fig. 4.65: Eimeria- Schizonts in the intestine H&E, x100

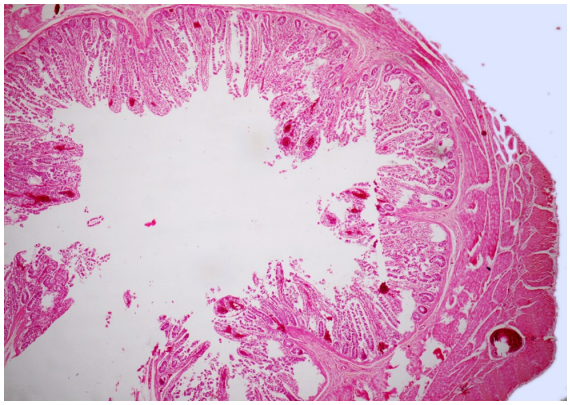


Fig. 4.66: Rectal haemorrhage- Congestion and haemorrhage mostly affecting tip of villi H&E, x40

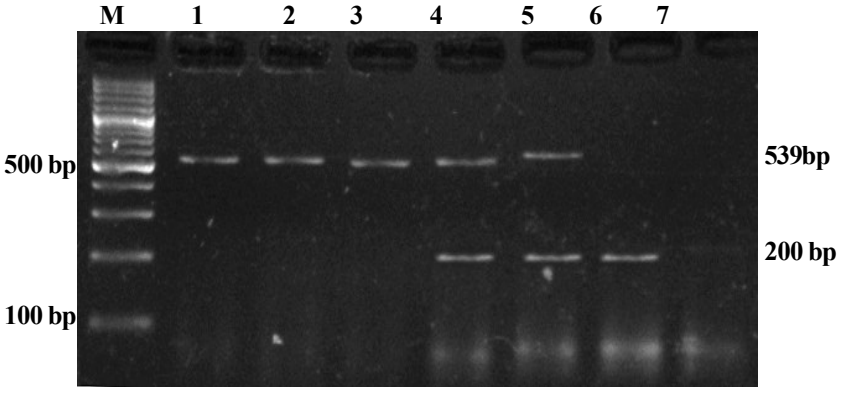


Fig. 4.67: Ethidium bromide stained 1.5% agarose gel showing amplification of *Eimeria tenella* (539bp) and *Eimeria necatrix* (200bp) duplex PCR

Lane M : 100 bp DNA ladder
Lanes 1-3 : Positive samples (539bp)
Lanes 4-5 : Positive samples (539bp and 200 bp)
Lane 6 : Positive samples (200bp)
Lane 7 : Negative control

Fig. 4.68: Necrotic typhlitis with caecal core formation

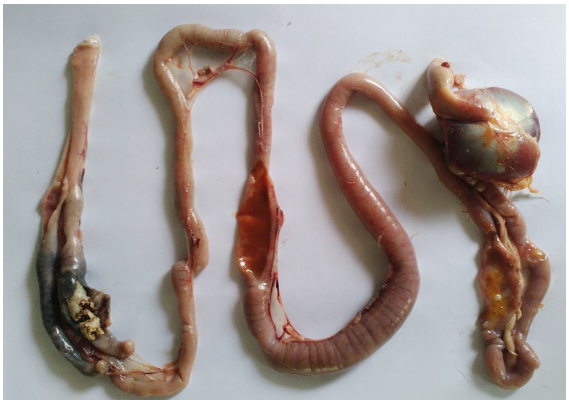


Fig. 4.69: Necrotic typhlitis along with haemorrhagic enteritis of small intestine

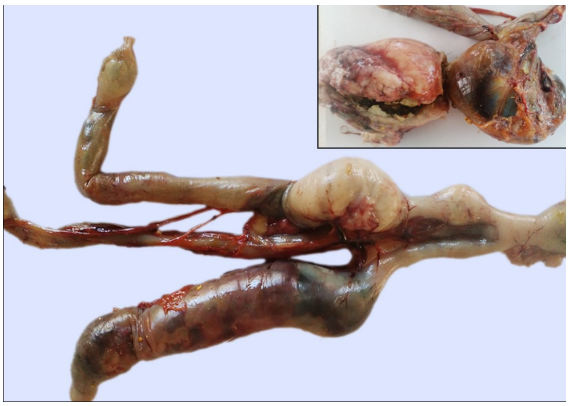


Fig. 4.70: Necrotic typhlitis along with neoplastic proventricular lesion (insert) in a PB2 adult bird

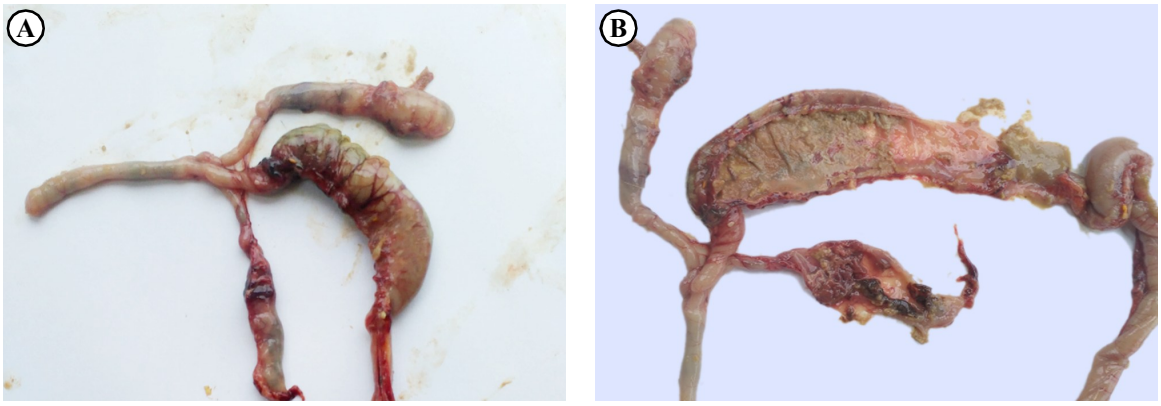


Fig. 4.71: Necrotic enteritis of distal ileum and caecum in a PB1 chick (A) serosal view (B) Diphtheritc membrane formation in the mucosa

Fig. 4.72: Necrotic enteritis with necrotic core in the middle intestine of a White leghorn adult bird. insert- necrotic core in the lumen

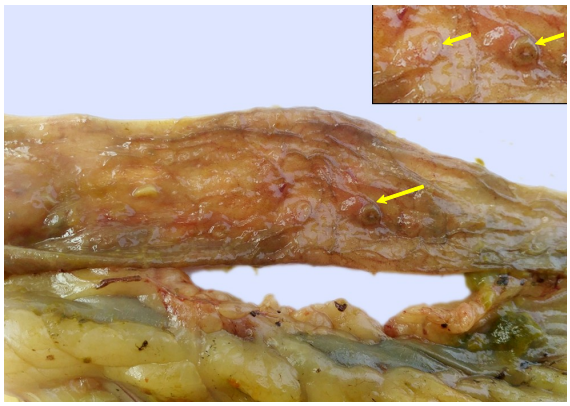


Fig. 4.73: Button ulcers in the caecum of control broiler adult bird (arrow). insert - enlarged view

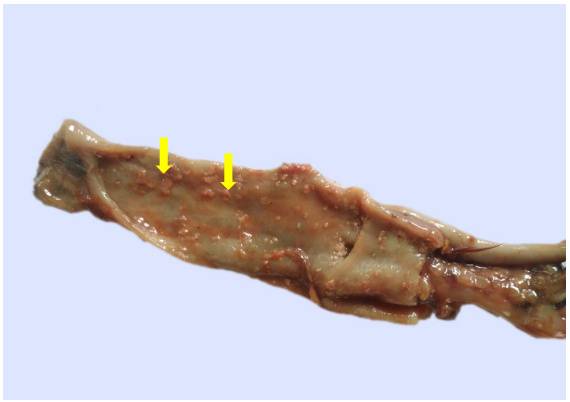
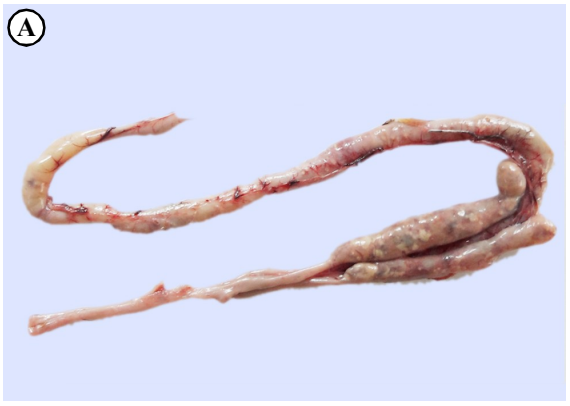


Fig. 4.74: Focal ulceration and diphtheretic membrane formation in the rectum (arrow) of a PD3 adult bird

Fig. 4.75: Mucosal necrosis and granular eruptions in the mucosa of distal ileum, rectum and mildly in caecum of a PD2 adult bird



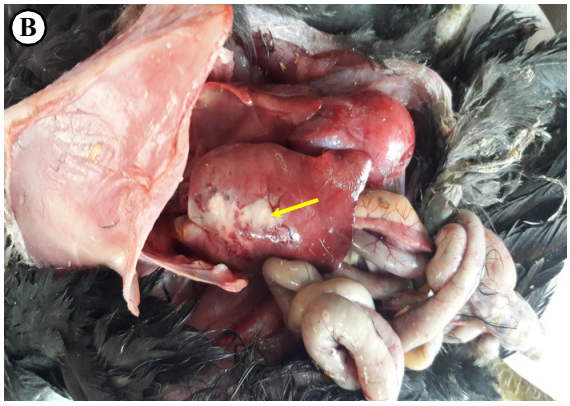


Fig. 4.76: Necrotic typhlitis (A) and white patchy area surrounded by haemorrhagic zone in the liver (arrow) of Aseel grower (B) (Histomoniasis)

(Fig. 4.74), severer form of granular necrosis affecting the distal ileum , rectum and mildly on caecum were noticed in PD2 adults (Fig. 4.75). The Aseel grower had focal button ulcers distributed along the length of distal ileum and rectum, while the caecum evinced severe diffuse necrosis with semisolid necrotic core. The liver of the bird was mildly enlarged with patchy white areas surrounded by haemorrhagic zone (Fig. 4.76 and 4.77).

Volvulus resulted in necrotic enteritis of ileum and caecum in a PD1 adult was characterised by brownish, coagulated foul smelling intestinal content and diphtheritic membrane formation in the mucosa.

4.3.2.2 Microscopic lesions

Moderate to severe infestation of coccidia was evident in 10 growers and 2 chicks with necrotic typhlitis. Various coccidial life stages and oocysts were evident in the mucosa and luminal necrotic contents (Fig. 4.78). The necrotic cores were composed of dead tissues, feed contents, heterophils and large number of oocysts. Whole thickness of the caecum was necrotised in varying degrees. Severe infiltration of inflammatory cells, predominantly mononuclear cells were observed in the mucosa and lamina propria. Infiltration extended into the muscularis externa in a few cases. The caecum of PB2 adult bird showed severe degree of necrosis with coccidal oocysts and lymphocytic proliferation in the whole layer including muscularis externa.

Histological examination of intestine with gross necrotic lesions in the distal ileum, caecum and rectum revealed focal necrosis. The Aseel grower had the extensive necrotic lesions in the caecum affecting all the layers of caecal wall. Numerous spherical or ovoid bodies were observed in the mucosal and even to submucosal and muscular layers in the caecum and rectum (Fig 4.79). Oedema and necrosis of the muscular wall with infiltration of mononuclear cells especially lymphocytes and small numbers of heterophils were evident in the intestinal wall. Liver of the Aseel grower revealed multiple areas of haemorrhage and hepatocellular necrosis with numerous degenerated and non-degenerated spherical bodies with lacunae, similar to that observed in the intestine were suggestive of trophozoites of histomonads (Fig. 4.80). Congestion, haemorrhage, and infiltration of mononuclear cells were also evident at the site of

necrosis. Characteristic shape of the bodies in the liver (Fig. 4.81 and 4.82) and intestine indicate the bodies were trophozoites of *Histomonas meleagridis*. Similar ovoid bodies with lacunae were observed in the mucosa, submucosa and muscle layer of rectum and intestine were observed in rectum, ileum and caecum of PD3 adult and PD2 adults (Fig. 4.83 and 4.84). Submucosal affection of protozoans observed in a GML grower with caecal necrotic core formation and presence of protozoans in the lower intestine (Fig.4.85)

4.3.2.3. Molecular diagnosis

Marek's Disease virus was identified by PCR method and histological lesions in the PB2 adult bird with necrotic typhlitis along with severe proventricular thickening and ulceration. Further, proventriculus was also affected by MDV in this chicken.

Eimeria tenella and *Eimeria necatrix* were detected in cecal coccidiosis cases by PCR using specific primers. *Clostridium perfringens* were cultured in the Robertson's cooked meat broth and confirmed by PCR using species specific primers in all the necrotic cases (Fig. 4.86). *Clostridium colinum* was detected in two cases from the caecum affected with protozoan infection and ulceration.

4.3.3. Catarrhal enteritis

Catarrhal enteritis was observed in 5.96% (1071/17978) chickens with maximum occurrence in the chicks (10.84%; 698/6,439) followed by grower (3.94%; 183/4,647) and least in adults (2.79%; 192/6, 892) (Table 4.18). Occurrence of catarrhal enteritis among different breeds and age group is presented in Table 4.18.

4.3.3.1. Macroscopic lesions

Catarrhal enteritis characterized by congestion of intestinal mucosa, excess mucus mixed or watery content with varying severity was observed. Pale intestinal wall with watery content and gas bubbles were noticed in chicks (Fig. 4.88, Fig. 4.89) and growers along with smearing of faecal materials in the perianal feathers. Birds with stunted growth were showing catarrhal enteritis with or without the proventricular thickening in most of the cases. In a few cases of chicks with stunted growth, intestine had dehydrated appearance. In adults, catarrhal

Table 4.17. Age and segment wise occurrence of necrotic enteritis

	Chick (N=4)	Grower (N=13)	Adult (N=7)
Necrotic typhlitis	2(50)	11(84.62)	3(42.86)
Ileum and caecum	1(25)	0	0
Ileum	1(25)	0	1(14.29)
Jejunum and ileum	0	1(7.69)	0.00
Distal ileum,caecum and rectum	0	1(7.69)	3(42.86)

(Figures in the parenthesis are percentage)

Table 4.18 Breed and age wise occurrence of catarrhal enteritis

Breed	Chick	Grower	Adult	Total
White leghorn	42 (10.37)	25 (5.39)	46 (3.75)	113 (5.39)
PB1	20 (3.87)	8 (4.85)	8 (5.33)	36 (4.33)
PB2	38 (12.18)	15 (7.25)	9 (4.92)	62 (8.83)
Krishibro	10 (9.90)	1 (14.29)	0 (0.00)	11 (10.19)
Control Broiler	35 (9.33)	10 (2.16)	4 (2.05)	49 (4.75)
Crosses	2 (4.26)	3 (7.14)	1 (5.00)	6 (5.50)
PD1	105 (11.86)	13 (3.62)	8 (1.71)	126 (7.36)
PD2	121 (12.49)	35 (4.66)	44 (2.04)	200 (5.15)
PD3	128 (12.18)	29 (2.38)	46 (2.36)	206 (4.81)
GML	92 (8.98)	12 (2.50)	8 (6.30)	112 (6.86)
Aseel	19 (15.32)	13 (13.98)	6 (4.84	38 (11.14)
Nicobari	44 (18.88)	3 (2.22)	1 (1.72)	48 (11.27)
Ghagus	22 (7.94)	3 (4.92)	1 (1.85)	26 (6.63)
Naked neck	12 (19.05)	9 (6.29)	5 (9.80)	26 (10.12)
Dwarf	8 (14.55)	2 (3.45)	5 (4.03)	15 (6.33)
total	698 (10.84)	181 (3.89)	192 (2.79)	1071(5.96)

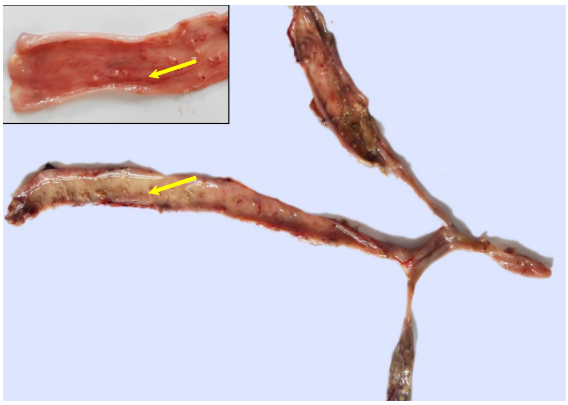


Fig. 4.77: Caecal necrosis and button ulcers in the mucosa of ileum and rectum (insert) of Aseel grower

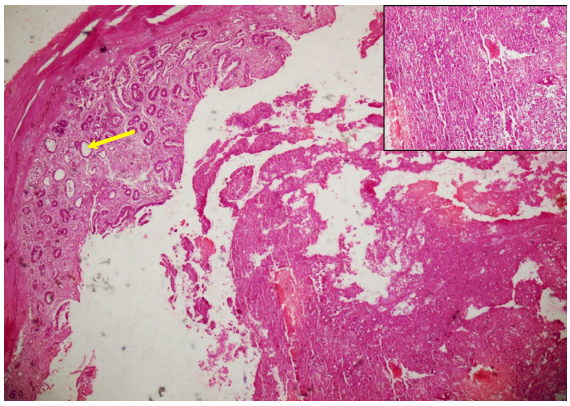


Fig. 4.78: Caecum- severe mucosal necrosis, cystic crypts formation (arrow) and large number of oocysts in the necrotic luminal content (insert) H&E, x40

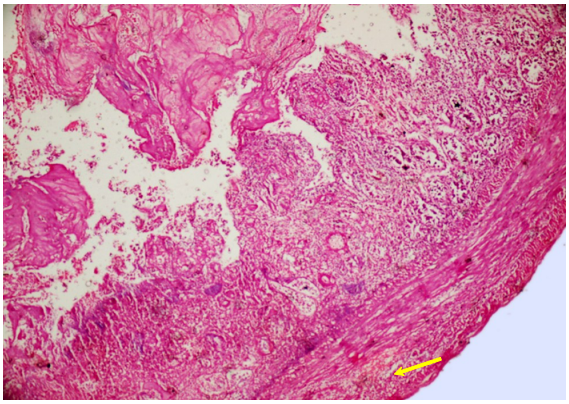


Fig. 4.79: Caecum of Aseel grower- Necrosis of all the layers with necrotic luminal content and infestation of histomonads in submucosa and muscular layer (arrow) H&E, x40

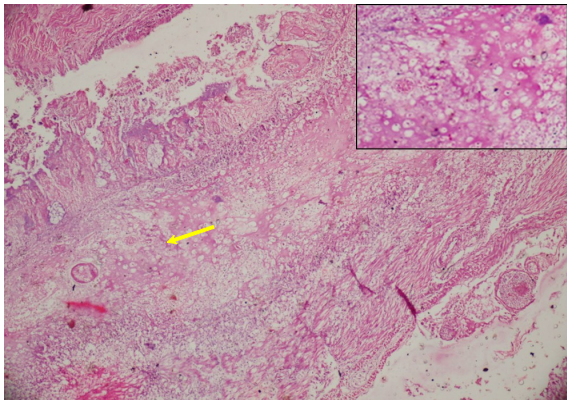


Fig. 4.80: Caecum of Aseel grower- Necrosis of all the layers and infestation of histomonads in submucosa and muscular layer H&E, x100. Muscular layer of caecum showing heavy infestation of protozoa (insert)

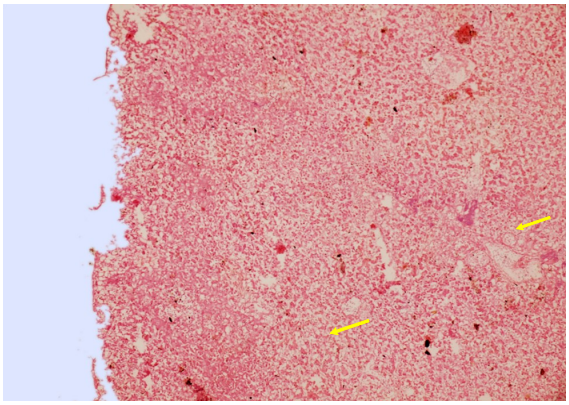


Fig. 4.81: Liver- Aseel grower- hepatocellular necrosis and accumulation of protozoa in the liver (arrow) H&E, x100

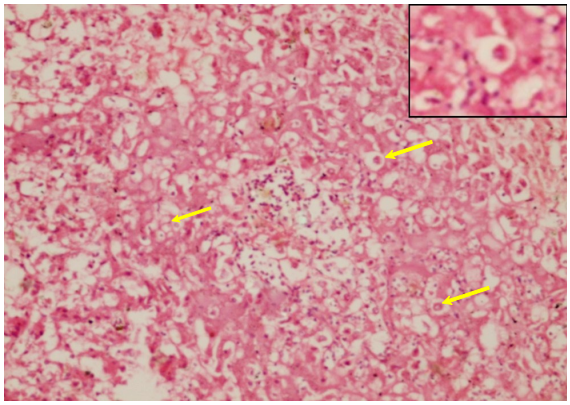


Fig. 4.82: Liver- Aseel grower- hepatocellular necrosis and accumulation of protozoa in the liver (arrow) H&E, x400 (insert -enlarged view)

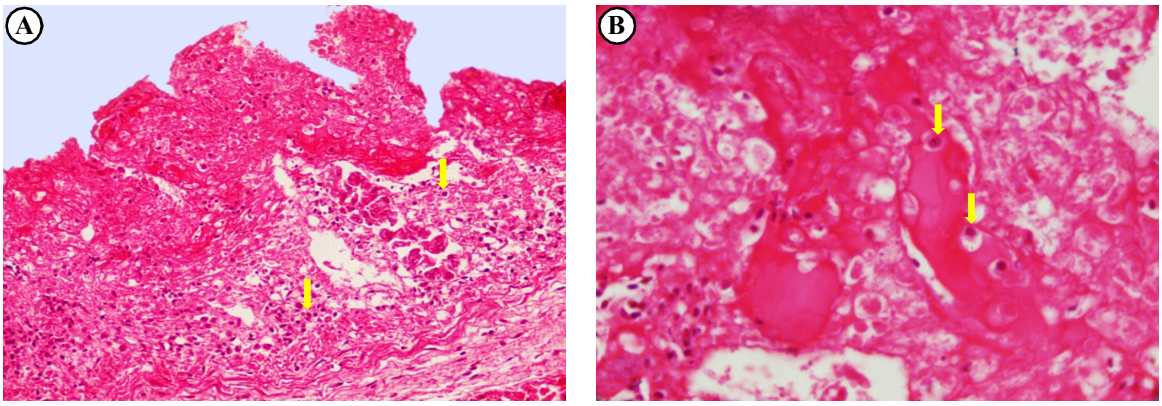


Fig. 4.83: Rectum of PD3 adult- Focal ulceration in the rectum with necrotic membrane formation and presence of oval nucleus with lacunae (arrows) (A) H&E, x100. Diphtheritic membrane with large number of oval shaped bodies of protozoa (arrows) (B) H&E, x600

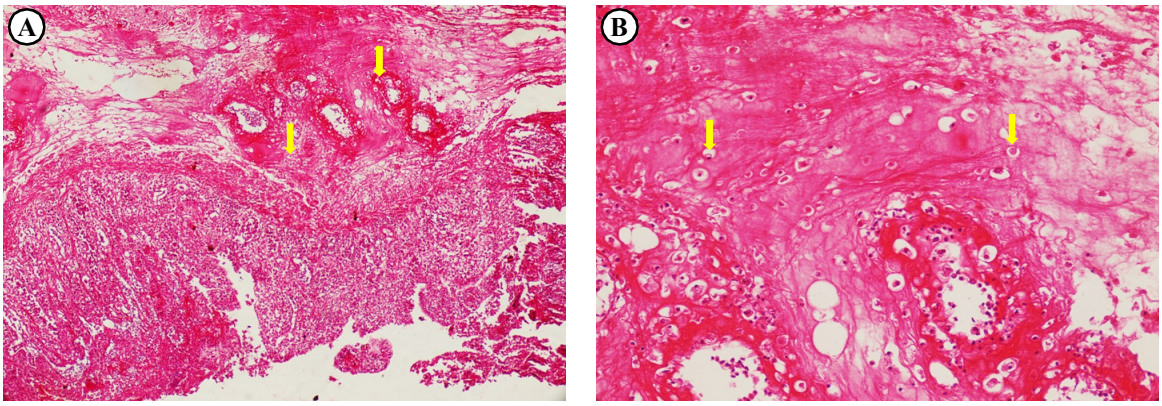


Fig. 4.84: Rectum PD2 adult birds. Necrosis of mucosa and submucosa with protozoa in the submucosa and muscle layer (arrows) (A) H&E, x100 (B) x400

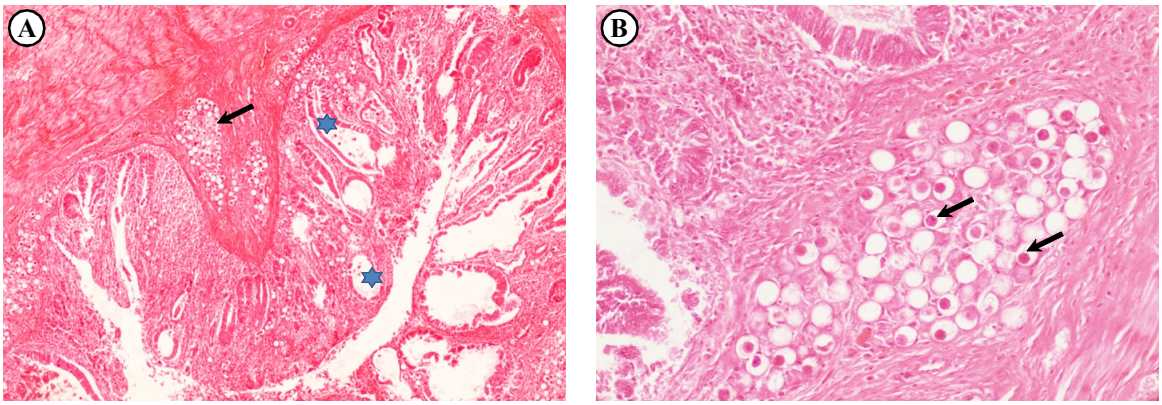


Fig. 4.85: Rectum of GML grower- mucosal necrosis, cystic dilatation of crypts (star) and heavy presence of protozoa in the submucosa. (A) H&E, x100 (B) x400

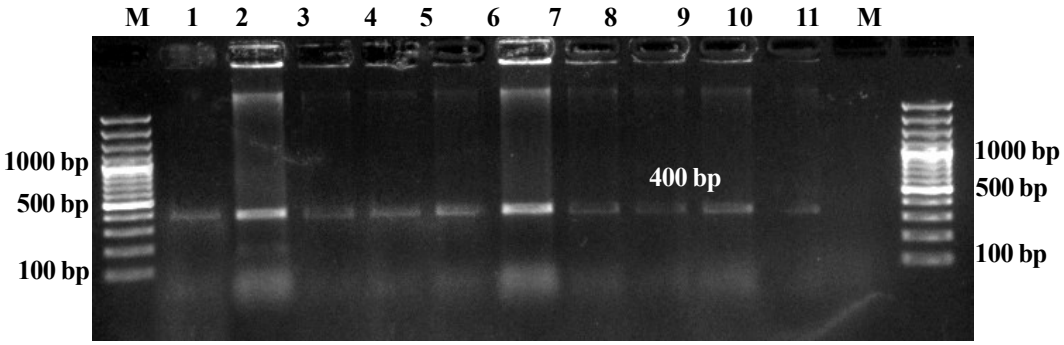


Fig. 4.86: Ethidium bromide stained 1.5% agarose gel showing amplification of *Clostridium perfringens* (400bp)
Lane M : 100 bp DNA ladder
Lanes 1-10 : Positive samples (400bp)
Lane 11 : Negative control

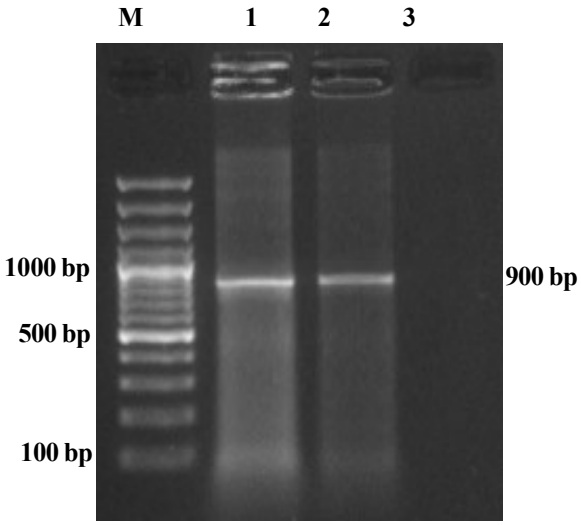


Fig. 4.87: Ethidium bromide stained 1.5% agarose gel showing amplification of *Clostridium colinum* (900bp)
Lane M : 100 bp DNA ladder
Lanes 1-2 : Positive samples (900bp)
Lane 3 : Negative control

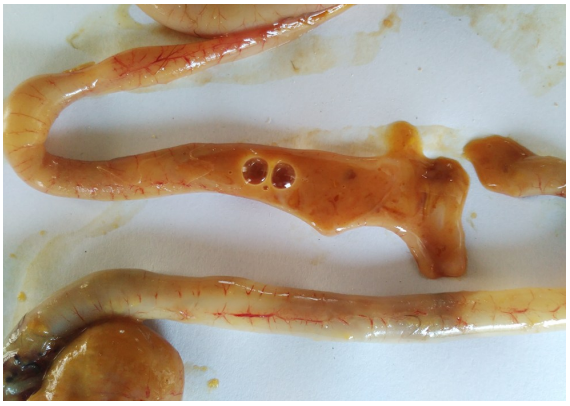


Fig. 4.88: Catarrhal enteritis with excess viscid mucus mixed fluidy intestinal content in a chick

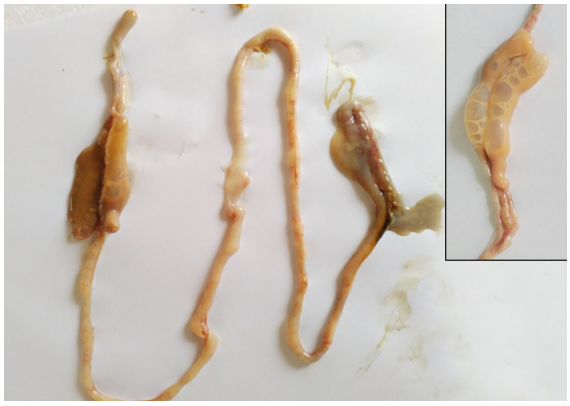


Fig. 4.89: Catarrhal enteritis with pale intestinal wall. Catarrhal entertis with gas bubbles in the caecum of a chick (insert)

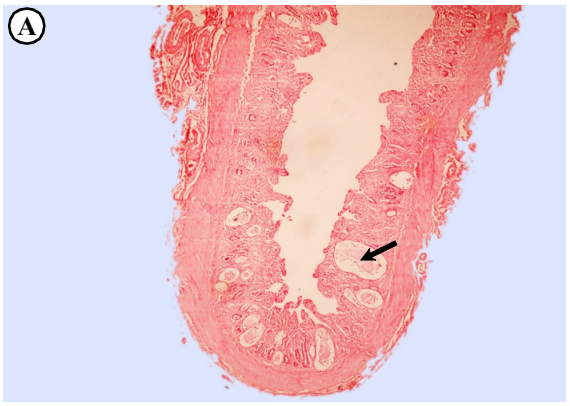


Fig. 4.90: Intestine – catarrhal enteritis along with proventricular thickening and RSS- cystic crypts and villi atrophy Duodinum (A) and Jejunum (B) H&E, x40

enteritis were mostly characterised by congested serosa and mucosa with excess mucus mixed contents.

4.3.3.2. Microscopic lesions

Histological examination revealed goblet cell proliferation, mild heterophilic and mononuclear cell infiltration in the mucosa and lamina propria. Cystic crypts were observed in the small intestine of chicks with runting-stunting syndrome (Fig. 4.90).

4.3.4. Molecular detection of enteric viruses

A total of 16 flocks were screened for 6 enteric viruses. The flocks comprises of 10 flocks with catarrhal enteritis and stunting with or without proventricular thickening, one catarrhal enteritis without stunting, two necrotic enteritis, two haemorrhagic enteritis and one without gross intestinal lesion. DNA and RNA isolated from the intestinal smaples were subjected to PCR and RT-PCR using specific primers. Age of sampled chickens varied from 1 week to 35 weeks. Details of the chickens with PM findings and screening results are given in Table 4.19 and and gel pictures in Fig. 4.91 to 4.96.

Atleast one virus were detected from all the samples. Most frequent enteric virus detected was ANV (87.5%, 14/16) ChPV (81.25%, 13/16), followed by, AvRtV(37.5%, 6/16), CAstV (18.75%, 3/16) and FAdV1(18.75%, 3/16). Most of the samples (87.5%; 14/16) revealed multiple viral infection (Table 4.20). ChPV alone was detected in two samples of PD3 growers showing runting-stunting syndrome (RSS) along with proventricular thickening and catarrhal enteritis.

RSS affected birds revealed 100% occurrence of ChPV (10/10). In two samples of stunted growth with catarral enteritis and proventricular enlargement, ChPV alone was detected. In the Krishibro flock with clinical signs of lameness and bone weakness, ChPV identified from both proventriculus and intestine and was found co-infected with ANV and CAstV. The ChPV was detected in native bird, Ghagus with stunted growth and was found co-infected with ANV. FAdV-1 was detected in a stunted chicken co-infected with ChPV, ANV, CAstV, and AvRtV.

4.3.4.1 Chicken parvovirus isolation

Enteric samples from which ChPV alone was detected were inoculated in to 7 day old chick embryo through yolk sac route after clarification and antibiotic treatment. Embryos collected 7th day PI, showed lesions like congestion, oedema and puffing along with runting (Fig. 4.97) on the first passage. All the embryos were runted on subsequent days of collection and passages with less prominent congestion and oedema of body (Fig. 4.98, 4.99). The embryos had runting with short neck and legs compared to control. The ChPV detected in the yolk and embryo visceral organ homogenates while other enteric viruses including IBV were not detected by PCR and Rt-PCR using species specific primers.

4.3.5 Neoplastic conditions in the intestine

Neoplastic lesions in the intestine were observed in a total of 36/17978 (0.2%) birds. All these intestinal tumors were identified in the adults only (Table 4.21).

4.3.5.1 Gross lesions

The tumours affecting intestine were grouped in to focal and diffuse types based on the gross appearance. In the diffuse type, miliary nodules were distributed diffusely all along the length of the intestinal serosa and mesentery where as in the focal type, localised and larger nodular tumors or thickening of intestinal wall were observed. The diffuse type was observed in 17 female adult chickens. The birds belonged to 7 flocks of 5 breeds, in which majority (9/16) were from a single flock of PD3 breeds. Miliary nodules in the intestinal serosa and thickened mesentery makes intestinal segments inseparable and thickened (Fig. 4.100). The wall of the intestine were uniformily thickened in many cases.. The miliary tumors present in the intestinal serosa were very small in size, 0.5- 2 mm diameter and firm in consistency. In a few cases the mesentery was smeared with yolk materials resembling nonneoplastic peritonitis (Fig.4.101). Concurrent gross lesions in other organs included, small white nodules in the liver (2cases), atrophy or cauliflower like growth of the ovary (14/17) (Fig. 4.102), tumor in the oviduct (Fig. 4.103) (1/17) tumors emerging from flat bones (3/17) (Fig 4.104) and pectoral muscles (1/17).

Table 4.19. Details of chicken flocks and results of PCR for enteric viruses screened

Sl. no.	Sample ID	Breed	Age (weeks)	RSS	Gross lesion	ChPV	AvRtV	ANV	ChAstV	AvRV	FAdV
1	Gut 105	Punjab Broiler	9	No	Necrotic enteritis and volvulus	-	-	+	-	-	+
2	Gut 206	White leghorn	7	Yes	Stunted growth, catarrhal enteritis	+	+	+	-	-	-
3	Gut 229	PD2	35	No	Haemorrhagic enteritis, coccidiosis	-	+	+	-	-	-
4	Gut 428	Ghagus	8	Yes	Stunted , gaseous, watery intestinal content	+	-	+	-	-	-
5	Gut 446	Ghagus	10	Yes	Stunted and dehydrated	+	-	+	-	-	-
6	Gut 450	PD3	9	Yes	Stunted, enlarged proventriculus, catarrhal enteritis	+	-	-	-	-	-
7	Gut 457	PD2	20	No	Congestion and ulceration of distal ileum and rectum, Histomoniasis	-	-	+	-	-	+
8	Gut 461	PD3	10	Yes	Stunted, enlarged proventriculus, catarrhal enteritis	+	-	-	-	-	-
9	Gut 465	Krishibro	6	Yes	Stunted and weak bone, enlarged proventriculus and watery intestinal contents	+	-	+	+	-	-
10	Gut 472	PD2	6	Yes	Stunted, enlarged proventriculus, watery intestinal content	+	+	+	+	-	+
11	Gut 473	White leghorn	1	Yes	stunted, watery intestinal content	+	+	+	-	-	-
12	Gut 475	Naked neck	4	Yes	Stunted growth and dehydration	+	+	+	+		
13	Gut 480	White leghorn	4	Yes	Stunted, enlarged proventriculus, watery gaseous intestinal contents	+	+	+	-	-	-
14	Gut 481	PD3	24	No	Rectal haemorrhage and nephritis	+	-	+	-	-	-
15	gut 483	PD3	10	No	No gross lesion in intestine	+	-	+	-	-	-
16	gut 484	Whitle leghorn	4	No	Mild catarrhal enteritis	+	-	+	-	-	-

Table 4.20. Occurrence of combination of enteric viral infections in 16 flocks

Virus detected	Inicdence (n=16)	
ChPV,ANV, ChAstV,AvRtV, FAdV1	6.25%	(n= 1)
ChPV,ANV, ChAstV,AvRtV,	6.25%	(n= 1)
ChPV,ANV,AvRtV	18.75%	(n= 3)
ChPV,ANV, ChAstV	6.25%	(n= 1)
ChPV,ANV,	31.25%	(n= 5)
ANV, FAdV	12.5%	(n= 2)
ANV, AvRtV	6.25%	(n= 1)
ChPV	12.5%	(n= 2)

Table 4.21. Age and breed wise occurrence of neoplastic lesions

Breed	Chick	Grower	Adult			Total (N=17978)
			Mileary nodules	Large nodules	Total (N=6892)	
White leghorn	0	0	4(0.33)	10(0.82)	14(1.14)	14 (0.67)
PB1	0	0	0	3(2.0)	3(2.0)	3 (0.36)
PB2	0	0	1(0.55)	3(1.64)	4(2.19)	4 (0.56)
Krishibro	0	0	0	0	0	0
Control Broiler	0	0	0	0	0	0
Crosses	0	0	0	0	0	0
PD1	0	0	1(0.21)	00	1(0.21)	1 (0.06)
PD2	0	0	1(0.05)	1(0.05)	2(0.09)	2(0.05)
PD3	0	0	9(0.46)	1(0.05)	10(0.51)	10(0.24)
GML	0	0	00	00	00	0
Aseel	0	0	1(0.81)	00	1(0.81)	1(0.29)
Nicobari	0	0	00	00	00	0
Ghagus	0	0	00	1(1.85)	1(1.85)	1(0.26)
Naked neck	0	0	00	00	00	0
Dwarf	0	0	00	00	00	0
Total	0	0	17(0.33)	19(0.27)	36(1.14)	36(0.20)

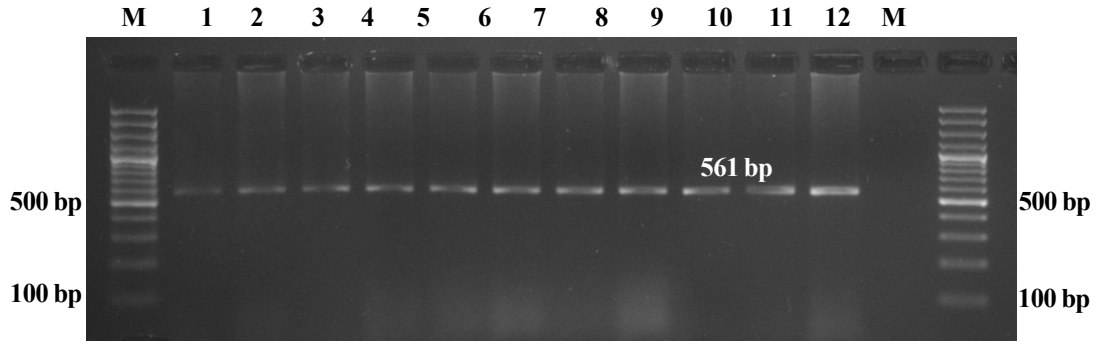


Fig. 4.91: Ethidium bromide stained 1.5% agarose gel showing amplification of 561 bp fragment of ChPV

Lane M : 100 bp DNA ladder
Lanes 1-11 : Positive samples (561bp)
Lane 12 : Negative control

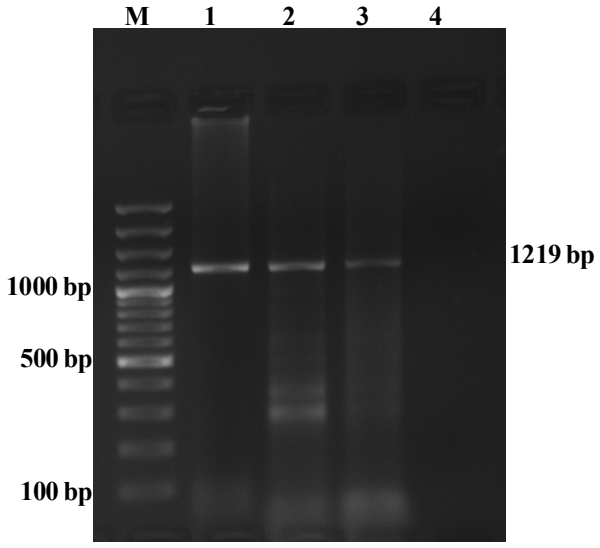


Fig. 4.92: Ethidium bromide stained 1.5% agarose gel showing amplification of 1219 bp fragment of Fowl adenovirus1

Lane M : 100 bp DNA ladder
Lanes 1-3 : Positive samples (1219bp)
Lane 4 : Negative control

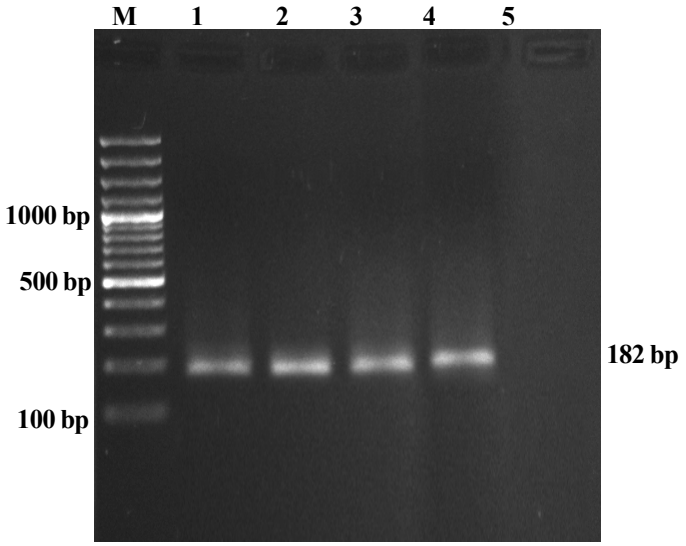


Fig. 4.93: Ethidium bromide stained 1.5% agarose gel showing amplification of 182 bp fragment of Avian nephritisvirus

Lane M : 100 bp DNA ladder
Lanes 1-4 : Positive samples (182bp)
Lane 5 : Negative control

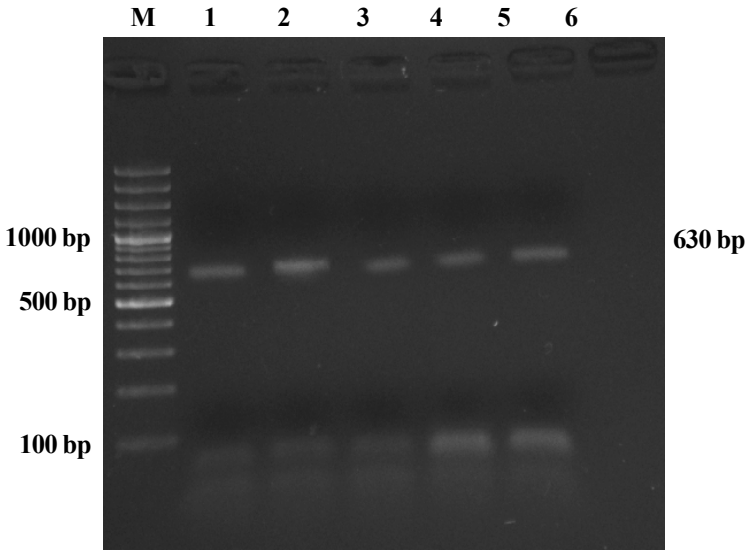


Fig. 4.94: Ethidium bromide stained 1.5% agarose gel showing amplification of 630 bp fragment of Rota virus- NSP4 primer

Lane M : 100 bp DNA ladder
Lanes 1-5 : Positive samples (630bp)
Lane 6 : Negative control

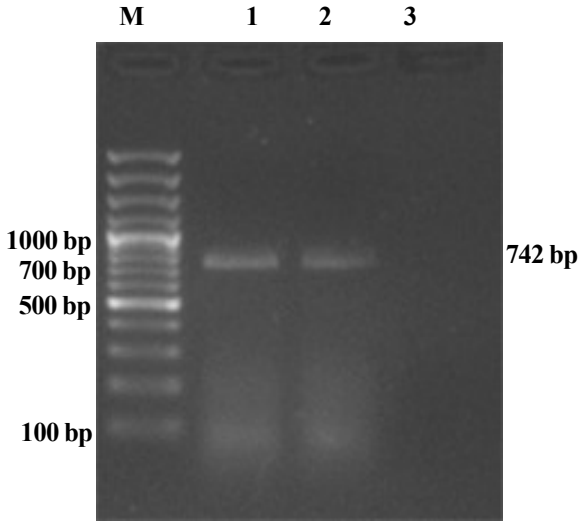


Fig. 4.95: Ethidium bromide stained 1.5% agarose gel showing amplification of 742 fragment of Avian Rota- D virus
Lane M : 100 bp DNA ladder
Lanes 1-2 : Positive samples (742bp)
Lane 3 : Negative control

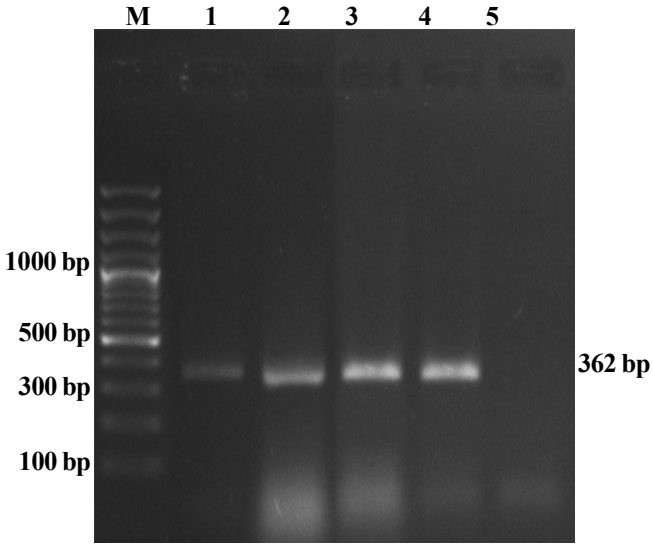


Fig. 4.96: Ethidium bromide stained 1.5% agarose gel showing amplification of 362 fragment of Chicken astrovirus
Lane M : 100 bp DNA ladder
Lanes 1-4 : Positive samples (362bp)
Lane 5 : Negative control

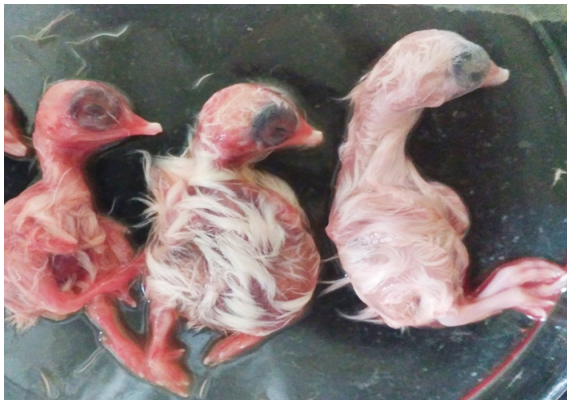


Fig. 4.97: Chicken parvovirus isolation – Intra yolk route- (7 day PI). Stunted embryo with control (right). Stunted embryos with congested (left) and oedematous (middle) body

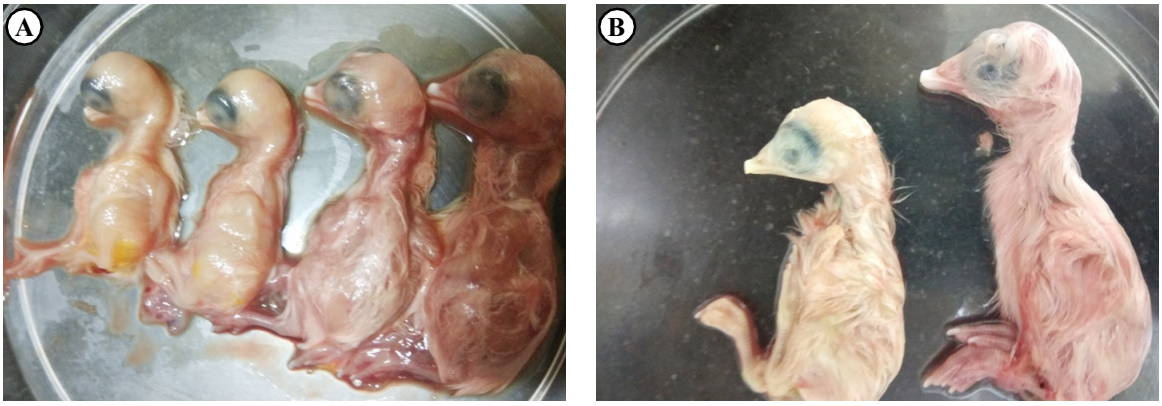


Fig. 4.98: Chicken parvovirus inoculation 7th day PI (A). Runting of inoculated embryos (left two) with controls (right two embryos) and (B) 9th day PI runted (left) with control (right)

Fig. 4.99: Chicken parvovirus inoculation-(13 day PI). Runted embryo (left) with control (right). Short legs and necks were seen in the inoculated one

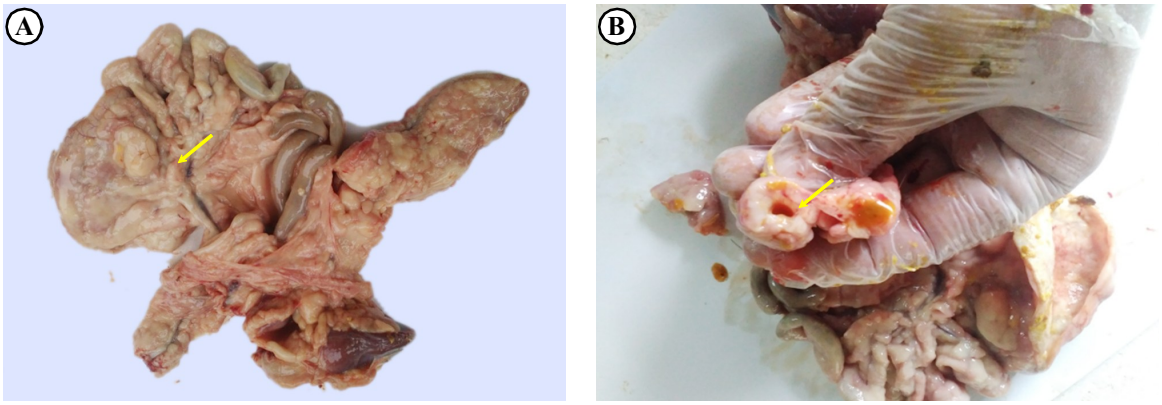


Fig. 4.100: Diffuse type of neoplastic lesions of intestine Miliary nodule formation in serosa of intestine (arrow) and proventriculus (A). Uniformly thickened intestinal wall (arrow) (B)

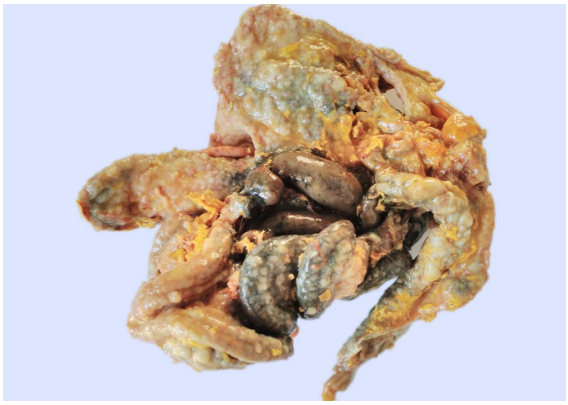


Fig. 4.101: Diffuse neoplastic lesion with miliary nodules in the intestinal serosa and mesentary and smearing with egg material

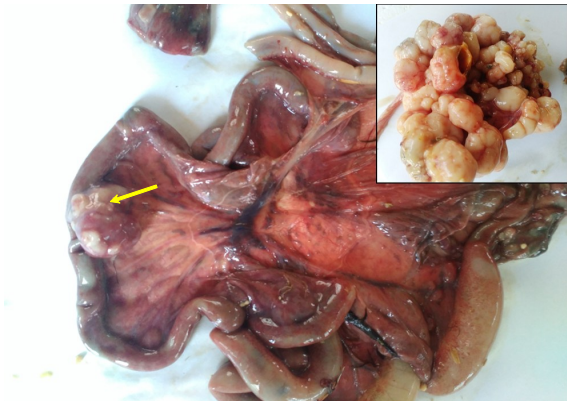


Fig. 4.102: Neoplastic condition of intestine. Intestinal wall thickening and multiple tumour nodules (arrow) in the intestine. Insert- ovarian tumour in the same bird

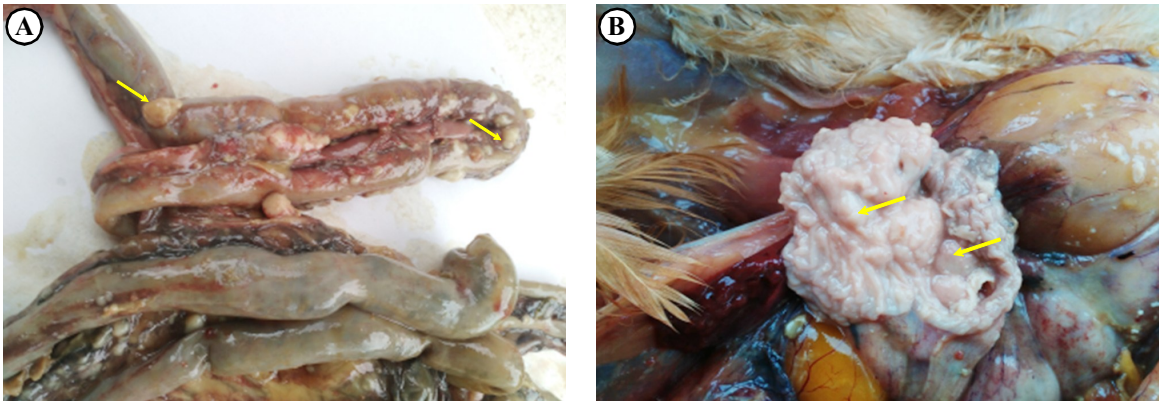


Fig. 4.103: Neoplastic conditions of intestine with tumor growth in the oviduct. Miliary nodules in the intestine (arrows) (A). Neoplastic growth in the oviduct (arrows) (B)

In the focal type, localized enlargement of the intestine with thickened wall and diphtheritic membrane formation in the mucosa of the affected part (Fig 4.105) were observed. Size of the engorged intestinal region varied from 4 cm to 10 cm in diameter. Large white and firm tumor nodules on the serosa were observed in a few cases (Fig 4. 106). These types of lesions were found in 19 birds belonging to 7 flocks and included 15 females and 4 males. Most frequently affected breed was white leghorn (9/19) belonged to two flocks. One of the White leghorn female with large thickening in the ileum revealed internal laying with 8 numbers of fully formed eggs in the abdomen (Fig.4.107). Grossly concurrent neoplastic lesions were evident in the ovary (n=8), spleen (n=2), liver (Fig.4.108) (n=5), mesentery (n=2), Kidney (n=3) and proventriculus (n=1).

4.3.5.2 Microscopic lesions

Histologically, the tumours were classified as pleomorphic lymphocytic tumours, myeloid tumours, fibroma and adenosarcoma. In diffuse type of tumours, two types of proliferating cells were observed in the serosa and muscularis externa. One type of proliferating cells were composed of pleomorphic lymphocytes of small and medium size, lymphoblasts , plasma cells and undifferentiated mononuclear cells (Fig.4.109). This type of cells were found in 10 birds, 4 from PD3, 4 White leghorn and one each of Aseel and PB2. The other types of proliferating cells were myeloid cells with almost uniform appearance with vesicular or nonvesicular nucleus and large eosinophilic cytoplasm (Fig. 4.110). Serosal tumor in the intestine of PD1 revealed adenosarcoma, where glandular epithelial cells were surrounded by fibrous tissue proliferation noted in the muscle layer of intestinal wall (Fig 4.111). The same bird showed tumor in the oviduct characterized by proliferation of glandular epithelial cells (adenocarcinoma). Fibrous tissue proliferation was observed in the serosa of intestine with or without the MNCs in 3 cases. Affection of the mucosa were mild and included loss of villi epithelium, crypt cell proliferation or cystic crypts and mild mononuclear cell proliferation predominantly of lymphocytes in the lamina propria.

The focal tumour of intestine showed necrosis of mucosa, necrotic diphtheritic tissue formation and proliferation of cells in all the layers of the intestine (Fig. 4.112). The proliferating cells were primarily composed of pleomorphic lymphocytes and lymphoblasts cells in 16 birds.

Severe fibrosis observed on the serosa and muscular layer in one bird (Fig.4.113), adenosarcoma in 1 bird and capillary hemangioma (Fig. 4.114) in another. Whole layers of intestinal wall of all the male birds were infiltrated with pleomorphic lymphocytes.

4.3.5.3 Molecular diagnosis

All the tumour samples were screened for MDV and ALV by PCR using specific primers. Marek's disease gave positive band for 12 birds from the first group with pleomorphic lymphocyte proliferation and 5 birds from second group birds. Three of the tumors with large eosinophilic cytoplasm were positive for ALV (Fig 4.115). PCR was performed from the tissue of intestine in all the cases. In a case of adenocarcinoma of ovary, where fibroplasia of the intestine was observed histolgically, PCR was positive for ALV in tissue sample from ovary but negative in the intestinal sample.

4.3.6. Miscellaneous conditions

Miscellaneous conditions include intussusception, volvulus, helmithiasis, cysts and diverticulums (Table 4.22)

4.3.6.1. Intussusception

Intussuception of intestine was noticed in 3(0.017%) out of 17,978 chickens belonging to growers of PB2, PD2 and Dwarf chicken.

4.3.6.1.1 Gross lesions

Intussusception occurred towards the distal end of jejunum in two birds while proximal part of the ileum in the third one. About 6 cm long proximal intestinal part telescoped into the distal part with severe haemorrhage, blood clot and necrosis in the intussusceptum (Fig. 4.116). Brownish fluid accumulated in the lumen of intestinal segments proximal to the intussusception giving ballooned up appearance of intestine in the dwarf (Fig 4.117).

4.3.6.1.2. Microscopic lesions

Histologically, telescoped parts revealed severe haemorrhage and necrosis in the whole layer in dwarf and PD2. Haemorrhage and moderate necrotic changes were observed in the

Table 4.22. Breed and age wise occurrence of miscellaneous conditions

Breed	Intussusception	Volvulus		Ileal diverticulum	Heterakis	Total
	Grower	Chick	Adult	Chick	Adult	
White leghorn					3(0.25)	3 (0.14)
PB2	1(0.48)					1(0.14)
PD1			1(0.21)			1(0.06)
PD2	1(0.13)	2 (0.21)				4(0.10)
PD3					1(0.05)	1(0.02)
Aseel				1(0.81)		2(0.59)
Dwarf	1 (1.72)					1(0.42)
total	**3 (0.07)**	**2 (0.03)**	**1 (0.02)**	**1(0.02)**	**4(0.06)**	**11(0.06)**

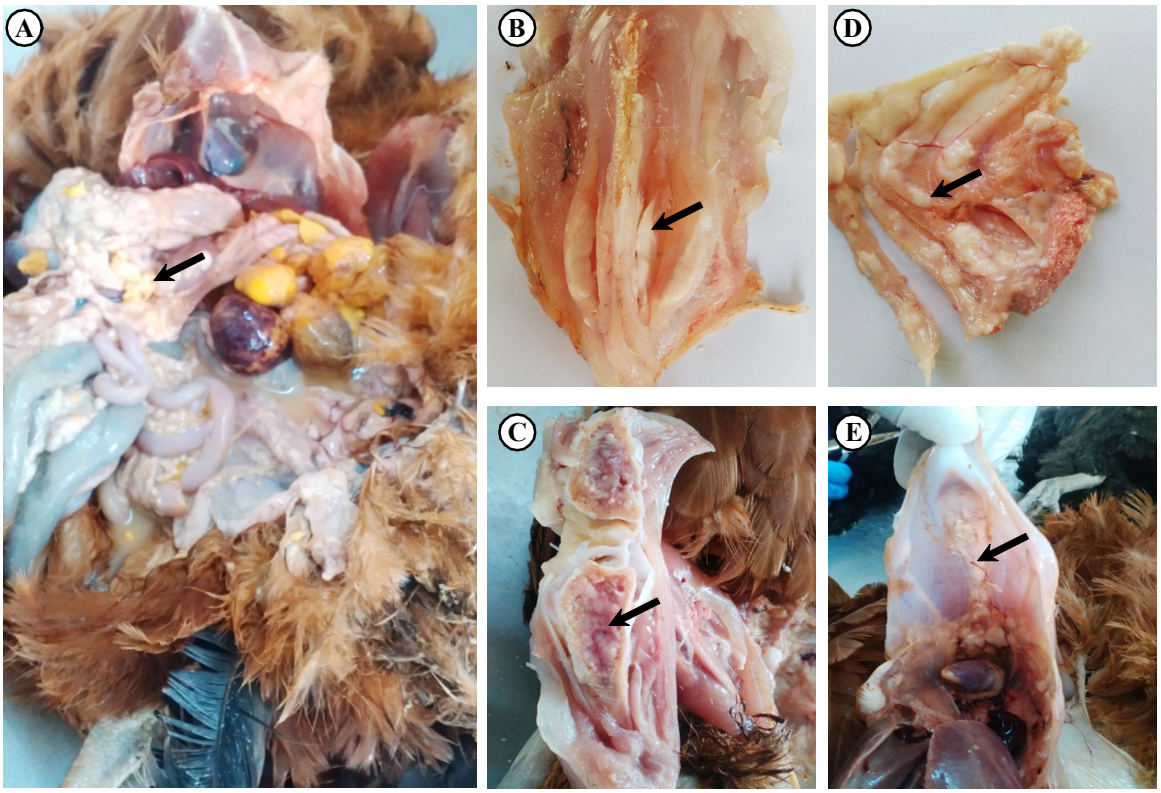


Fig. 4.104: Miliary nodules in the intestinal serosa and mesentery (arrow) (A) with tumours in the muscle (B) bone marrow (C) ribs (D) keel bone (E). ALV infection

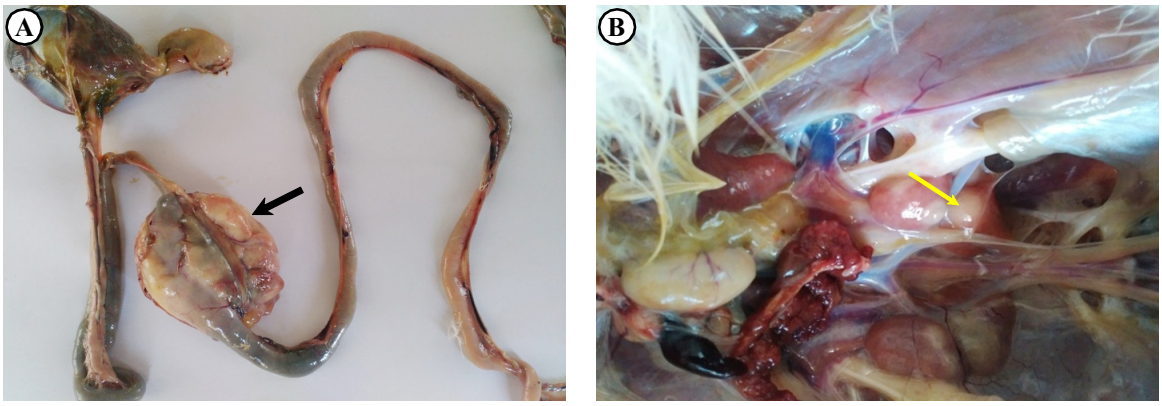


Fig. 4.105: Tumour nodule in the jejunum (arrow) (A) tumour nodules in the kidney (arrow) in the bird (B)

Fig. 4.106: Tumour nodules in the intestinal serosa, pancreas and mesentery (arrows)

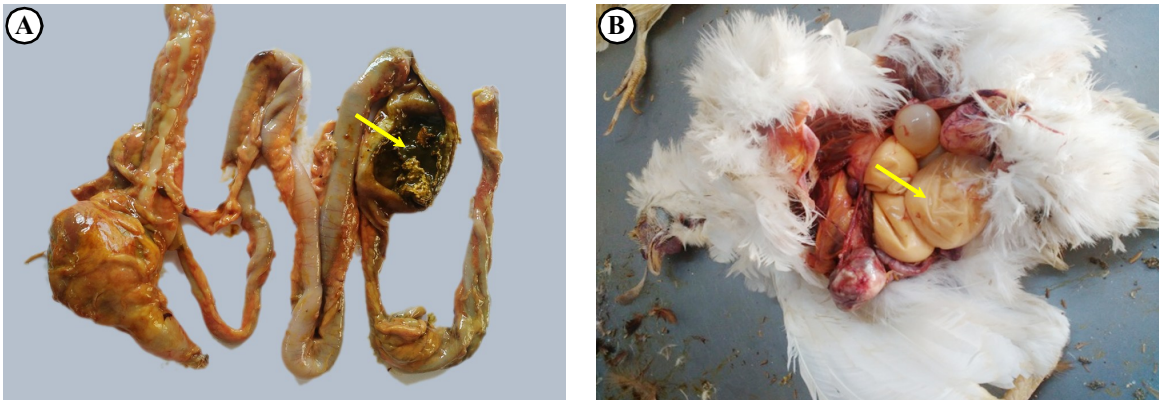


Fig. 4.107: Tumour in the ileum with diphtheretic membrane formation (arrow) (A). Internal laying with multiple eggs (arrow) in the abdomen (B)

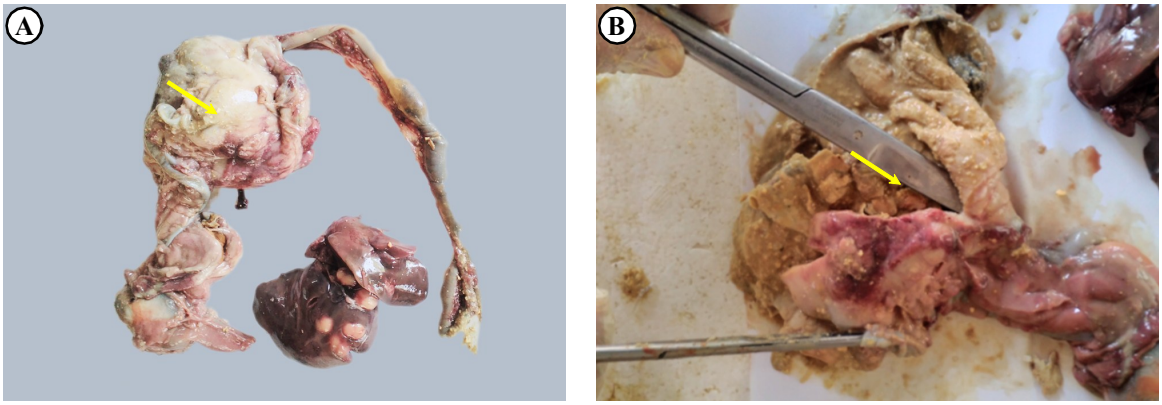


Fig. 4.108: Neoplastic enlargement on the duodenal wall (arrow) with nodules in the liver (A) Diphtheritic membrane in the duodonal mucosa (arrow) (B)

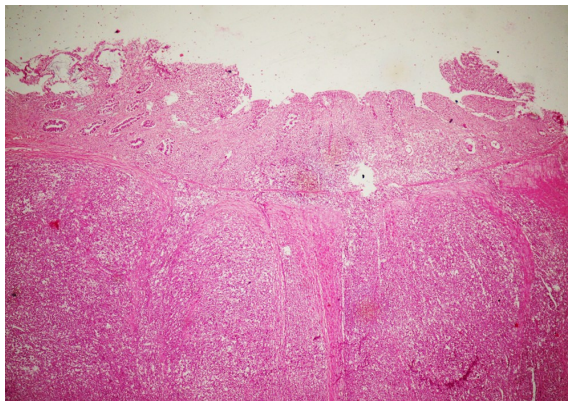


Fig. 4.109: Proliferation of pleomorphic lymphocytes in all the layers of intestine H&E, x40

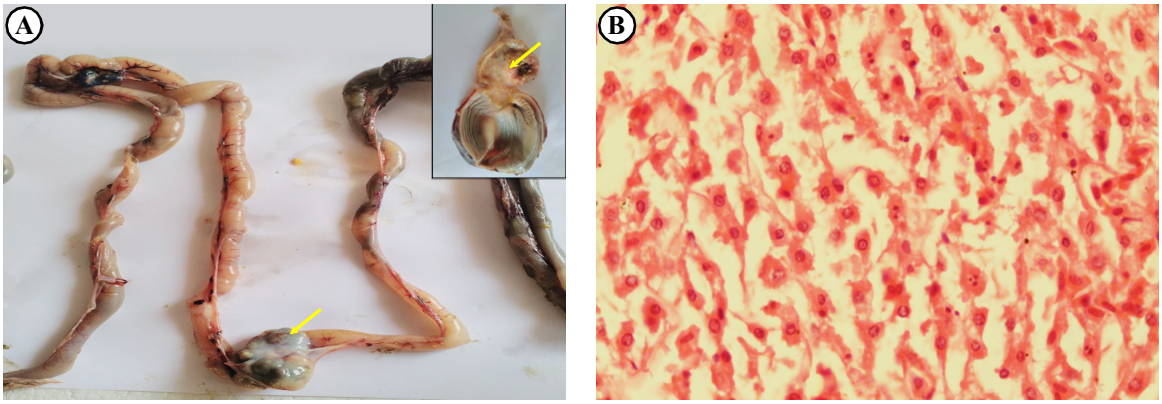


Fig. 4.110: Neoplastic lesions of intestine tumour nodules in the ileum (arrow). Nodular formation and ulceration in the proventricular mucosa (arrow) (insert) (A). Proliferating myeloid cells H&E, x1000 (B)

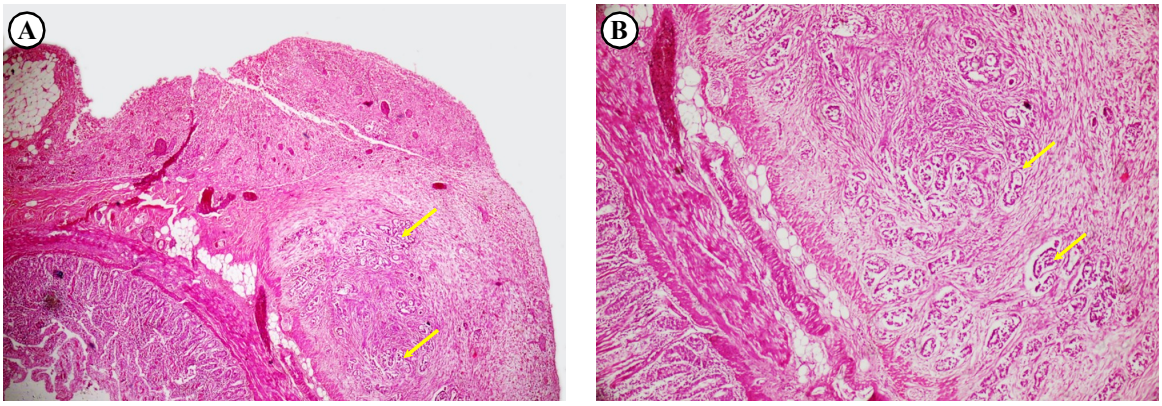


Fig. 4.111: Proliferation of glandular epithelium (arrows) in a fibrous tissue stroma located on the serosal surface of the intestine. H&E, (A) x100 (B) x400

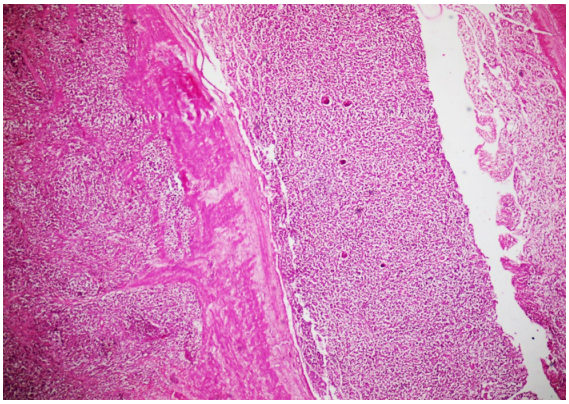


Fig. 4.112: Severe lymphocytic proliferation and necrosis of all layers of intestine H&E, x40

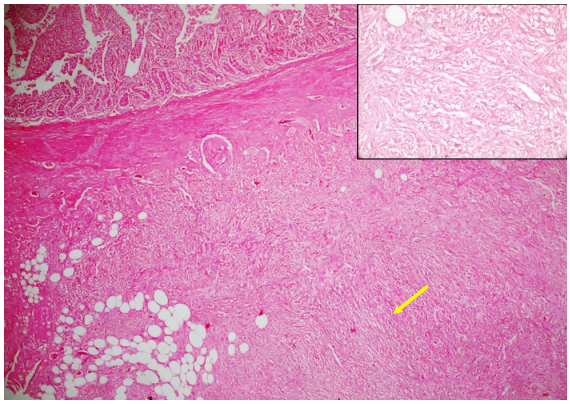


Fig. 4.113: Thickening of muscle layer by fibroplasia (arrow) H&E, x40 insert x400

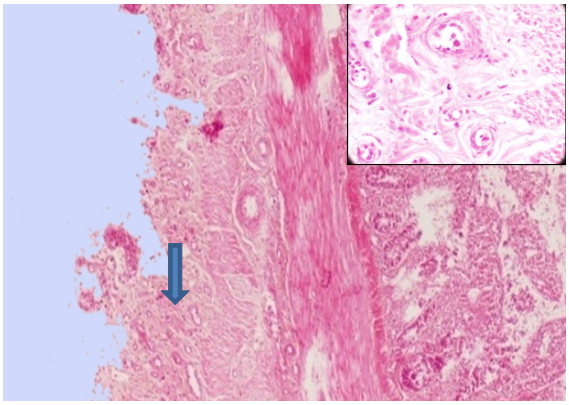


Fig. 4.114: Proliferation of capillaries (arrow) in the serosal surface of intestine. Capillary haemangioma H&E, x40 insert x400

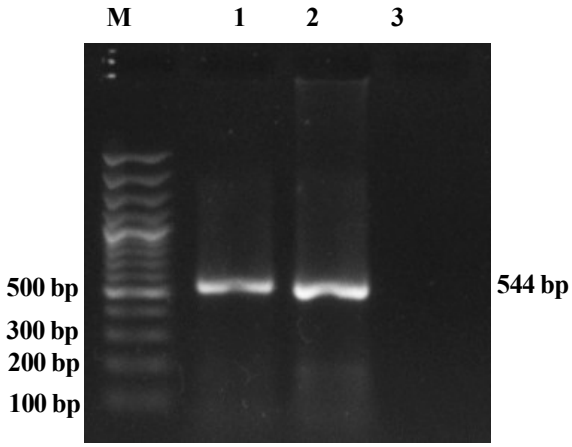


Fig. 4.115: Ethidium bromide stained 1.5% agarose gel showing amplification of 544 bp fragment Avian Leucosis virus

Lane M : 100 bp DNA ladder
Lanes 1-2 : Positive samples (544bp)
Lane 3 : Negative control

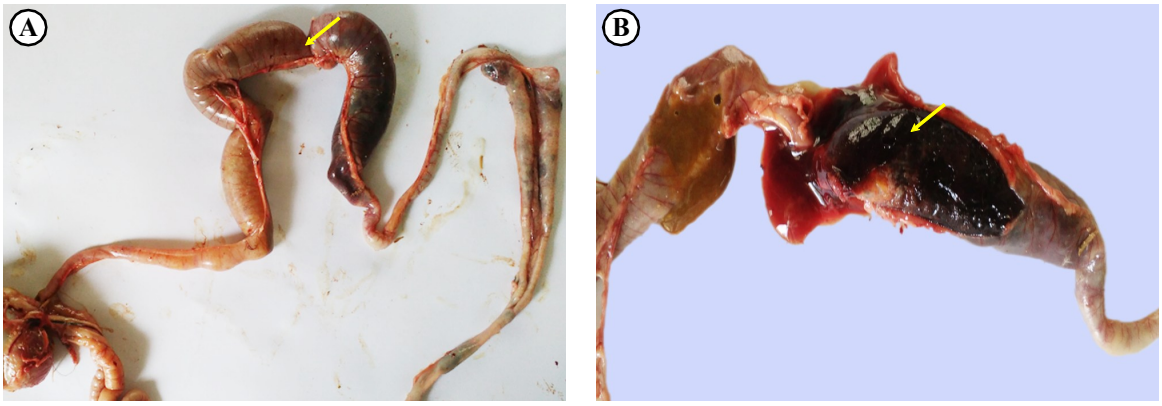


Fig. 4.116: Intussusception (arrow) in a Dwarf grower (A), severely haemorhagic intussusceptum (arrow) (B)

intussusceptum of PB2 grower (Fig 4.118). Coccidial life stages were detected in the intestinal mucosa of the jejunum and ileum in PD2 and PB2 birds, while no coccidia were detected in the intestine of dwarf bird.

4.3.6.2 Volvulus

Volvulus identified in two PD2 chicks of different flocks and in one adult PD1 male chickens.

4.3.6.2.1. Gross lesions

In PD 1 adult male chicken, volvulus occurred at middle part of ileum along with the blind end of caecum (Fig. 4.119). Both ileum and part of caecum got strangulated in the case. Grossly the strangulated parts of the ileum and caecal sac were severely congested and haemorrhagic. Ileal mucosa had diphtheritic membrane formation and bloody watery content. The strangulated caecal sacs were ballooned up with blood mixed necrotic content and gas. In the PD2 chick, volvulus observed at the middle of the ileum. The entire length of the ileum and jejunum got severely congested and haemorrhagic (Fig. 4.120) and engorged with gas. The intestinal mucosa had yellowish necrotic membrane in strangulated part of both jejunum and ileum. In the PD2 chick with prevailing coccidiosis in the flock, the strangulation occurred at distal jejunum (Fig. 4.121). Engorged mesenteric blood vessels, tearing of mesentery occurred in all these cases.

4.3.6.2.2.Microscopic lesions

The strangulated part of intestinal segment exhibited severe haemorrhage and necrosis of the mucosa (Fig.4.122) as well as in submucosa and muscularis externa in all the three cases. Submucosa exhibited mild to moderate oedema. In one PD2 chick, coccidia was detected in sections of intestine.

4.3.6.2.3 Molecular diagnosis

The intestinal contents from the necrotic parts were screened for enteric viruses and CAV. Intestinal contents of both PD2 chicks were positive for CAV. No other enteric viruses were detected in any of the three.

4.3.6.3. Diverticulum at the ileum

One Aseel chick aged 4 weeks, was found with distended abdomen and gross stunted growth.

4.3.6.3.1. Gross lesions

On opening the carcass, an engorged cyst like structure was evident on the right side of the abdomen, occupying a major portion of the abdomen. On detailed examination, the structure was found to be a diverticulum attaching to the middle of the ileum at its mesenteric part, nearly half way from the Meckel's diverticulum and ileo-caecal junction. Mesenteric part and serosa of the ileum could be observed grossly continuing with the diverticulum with the congested blood vessels. Other segments of the intestine were grossly normal. The diverticulum had 0.5cm long patent stalk and a distal engorged blind sac. Lumen of the stalk was continuing with lumen of the ileum (Fig. 4.123). The sac had 5cm diameter size and was distended with gas and brownish semisolid contents. The inner wall of the sac was lined with loosely attached yellow coloured leathery diphtheritic membrane.

4.3.6.3.2 Microscopic lesions

Histologically the diverticulum wall had outer serosa, middle oedematous and atrophied muscular layer, inner cornified epithelium and diphtheritic membrane (Fig. 4.123). Diphtheritic membrane was detached from the epithelial layer in most of the areas. The diphtheritic membrane contained heterophils and necrotised cell debris. The normal mucosa and muscularis mucosa of the intestine were absent in the diverticular wall. Blood vessels were engorged in the muscular layer. Scattered areas of inflammatory cells composed of heterophils, plasmacytes and lymphocytes were evident in the muscular layer towards the mucosa (Fig 4.124).

4.3.6.4. Helminthiasis

Adult stage of *Heterakis gallinarum* was observed in the caecum of 3 White leghorn and one PD3 adult birds. Large numbers of adult stage of *Heterakis gallinarum* was detected in the caecum. (Fig. 4.125). No major gross lesions noticed in these caecum or other segments of the intestine. Histologically mild degeneration of the epithelium of villi and mild proliferation of lymphocytes in the lamina propria were noted.

Fig. 4.117: Intussusception PB2 grower. Ballooning of proximal part of intussusception with fluid content. Insert –enlarged view

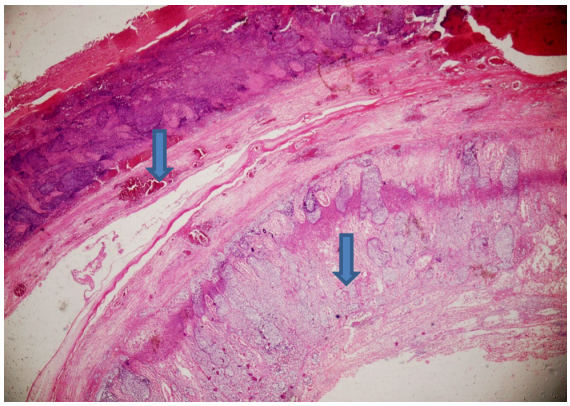


Fig. 4.118: Intestinal intussusception-Severe congestion and haemorrhage, infiltration of inflammatory cells in the inner mucosa, necrosis of mucosa of both layers (arrow) H&E, x40

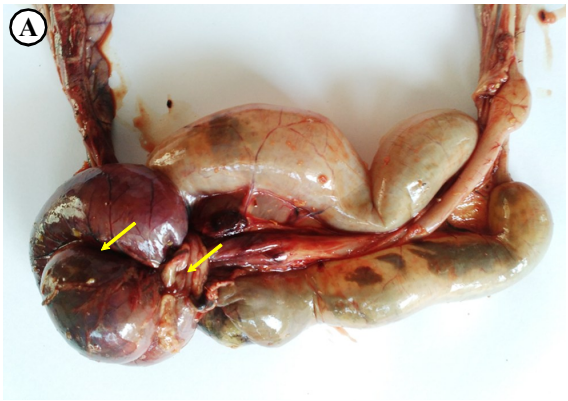


Fig. 4.119: Volvulus in a PD1 adult bird-Strangulation of ileum and blind part of the caecum (arrows) (A). Necrosis of the ileal and caecal mucosa (arrows)

Fig. 4.120: Volvulus in a PD2 chick (A). Strangulation at distal end of the intestine (arrow) (B)

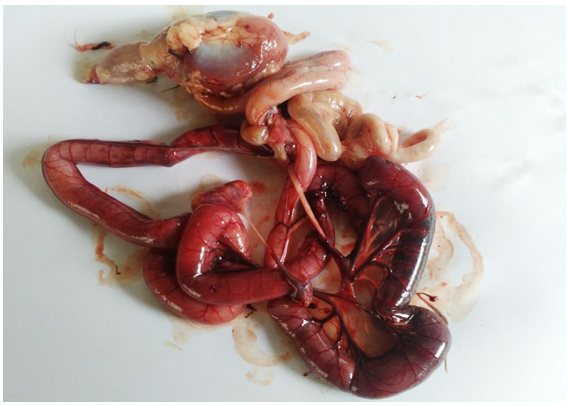


Fig. 4.121: Volvulus in a PD2 chick

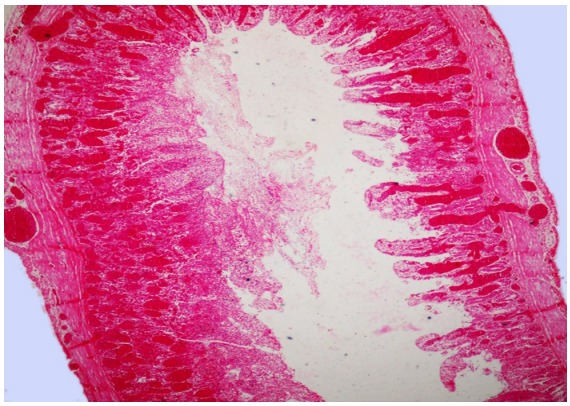


Fig. 4.122: Volvulus PD2 chick – Severe haemorrhage and congestion in all layers, mild necrotic changes in the mucosa, and muscular layer. H&E, x40

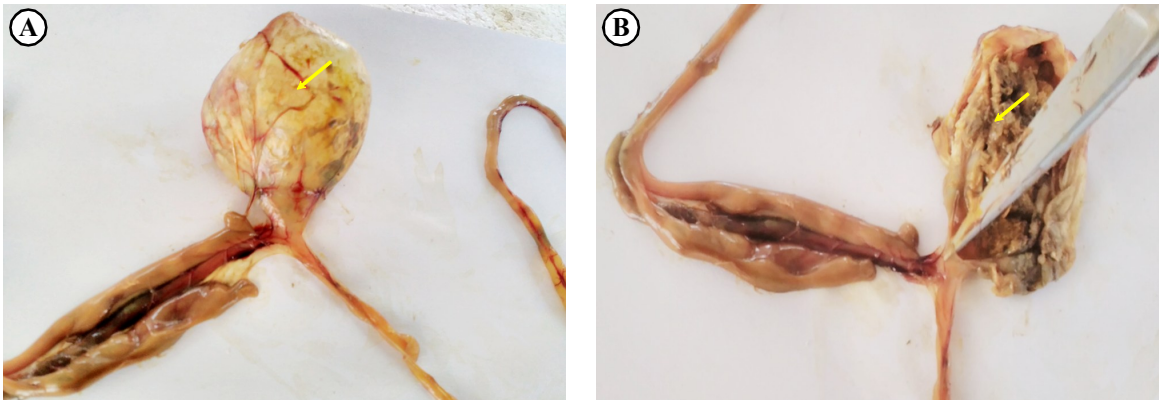


Fig. 4.123: Ileal diverticulum (arrow) (A). Patent lumen connecting ileum (pointed) and necrotic content in the ileum (arrow) (B)

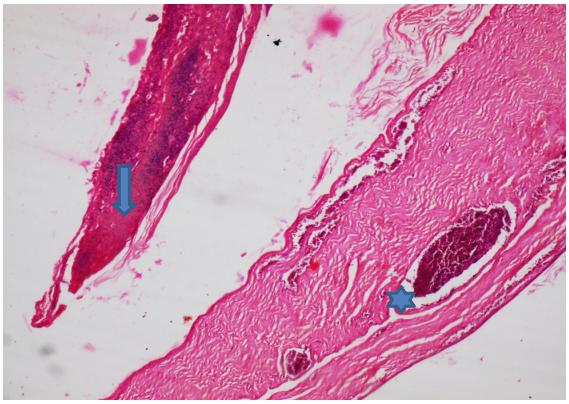


Fig. 4.124: Wall of the ileal diverticulum. Diphtheritic membrane forma-tion (arrow) and congested muscular wall (sar)

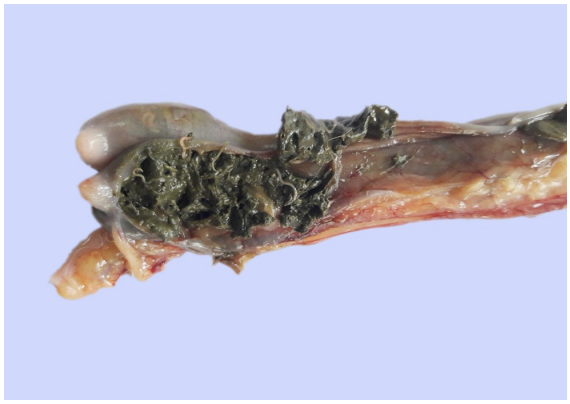


Fig. 4.125: Heterakis infestation. Worms in the caecal lumen

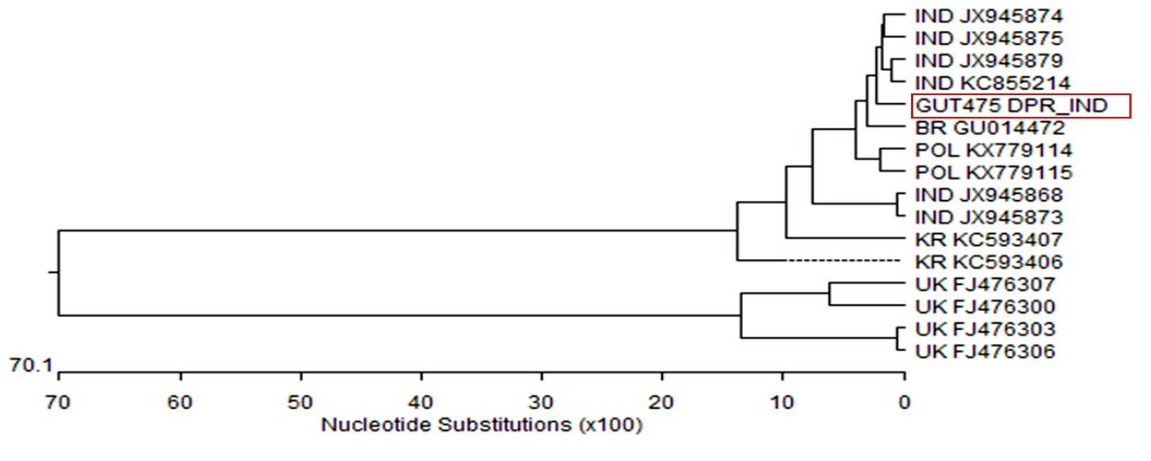


Fig. 4.126: ChAstrovirus phylogenetic tree. GUT475 DPR_IND from this study

Percent Identity

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16		
1		36.4	94.4	84.5	84.5	94.9	96.3	95.8	96.0	79.4	78.5	86.7	84.7	36.2	36.4	36.7	1	GUT475 DPR_IND
2	135.3		29.1	36.9	37.1	36.9	37.1	36.9	37.1	29.4	29.4	36.4	36.9	88.7	77.9	77.7	2	UK FJ476307
3	5.9	127.3		86.2	85.1	93.6	94.5	94.2	94.5	77.3	77.1	85.6	83.7	35.9	35.4	35.6	3	BR GU014472
4	17.9	150.0	15.6		98.8	87.5	87.5	88.0	86.5	45.0	46.3	72.2	70.0	27.8	28.0	27.8	4	IND JX945868
5	17.9	147.4	17.1	1.2		86.3	86.3	86.8	85.3	44.7	46.3	71.5	69.3	27.7	27.8	27.7	5	IND JX945873
6	5.3	148.1	6.7	13.9	15.4		96.8	96.2	94.7	45.7	45.7	75.8	74.5	27.0	27.2	27.3	6	IND JX945874
7	3.8	144.7	5.8	14.0	15.5	3.3		98.3	96.5	46.8	46.0	75.7	75.2	27.5	27.5	27.7	7	IND JX945875
8	4.4	147.1	6.1	13.4	14.8	4.0	1.7		97.8	46.8	46.3	75.2	74.7	28.0	27.8	28.0	8	IND JX945879
9	4.1	144.7	5.8	15.0	16.5	5.4	3.4	2.0		46.0	45.8	74.2	73.7	27.4	27.4	27.6	9	IND KC855214
10	17.0	115.0	17.4	22.0	22.9	19.9	17.1	17.1	17.5		86.7	80.9	79.7	39.4	40.0	40.0	10	KR KC593406
11	18.2	114.4	17.9	18.5	18.5	19.9	19.1	18.3	17.9	15.4		81.2	78.5	38.2	38.5	38.5	11	KR KC593407
12	7.7	152.4	6.7	12.2	13.2	6.9	7.2	7.9	7.7	19.0	18.6		96.1	34.1	35.1	35.3	12	POL KX779114
13	10.2	145.4	9.2	15.6	16.6	8.8	7.9	8.6	8.4	20.8	22.7	4.0		33.7	34.7	34.5	13	POL KX779115
14	137.1	12.4	133.4	150.0	152.8	165.8	154.6	146.5	149.0	121.4	130.5	149.7	153.1		77.0	78.1	14	UK FJ476300
15	142.3	26.6	145.9	146.5	149.0	158.3	153.5	148.1	148.1	119.8	131.6	138.7	141.6	27.8		98.9	15	UK FJ476303
16	139.0	26.9	142.3	151.1	153.9	157.6	152.8	147.4	147.4	119.8	131.6	138.1	145.6	26.2	1.1		16	UK FJ476306
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16		

Divergence

Fig. 4.127: Chicken astrovirus nucleotide percent identity. GUT475 DPR_IND from this study

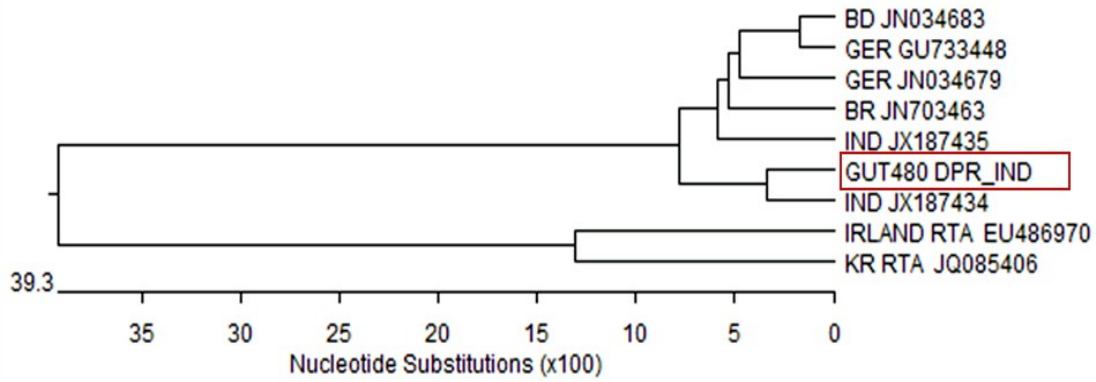


Fig. 4.128: Avian Rotavirus phylogenetic tree. GUT480DPR_IND from this study

Percent Identity

Divergence

	1	2	3	4	5	6	7	8	9	
1		99.6	89.2	88.8	89.2	90.2	93.9	55.3	53.9	1
2	0.0		84.3	84.3	84.2	85.3	89.3	51.5	49.6	2
3	11.8	17.9		49.3	96.7	91.3	81.0	44.9	47.6	3
4	12.4	17.6	11.4		90.8	90.4	89.8	52.7	53.4	4
5	11.8	18.1	3.4	10.0		91.3	80.5	44.9	47.9	5
6	10.6	16.6	9.5	10.5	9.4		81.9	44.7	47.9	6
7	6.3	11.7	12.7	11.4	13.3	11.3		49.2	49.0	7
8	66.9	77.0	79.4	73.9	79.4	80.0	79.0		78.3	8
9	70.4	82.3	88.9	71.8	87.9	87.6	84.5	26.1		9
	1	2	3	4	5	6	7	8	9	

1 GUT480 DPR_IND
2 IND JX187434
3 BD JN034683
4 BR JN703463
5 GER GU733448
6 GER JN034679
7 IND JX187435
8 IRLAND RTA EU486970
9 KR RTA JQ085406

Fig. 4.129: Rotavirus identity. GUT480DPR_IND from this study

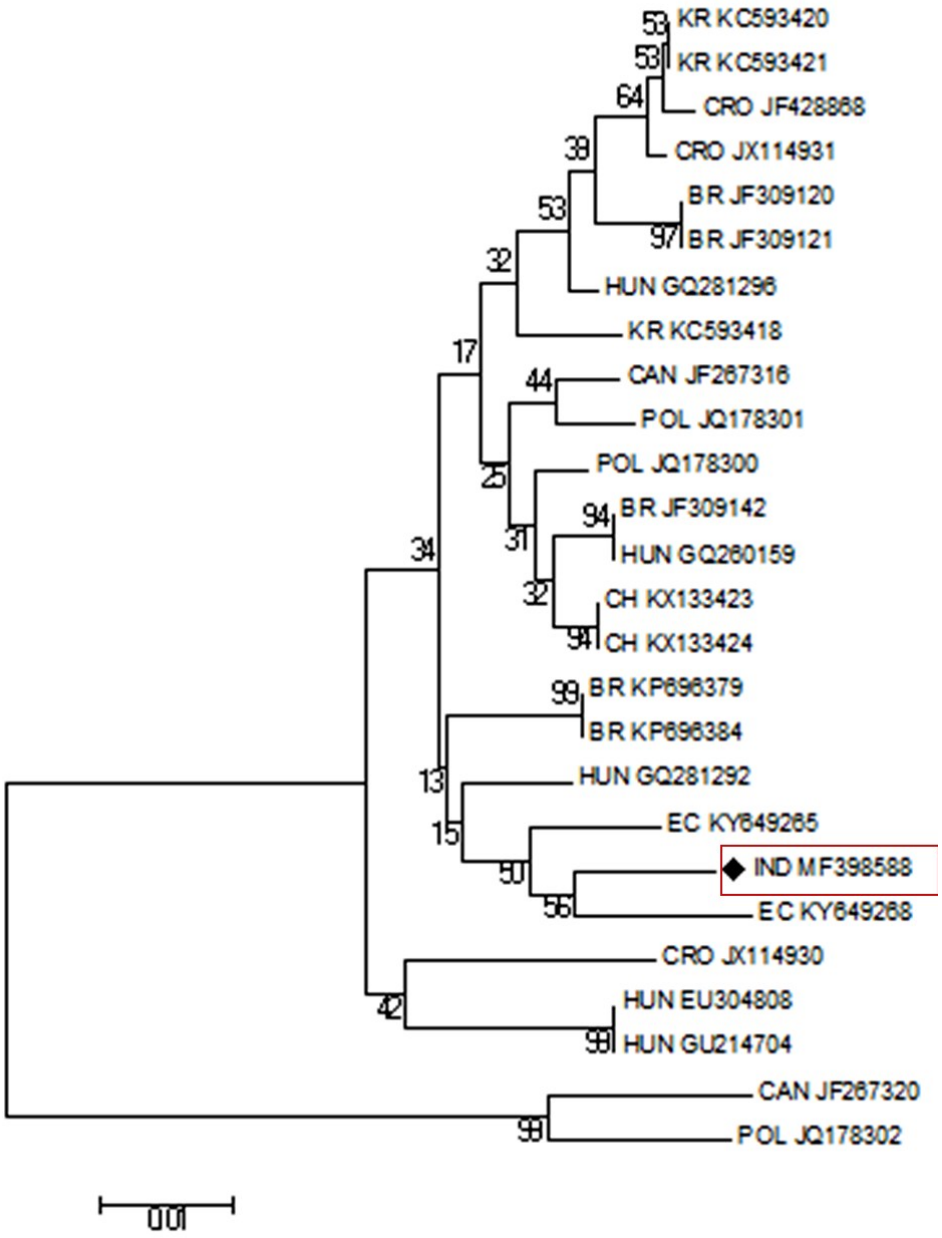


Fig. 4.130: ChPV nucleotide percent identity. IND MF398588 from this study

Sequences	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
1.IND MF398588	***	89.2	82.7	82.7	84.3	96.5	96.5	93.4	88.5	96.5	96.5	95.4	95.4	95.6	97.1	97.1	94.9	96.7	96.2	95.8	94.9	91.2	90.9	90.9	96.9	96
2.POL JQ178302	11.8	***	77.4	77.4	76.7	89.7	89.7	86.2	96.9	88.6	88.6	89.5	88.8	89.5	88.6	88.4	89.7	88.8	89.2	89.7	89.7	85.3	85.5	85.5	89.5	89.7
3. BR JF309120	4.5	10.8	***	100	96.9	96.7	96.7	96.4	89.3	96.7	96.7	98	95.4	98.2	95.7	95.4	95.7	96.9	96.4	98.2	95.7	95.4	96.4	96.4	96.9	96.9
4. BR JF309121	4.5	10.8	0	***	96.9	96.7	96.7	96.4	89.3	96.7	96.7	98	95.4	98.2	95.7	95.4	95.7	96.9	96.4	98.2	95.7	95.4	96.4	96.4	96.9	96.9
5. BR JF309142	2.9	11.9	2.9	2.9	***	96.7	96.7	98.5	89	98.5	98.5	96.9	95.7	97.2	96.9	97.2	95.4	100	96.9	97.7	95.4	95.7	95.2	95.2	98.7	97.7
6. BR KP696379	3.4	10.3	2.9	2.9	3.1	***	99.8	93.4	89.8	97.1	97.1	96.7	95.6	96.7	96.9	96	95.4	96.2	97.1	96.9	95.4	92.7	92.5	92.5	97.8	97.1
7. BR KP696384	3.6	10.5	3.1	3.1	3.4	0	***	93.4	89.8	97.1	97.1	96.7	95.6	96.7	96.9	96	95.4	96.2	97.1	96.9	95.4	92.7	92.5	92.5	97.8	97.1
8. CAN JF267316	3.5	11.2	3.4	3.4	1.5	3.3	3.5	***	89.9	98.2	98.2	97	95	97.5	96.8	96.3	95.7	97.9	96.8	97.9	95.7	96.1	95.4	95.4	98.2	98.6
9. CAN JF267320	12.4	2.9	11.4	11.4	11.9	10.6	10.8	10.9	***	88.8	88.8	89	89.4	89	89	88.5	89.4	88.8	89.2	89.2	89.4	85.5	85.2	85.2	89.4	89.6
10. CH KX133423	3.6	11.8	3.1	3.1	1.5	2.7	2.9	1.9	11.6	***	100	96.7	95.8	97.1	97.6	96.5	95.6	98.2	97.8	97.8	95.6	92.9	92.5	92.5	98.5	98.2
11.CH KX133424	3.6	11.8	3.1	3.1	1.5	2.7	2.9	1.9	11.6	0	***	96.7	95.8	97.1	97.6	96.5	95.6	98.2	97.8	97.8	95.6	92.9	92.5	92.5	98.5	98.2
12. CRO JF428868	4.6	10.5	1.6	1.6	2.9	3	3.2	2.8	11.1	3.2	3.2	***	97.3	99.6	96	95.3	97.1	96.9	96.5	98.7	97.1	93.6	95.1	95.1	97.6	97.6
13. CRO JX114930	4.8	11.6	4.5	4.5	4.5	4.3	4.6	5.2	10.8	4.3	4.3	2.7	***	96.9	96	94.7	96.2	95.8	96.7	95.8	96.2	91.4	92.3	92.3	96.2	95.4
14. CRO JX114931	4.6	10.8	1.6	1.6	2.9	3.2	3.4	2.6	11.3	2.9	2.9	0.4	3.2	***	96.5	95.4	96.9	96.9	96.7	98.9	96.9	93.8	94.9	94.9	97.3	97.8
15. EC KY649265	2.9	11.8	4.2	4.2	3.1	2.9	3.2	3.3	11.3	2.5	2.5	4.1	4.1	3.6	***	97.6	96.7	96.7	97.1	96.7	96.7	91.6	91.6	91.6	97.3	97.1
16. EC KY649268	2.9	12.1	4.5	4.5	2.9	3.9	4.1	3.8	11.8	3.6	3.6	4.8	5.5	4.8	2.5	***	94.7	96.7	95.6	95.6	94.7	91.2	90.9	90.9	96.5	96
17. HUN EU304808	5.3	10.5	4.2	4.2	4.7	4.6	4.8	4.5	10.8	4.6	4.6	2.9	3.9	3.2	3.4	5.5	***	95.4	96	96.5	100	92	92.3	92.3	95.8	96.5
18. HUN GQ260159	3.4	11.5	2.9	2.9	0	3.6	3.9	2.1	11.6	1.8	1.8	3.2	4.3	3.2	3.4	3.4	4.8	***	97.3	97.6	95.4	92.5	92.3	92.3	98.2	97.3
19. HUN GQ281292	3.9	11.1	3.4	3.4	3.1	2.7	3	3.3	11.1	2.2	2.2	3.6	3.4	3.4	2.9	4.6	4.1	2.7	***	97.3	96	92.5	92	92	97.1	96.9
20. HUN GQ281296	4.3	10.5	1.6	1.6	2.3	2.9	3.2	2.1	11.1	2.3	2.3	1.3	4.3	1.1	3.4	4.6	3.6	2.5	2.7	***	96.5	93.4	94	94	98	98.5
21. HUN GU214704	5.3	10.5	4.2	4.2	4.7	4.6	4.8	4.5	10.8	4.6	4.6	2.9	3.9	3.2	3.4	5.5	0	4.8	4.1	3.6	***	92	92.3	92.3	95.8	96.5
22. KR KC593418	4.3	10.5	2.1	2.1	2.1	2.4	2.6	1.9	10.5	2.4	2.4	1.9	4.1	1.4	3.8	4.3	3.3	2.9	2.9	1.9	3.3	***	98.4	98.4	97.7	98.1
23. KR KC593420	4.6	10.3	1.1	1.1	2.7	2.6	2.9	2.6	10.8	2.9	2.9	0.2	3.1	0.2	3.8	4.6	3.1	3.1	3.3	1.2	3.1	1.6	***	100	97.9	97.9
24. KR KC593421	4.6	10.3	1.1	1.1	2.7	2.6	2.9	2.6	10.8	2.9	2.9	0.2	3.1	0.2	3.8	4.6	3.1	3.1	3.3	1.2	3.1	1.6	0	***	97.9	97.9
25. POL JQ178300	3.2	10.8	2.9	2.9	1.3	2	2.3	1.9	10.8	1.6	1.6	2.5	3.9	2.7	2.7	3.6	4.3	1.8	2.9	2	4.3	2.4	2.1	2.1	***	98.5
26. POL JQ178301	4.1	10.5	2.9	2.9	2.3	2.7	2.9	1.4	10.5	1.8	1.8	2.5	4.8	2.2	2.9	4.1	3.6	2.7	3.2	1.6	3.6	1.9	2.1	2.1	1.6	***
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26

Fig. 4.131: Chicken parvovirus nucleotide identity. IND MF398588 from this study

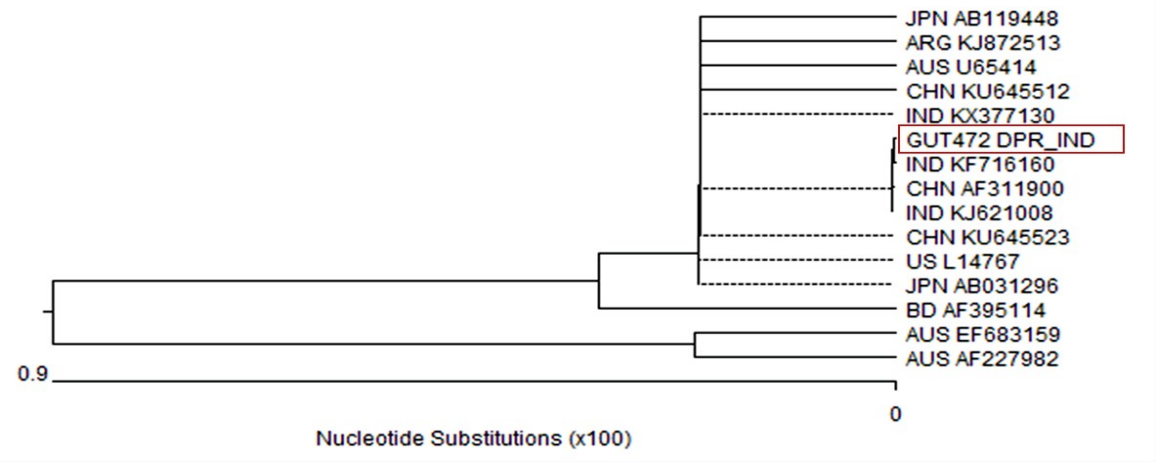


Fig. 4.132: Chicken infectious anaemiavirus. GUT 472 DPR_IND from this study

Percent Identity

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	
1		98.3	100.0	99.6	97.9	100.0	99.6	99.6	100.0	97.9	100.0	100.0	100.0	100.0	100.0	1
2	1.7		98.3	97.9	99.6	98.3	98.8	97.9	98.3	96.3	98.3	98.3	98.3	98.3	98.3	2
3	0.0	1.7		99.6	97.9	100.0	99.6	99.6	100.0	97.9	100.0	100.0	100.0	100.0	100.0	3
4	0.4	2.1	0.4		97.5	99.6	99.2	99.2	99.6	97.5	99.6	99.6	99.6	99.6	99.6	4
5	2.1	0.4	2.1	2.5		97.9	98.3	97.5	97.9	95.9	97.9	97.9	97.9	97.9	97.9	5
6	0.0	1.7	0.0	0.4	2.1		99.6	99.6	100.0	97.9	100.0	100.0	100.0	100.0	100.0	6
7	0.4	1.3	0.4	0.8	1.7	0.4		99.2	99.6	97.5	99.6	99.6	99.6	99.6	99.6	7
8	0.4	2.1	0.4	0.8	2.5	0.4	0.8		99.6	97.5	99.6	99.6	99.6	99.6	99.6	8
9	0.0	1.7	0.0	0.4	2.1	0.0	0.4	0.4		97.9	100.0	100.0	100.0	100.0	100.0	9
10	0.0	1.7	0.0	0.4	2.2	0.0	0.4	0.4	0.0		95.5	95.5	95.5	95.5	95.5	10
11	0.0	1.7	0.0	0.4	2.1	0.0	0.4	0.4	0.0	0.0		100.0	100.0	100.0	100.0	11
12	0.0	1.7	0.0	0.4	2.1	0.0	0.4	0.4	0.0	0.0	0.0		100.0	100.0	100.0	12
13	0.0	1.7	0.0	0.4	2.1	0.0	0.4	0.4	0.0	0.0	0.0	0.0		100.0	100.0	13
14	0.0	1.7	0.0	0.4	2.1	0.0	0.4	0.4	0.0	0.0	0.0	0.0	0.0		100.0	14
15	0.0	1.7	0.0	0.4	2.1	0.0	0.4	0.4	0.0	0.0	0.0	0.0	0.0	0.0		15
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	

Divergence

1 GUT472 DPR_IND
2 AUS EF683159
3 JPN AB031296
4 JPN AB119448
5 AUS AF227982
6 CHN AF311900
7 BD AF395114
8 AUS U65414
9 US L14767
10 IND KF716160
11 IND KJ621008
12 ARG KJ872513
13 CHN KU645512
14 CHN KU645523
15 IND KX377130

Fig. 4.133: Chicken infectious anaemiavirus percent identity . GUT 472 DPR_IND from this study

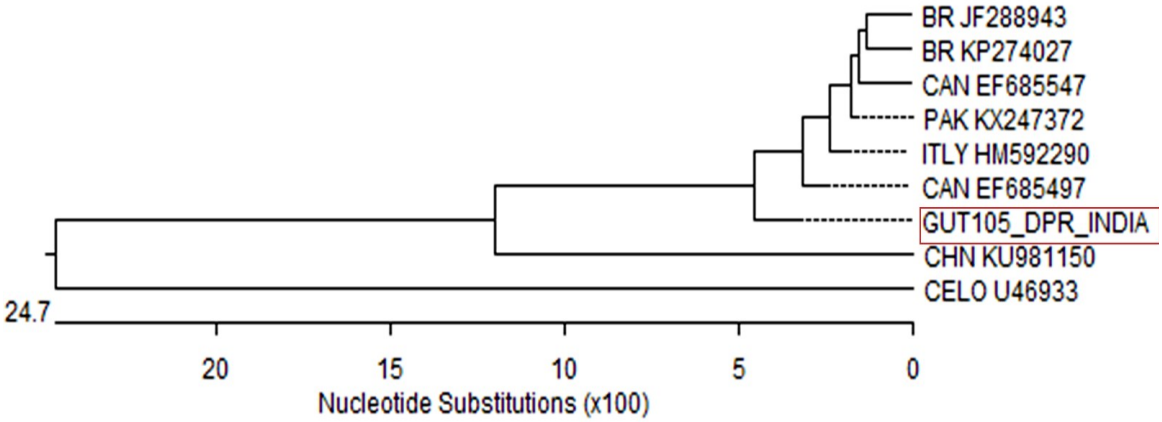


Fig. 4.134: Phylogenetic analysis of Fowl Adenovirus1. Nucleotide sequence GUT 105_DPR_INDIA from this study

Percent Identity

Divergence

	1	2	3	4	5	6	7	8	9	
1		99.8	59.3	66.2	95.1	43.0	44.1	43.6	85.8	1
2	0.1		59.4	66.2	95.0	43.1	44.0	43.5	85.9	2
3	0.3	0.2		62.9	99.7	72.3	73.8	73.3	81.0	3
4	43.6	43.7	50.4		63.4	29.2	25.7	26.0	65.9	4
5	0.4	0.5	0.3	44.6		45.1	46.2	45.7	85.0	5
6	0.2	0.0	0.2	41.9	0.2		68.4	65.6	81.6	6
7	3.0	3.2	3.4	65.3	3.0	3.8		93.3	75.0	7
8	2.4	2.6	2.4	61.5	2.4	2.7	2.7		77.2	8
9	15.7	15.7	22.0	45.6	16.8	20.5	29.8	27.3		9
	1	2	3	4	5	6	7	8	9	

1 GUT105_DPR_INDIA
2 CAN EF685497
3 CAN EF685547
4 CELO U46933
5 PAK KX247372
6 ITLY HM592290
7 BR JF288943
8 BR KP274027
9 CHN KU981150

Fig. 4.135: Nucleotide identity-Fowl Adenovirus1. GUT 105_DPR_INDIA from this study

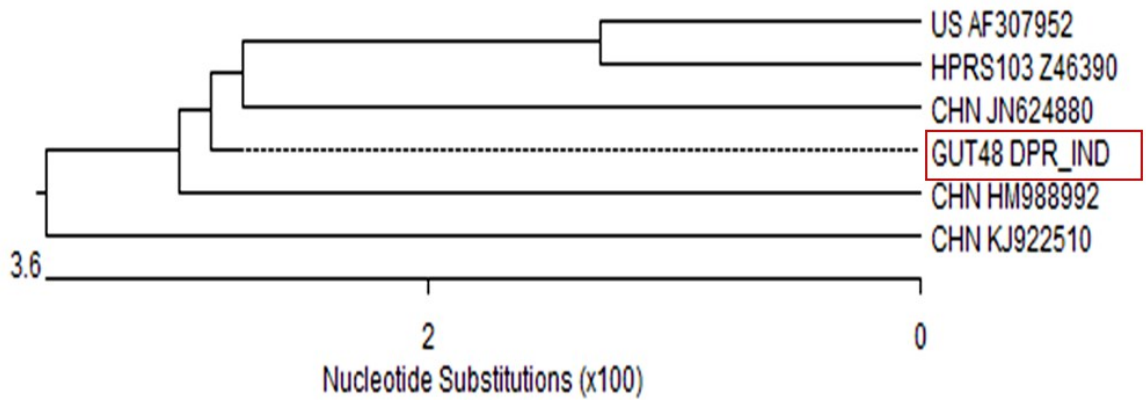


Fig. 4.136: Avian leucosis virus phylogenetic tree. GUT48DPR_IND from this study

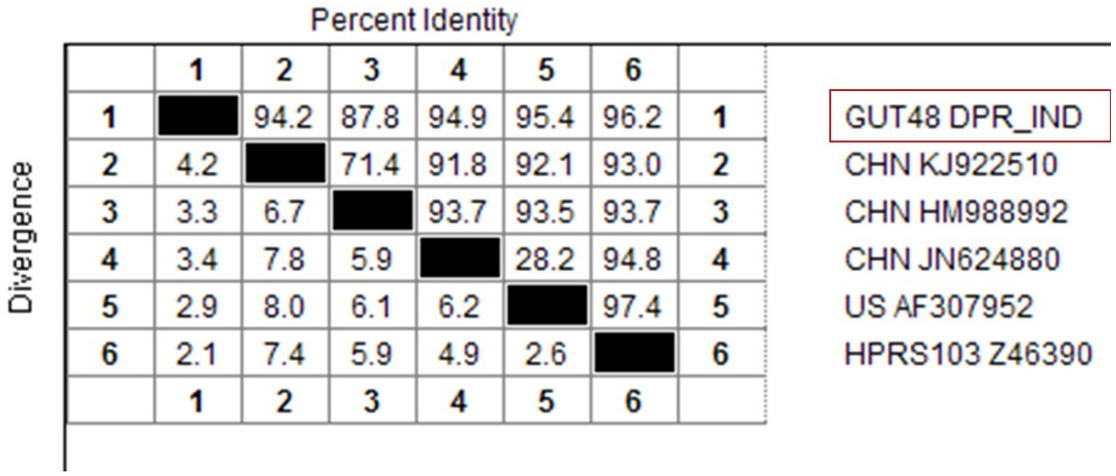


Fig. 4.137: Avian Leucosisvirus nucleotide identity. GUT48 DPR_IND from this study

4.3.7 Phylogenetic analysis

4.3.7.1 Chicken astrovirus

Pairwise comparison and phylogenetic tree of sequenced polymerase gene (354bp) showed 95.8 5 to 96.3 % (Fig. 4.126) nucleotide homology with the Indian group1 nephro pathogenic strains (JX945874,75,79) and 84.5% and 94.9% similarity with Indian group 2 strain. When Brazilian (94.4%) and Poland (84.7%) strains exhibited higher identity, Korean(79.4%) and UK (36.2%- 36.7%) strains showed least identity. Phylogenetic tree the present isolate clustered with nephro-pathogenic Indian strain while UK strains formed a separate clad (Fig. 4.127).

4.3.7.2 Avian rota -D virus

The partial VP-6 gene nucleotide sequence of present study revealed high homology of 88.8% to 99.6% (Fig. 4.128) with other VP-6 gene strains of India, Bangladesh, Brazil and Germany. It shared a homology of 55.3% and 55.9% with avian rota- A virus strains from Korea and Ireland. The present isolate, clustered (Fig. 4.129) with one Indian isolate and away from Bangladesh, Germany, Brazil and another Indian Avian rotavirus D isolate.

4.3.7.3. Chicken parvovirus

The partial sequence of NS1 gene of the chicken parvovirus from the present study showed homology of 82.7%- 97.1% (Fig. 4.130) with the strains from different countries (the sequence deposited the GenBank -accession no. MF398588). The isolate clustered with Ecuador strain rather than the strains from geologically proximal countries like China and Korea (Fig. 4.131).

4.3.7.4. Chicken infectious anaemia virus

The partial sequence of ORF3 region of chicken infectious anaemia virus strain isolated in the present study exhibited a homology of 97.9% to 100% (Fig. 4.132) with isolates from India and other countries. Strains from India and other countries clustered with the present isolate except a Bangladesh and Australian strains (Fig. 4.133).

4.3.7.5 Fowl adenovirus1

The partial sequence of Hexon region of fowl adenovirus 1 showed a wide homology of 43% to 99.8% (Fig. 4.134) with different strains of viruses. Phylogenetically it was close to Canadian strain (Fig. 4.135).

4.3.7.6 Avian leucosis virus

The partial sequence of envelop gene nucleotide sequence showed a homology of 87.8%-96.2% (Fig. 4.136) between the strains from US and China. HPRS strain has shown maximum homology (Fig. 4.137).

Discussion

5 DISCUSSION

An overall occurrence of gut lesions, which included lesions in proventriculus and intestine, was found to be 9.22%. Occurrence of gross intestinal lesions was higher than the proventricular lesion in all breeds and age groups. Chicks group had higher occurrence of both proventricular and intestinal lesions than the other age groups. Even though proventricular and intestinal lesions found together in a few cases (0.422%, 76/17978), independent occurrence of lesions (1.40 % of proventriculus and 7.39% of intestine) in these two sites were more frequent. The present study was based on the prominent gross lesions. In contrast to gross lesions, histologic screening of intestine performed by a few workers (Hoerr, 1998; terVeen *et al*., 2017) revealed lesions in 100% of the samples including clinically healthy birds.

Proventricular lesions were classified into proventricular thickening, proventricular dilatation, proventricular haemorrhage, proventricular ulceration and miscellaneous conditions. Occurrence of proventricular haemorrhage found to be higher than the other proventricular lesions in most of the breeds and age groups and haemorrhage alone was the major lesion in adult chickens.

Provenetricular thickening was not affecting all the breeds and all the flocks of the same breeds. Thickening was appearing as sporadic cases in most of the flocks except in a few, in which higher number of birds died with proventricular thickening in a shorter period. The proventricular thickening started from as young as 1 week. Transmissible viral proventriculitis reported in western countries and China was found in 3-4 weeks old broiler chicks (Kouwenhoven *et al.,* 1978b; Goodwin *et al.,* 1996, Reece, 2002). Occurrence of TVP even

in adult birds was reported recently (Marusak *et al.,* 2012). On flock wise examination, a Nicobari flock had the maximum occurrence (33.33%, 4/60). Higher occurrence of the proventricular thickening in the month of July, November and December may be due to change in weather to rainy and cold environment. The difference in occurrence between the months was not statistically significant.

By microscopic examination and PCR testing, proventricular thickening were grouped in to two types, neoplastic and non-neoplastic thickening. Neoplastic thickening was caused by MDV and ALV and were seen mostly in severely thickened proventriculus. This condition was characterized microscopically by proliferation of neoplastic cells in all layer of the proventriculus. This type of thickening was primarily seen in adult birds and a few in growers. This proventriculus may have focal or diffuse ulceration. The non-neoplastic enlargement chiefly affecting chicks and growers had gross lesions similar to that been described for TVP (Goodwin *et al.,* 1996; Noiva *et al.,* 2015) such as thickened proventriculus with pale serosa, with or without haemorrhage in the mucosa, excess mucus production and oozing of white viscous materials on pressing. During IBV outbreaks also similar lesions were reported (Toffan *et al.,* 2013). The major microscopic lesions found in the non –neoplastic proventricular thickening in the present study were duct cell hyperplasia, thickening of muscularis mucosa, glandular ductal dilatation and necrosis of glandular epithelium in mild to moderate severity. These lesions were similar to that described for TVP (Bayyari *et al.,* 1995, Goodwin *et al.,* 1996, Noiva *et al.,* 2015) except for severe glandular infiltration of lymphocytes, characteristic of TVP. Severe proliferation of mononuclear cells was seen mostly in neoplastic conditions like MD in the study. The microscopic lesions were more similar to that described by Maas and Van De Venne (1985) and Toffan *et al.* (2013). They opined that the thickening might be secondary to infection. Maas and Van De Venne (1985) measured the relative thickness of each layer of proventriculus and found slight change in the ratio of the thickness of muscular layers, muscularis mucosae in affected proventriculi. The ratio in thickened proventriculus was 1:0.3 as compared to 1:0.4 in normal organs. They could not identify any specific cause for the development of the same. In molecular analysis of non- neoplastic proventricular thickening, CAV and ChPV were identified in a few cases. In the present study, proventricular haemorrhage was common finding in which CAV

alone was detected. The relation between CAV and proventricular haemorrhage were already been established (Schat and van Santen, 2013). The proventricular haemorrhages were reported to be associated with various infectious conditions such as NDV, avian influenza, IBD, IBV and non-infectious conditions such as over dosage of drugs (Randall *et al*., 1985), dehydration (Randall *et al*., 1985). Such viruses were not detected in the proventriculus in the present study using molecular methods, chicken embryo inoculation and haemagglutination tests. Electron microscopic studies in the present study revealed virus like particles (VLP) of size 100-120nm in a case. However, no virus of corresponding size such as adenovirus was detected by PCR examination using specific primers. Pantin-Jackwood *et al*. (2005) reported intracytoplasmic and large (100 nm diameter) virus particles in TVP cases; but later Guy *et.al*., (2007) confirmed presence of 75nm sized novel birna viruses, CPNV in TVP. Moreover, Guy *et al*. (2007) reproduced TVP by intraamniotic route inoculation in chicken embryos. The CPNV were not detected in any of the proventricular samples utilizing the Rt-PCR using specific primers as described by Guy *et al*. (2011). Further, proventricular thickening could not be reproduced by chicken embryo inoculation in any of the routes attempted. Proventricular thickening in P1 passage were not having lesions typical of TVP. Oro-nasal administration of proventricular homogenate to day old chicks did not reproduce the lesion even after rearing for 7 weeks. For all these inoculation, proventriculus with non- neoplastic type of thickening were utilized.

The results indicate that the proventricular thickening found during the study was not infectious type or the viruses present in the proventriculus were not in enough quantity to reproduce the lesion. Viruses such as CAV, chicken parvovirus and other enteric viruses detected from the intestine along with proventricular thickening may have a secondary effect in the development of the proventricular thickening. Proventricular thickening by neoplasia causing viruses were similar to the earlier studies (Schat and Venugopal, 2013). In neoplastic conditions, thickness is due to proliferating lymphocytes/ neoplastic cells within and between the glands.

Runting- stunting syndrome was noticed in 40% of the birds with proventricular thickening during the current study. This can be read along with the ductal cell hyperplasia, one of the major histological lesions of proventriculus in non-neoplastic thickening. In chicken, both pepsin

and acids are secreted by the glandular epithelial cells. In the metaplasia of glandular epithelium to duct cell epithelium, it loses its function of secretion, which will subsequently affect digestion, and absorption of nutrients at intestine (Hoerr, 1998).

Proventricular dilatation was found in fewer breeds (9/15) compared to proventricular thickening. Difference in occurrence between different age group and breeds were not significant. The proventricular dilatation was accompanied by thinning of the wall and accumulation of feed materials in nearly half of the cases. Histological lesions were not apparent in most of the cases. Proventricular dilatation without microscopic changes were noted in earlier studies (Riddell, 1976; Taylor and Jones, 2004) due to low fibre diet and not associated with any clinical signs (Taylor and Jones, 2004). In the present study, the dilated proventriculus was screened for MD and Borna virus. Borna virus has been recently identified as causative agent of proventricular dilatation syndrome (PDS) in psittacine birds (Honkavuori *et al.,* 2008), but the virus has not been identified in chickens so far. The virus could not be identified either in the proventriculus or brain tissue of the birds with proventricular dilatation in the current study using Rt-PCR method with species-specific primers. MDV was also not detected in the tissue samples from these birds by PCR.

Proventricular ulceration was observed in 0.09% (16/17978) birds belonging to 10 breeds. Highest occurrence of 0.92% was found in Crosses line. Proventricular ulcerations were seen mostly (13/16) in adult birds. Many factors were attributed to cause proventricular ulceration such as starvation (Bierer *et al*., 1966), Mycotoxins ((Kamalavenkatesh *et al*., 2005), bacterial infections (Dinev, 2010) and neoplastic conditions. The ulceration in the current study was found to associate with MDV in 13 birds and with ALV in one bird. Ulceration of proventriculus in MDV infection was reported earlier (Carvallo *et al.,* 2011). Tissue tropism of ALV in proventriculus was demonstrated before (Arshad *et al*., 1999).

Another interesting finding in the proventriculus was death of broiler breeder birds with severe flaccidity of proventriculus and gizzard in a commercial farm. Moderate mortality was evident in the farm with this problem. Microscopically degeneration and mild necrosis of the epithelial cells and muscular layers were seen. Viruses could not be detected in these organs. Feed examination by the farm revealed mycotoxin content within the limit. Hoerr

(1998) pointed out the role of vitamin E and selenium deficiency in the development of necrosis of muscle of gizzard and intestine resulting in flaccidity. The current problem supports this finding as change in feed and supplementation of vitamin and mineral mixture containing viamin E and selenium completely cured the condition.

Proventriculo-ventricular intussusception in 2 PD2 chicks and one Nircobari grower was observed during the study period. Even though the intussusception of intestine is rather common in poultry (Crespo and Shivaprasad, 2013), intussusception of proventriculus, very rarely reported (Sharma, 1972; Shrivastava *et al*., 1989). As the occurrence was very less, no causative studies conducted on this so far. Multiple predisposing conditions like coccidiosis, mucosal injury and increased intestinal motility were considered as the possible causes of the intestinal intussusception (Williams, 1986). Except in one case, coccidial oocysts were not found in the present cases and hence coccidiosis cannot be a cause for development of intussusception in the present cases. Similar to the findings of Sharma (1972), proventriculi were devoid of any significant gross and histological lesions in the two chicks of Vanaraja. This may be due to the acute development of the condition. However, the lesions in the Nicobari were different with its severely congested and haemorrhagic everted part with pealed koilin layer of ventriculus. Absence of fibrous tissue adhesions indicates a subacute nature of the condition. Chronic dyspepsia, chronic inspiratory difficulty due to upper airway obstruction and increased abdominal pressure were attributed for gastro-oesophageal intussusception in mammals (Guilford, 1996; McGill *et al*., 2009). However, such causes could not be ascertained in the present case. A close follow up with other chickens of the same flocks showed no recurrence of the condition which excludes any infectious cause. Unknown cause of loss of musculuar tonicity of proventricular wall posterior to the oeophageo-proventricular junction may be a possible cause for the proventriculo-ventricular intussusception.

Serosal affections of proventriculus found in the present study were part of the tumours affecting ovary or flat bones and its metastasize to the intestine and proventriculus.

Occurrence of haemorrhagic enteritis was not significantly different between the age groups and breed groups. The study revealed that major reason for haemorrhagic enteritis

especially the one that diffusely affects one or more segment of the intestine was due to coccidiosis (166/293). Out of this, coccidiosis occurrence in the caecum was high compared to other segment. Asexual stages on the mid intestine along with gross ballooning and haemorrhage are characteristic of *E. necatrix*. Clustering of large schizonts in the mucosa, lamina propria and submucosa were observed in the current study, which are characteristic of *E. necatrix*. Presence of asexual stages of coccidia in the caecum is characteristic of *E. tenella*. While sexual stages of coccidia and oocysts formation in the caecum occur for both *E tenella* and *E necatrix*. Presence of *E necatrix* and *E tenella* in the present study either as single or mixed type were confirmed by PCR using the species specific primers. Most pathogenic coccidia of the chicken are *E. necatrix* along with E. tenella which can produce 25% mortality in commercial flocks and 100% mortality in experimental infection (McDougald and Fitz-coy, 2013). The low occurrence of death due to coccidiosis in the study group may be due to proper use of anticoccidial drugs and timely detection and treatment. Screening of coccidial oocysts in the birds with non- enteric lesion were not part of the study, hence occurrence of coccidiasis were not studied here. Higher occurrence of coccidiosis was found in the chick and grower age. High density of chicks and grower housing may be a cause for coccidiosis in this group. Rearing of adult birds in cages might be reason for very low occurrence of coccidiosis in that age group.

Another interesting finding in the present study was observation of linear haemorrhage in the rectum without any haemorrhagic lesions in small intestines or caecum. Microscopically, the haemorrhage was limited mostly to the villi tips especially at the longitudinal folds region and the inflammatory reactions were found to be minimal. Ambali (1992) in an experimental study with IBV infection observed denudation of rectal mucosa sparing other segments of intestine. No IBV were identified in the screened samples under present study. Identification of ANV and ChPV from the rectum along with kidney tissue having nephritis needed to be further probed. No reference regarding such linear haemorrhage in the rectum of poultry could be traced. The rectal haemorrhage may be of non-infectious in nature and could be as a result of mechanical irritation like handling and straining during lay.

Haemorrhagic enteritis in the adult White leghorn bird concurrently with necrotic spots on liver and presence of bipolar organisms in the heart blood were suggestive of fowl cholera.

We could not observe the bipolar organism in ailing birds of the same flock. This is similar to findings of Glisson *et al*. (2013) that the organism in fowl cholera outbreak cannot be demonstrated in chronic ailing birds.

Out of the 24 necrotic enteritis observed in the study, involvement of coccidia was evident in 12 cases of necrotic typhlitis. Molecular screening confirmed mixed infection of *E tenella* and *E necatrix* in these cases. The problems occurred mostly in growers and chicks as they were reared in litter while the adults were reared in cages. Similar findings in young age group was reported by Lobago *et al*. (2005). Coccidiosis was found in a case with MDV infection. However, coccidiosis was not found in other cases of intestinal MDV infection of the present study. Though immunosuppression may cause coccidiosis, no relation found between MDV and coccidiosis in the present study. The study by Biggs *et al.*(1968) revealed lack of correlation between MD and coccidial infection.

Spherical bodies with lacunae in the lower intestine and liver indicate infection of *Histomonas meleagridis*. *Candida albicans* in the liver can be misdiagnosed as *Histomonas* (Kemp and Reed, 1966), but presence of the organism in both liver and caeca in the present study rule out the chance of *C. albicans*. The caecal and hepatic lesion due to *Histomonas* vary widely in chicken (McDougald, 2005). Liver lesions may not be characteristic in chicken in all the cases. Unlike the disease in turkey, the caecal lesion may contain blood clot, causing confusion with *E. tenella* infestation (McDougald, 2005). In the present case of histomoniasis in Aseel birds, *Heterakis* adult worms could not be detected in the caecum. Reports of *Histomonas meleagridis* infection in India is rare (Kalia, 1958; Banerjee and Yadav, 2001, Sawale *et al*., 2011). Histomoniasis usually occurs at young age group of poultry (Hu *et al*., 2006) as the adult chicken acquires resistance (Reis Júnior *et al*., 2009). Morbidity and mortality due to histomoniasis are less in chicken (Van der Heijden and Landman, 2011) and even act as asymptomatic parasitic carrier (Hess *et al.*, 2006). Liver lesions were mostly mild in chickens (Esquenet *et al*., 2003) when compared to turkeys. In an experimental study, chickens showed no clinical signs or mortality even with significant caecal necrosis (Zahoor, *et al*, 2011). Concurrent conditions like coccidiosis, bacteria infections like *E coli* and *Clostridium* complicate the *Histomonas* infection (Ganapathy *et al*., 2000; McDougald and Hu, 2001).

The present study identified *Clostridium* in all these cases of caecal necrosis. This indicates chances of prevalence histomoniasis in the farms under the present study, with milder affections, or misdiagnosis of the condition with coccidiosis. The lesions found in the adults were not very severe to cause mortality by the histomonads alone. Further study utilizing the molecular method is needed for confirming the prevalence of the histomoniasis infection in the farms.

Catarrhal enteritis was significantly higher in chicks when compared to other age groups. Catarrhal enteritis was seen along with runting-stunting syndrome with or without proventricular thickening in many cases. Many viruses, suspected to have role in the development of enteric disorders, such as Chicken astrovirus with suspected role in diarrhoea (Baxendale and Mebatsion, 2004), Avian nephritis virus in growth depression (de Wit *et al.*, 2011), Avian rotavirus in caecal engorgement with watery content and gas (McNulty *et al.*, 1983) and Chicken parvovirus in runting-stunting syndrome (Kisary, 1985; Zsak, 2013). These viruses were screened in randomly selected flocks. At least one virus was detected in all the samples and mostly as multiple infections. The viruses detected include ChPV, ANV, CAstV, AvRtV and FAdV.

Chicken parvovirus was first identified and isolated by Kisary *et al*. (1984) using CsCl separation method. There after Nunez *et al*. (2015) isolated ChPV from a single intestinal sample identified with infection of ChPV alone. Presence of stunting of the chick embryos was similar to findings of Nunez *et al*. (2015). Congestion and haemorrhages of embryos were prominent in the early stages, similar to findings of Kisary (1985) and Nunez *et al*. (2015). On the subsequent days of harvest or passages runting was characteristic but congestion were not prominent or rather absent. Nunez *et al.* (2015) harvested the embryo on 5th day PI and had not reported changes of the embryos on subsequent passages.

Avian rotavirus has been reported worldwide including India (Wani *et al*., 2003, Minakshiprasad *et al*., 2004; Niture *et al*., 2010; Kattoor *et al*., 2013b). In the present study, we found occurrence of AvRtV from 1 week to 35 weeks of age. Majority of occurrence on the young age group of 1-6 weeks of age was in accordance with the earlier reports (Bezerra *et al*., 2014; Dhama *et al*., 2015). Occurrence of AvRtV in 35 weeks old chickens support the

findings of Jones *et al.* (1979) and Niture *et al.* (2010) who detected the virus in older birds of age in 32 and 92 week old commercial layers and in 60-65 weeks layer chicken respectively. The present study found occurrence AvRt-D virus in18.75% (3/16) flocks with enteritis which is higher than the findings of Kattoor *et al.* (2013a). Gross lesions like pale and flaccid intestinal wall with fluidy contents observed in the present case is in accordance with the earlier findings (Mori *et al.*, 2002; Day, 2013). However, in the present study AvRtV found only along with other virus not as a single entity.

Chicken astrovirus (CAstV) was identified from intestine in cases of catarrhal enteritis and runting-stunting syndrome. This finding supports the finding of the McNeilly *et al*., (1994), who found association of CAstV with RSS. But the association of the CAstV with the RSS in the present study was only 18.75(3/10 RSS samples) which is far less than the previous prevalence study by Smyth *et al*. (2009). Even in the RSS, the CAstV was found in association with other viruses in the present study agrees to the findings of Koo *et al*. (2013a), Koo *et al*, (2013b), Smyth *et al.* (2010) and Day *et al*. (2007).

ANV is the most commonly found enteric virus in the present study. The study by Roussan (2012a) and Koo *et al*. (2013a) also revealed presence of ANV higher than the other enteric viruses. The higher detection of ANV in the present study than the earlier reports mentioned above may be because of more sensitive primer used in this study (Todd *et al.*, 2010) and presence of enteric disorders in most of the samples.

Present study detected chicken parvovirus in various enteric conditions next to the ANV. Chicken parvovirus detected in the intestine of chicken from various countries such as US (Zsak *et al.,* 2009), Hungary (Palade *et al.*, 2011), Croatia (Bidin *et al.,* 2012), Poland (Tarasiuk *et al*., 2012), South Korea (Koo *et al*., 2013), Brazil (Mettifogo *et al*., 2014) and latest from China (Feng *et al*., 2016). In India, no studies were reported on the chicken parvovirus so far. Intestinal samples from birds with RSS revealed 100% (10/10) occurrence of ChPV in which the virus alone was detected in two flocks. The association of ChPV with the RSS has been reported by earlier studies (Kisary *et al*., 1984; Zsak, 2013; Nunez *et al*., 2015). The earlier occurrence were 77.77% (7/9 flocks, Bidin *et al*., 2011). ChPV and ANV were detected

from a broiler breed with bone deformities in the present study. Palade *et al.* (2011) made similar findings. Few workers could not find any direct relation between the presence of virus and disease (Zsac *et al*., 2009). In this study, we found ChPV in a bird without any gross enteric lesion. The occurrence of ChPV in the present study is high when compared to earlier reports like Koo *et al*. (2013) but similar to studies like Palade *et al*. (2011) and Finkler *et al* (2016).

FAdV1 was identified in 18.75% (3/16) flocks in the present study. This finding is a little higher than the previous finding of 2.9 % by Koo *et al*. (2013a) and 5% by Mettifogo *et al.* (2014) . FAdV1 detected in two intetstinal samples with necrotict enterits, co-infected with ANV. Histomoniasis and volvulus were the primary cause for enteritis in these cases. The other sample was from RSS affected chick and the virus found co-infected with ChPV, ANV, CAstV and AvRtV. FAdV1 considered as one of the major cause for gizzard erosion (Hess, 2013) that was not observed in any of these FAdV1 positive samples of present study. This may be because of difference in pathogenicity within the virus (Okuda *et al.,* 2006) or simple presence of virus without any harmful effect (Mettifogo *et al.,* 2014).

High prevalence of ChPV and ANV along with AvRtV and CAstV were seen in the intestinal samples of birds with RSS. Earlier studies by Smyth *et al*. (2010), Koo *et al*. (2013a and b) revealed higher occurrence of enteric viruses in the intestine of birds affected with RSS indicating the role of concomitant infection of this viruses in the development of the condition. By experimental inoculation, we could reproduce runting in the chick embryo inoculated with ChPV positive intestinal sample. The similar studies were made earlier in other western countries by a few workers (Kisary, 1985; Nunez, 2015; Zsak, 2013). Still, involvement of a single organism in the development of the RSS is not proved beyond doubt.

The study revealed intestinal tumours only in adults. Presence of tumour in the serosa of intestine and its growth towards the lumen indicate that all the tumours in the intestine might be metastasized from other organs. Ovary was found to be affected with most of the neoplastic conditions of the intestine and the tumours might have metastasized from the ovary. This may be the reason for higher occurrence of tumour in females in the present study. Retro virus - immunohistochemistry

studies (Arshad *et al*., 1997) showed extensive tropism for the retrovirus (ALV-J) replication in the smooth muscles particularly of intestine and blood vessels. Adenocarcinoma of the ovary is the most common tumour in chicken and its metastasize to different organs were reported before (Fredrickson, 1987; Johnson and Giles, 2006). Intestinal metastasis of adenocarcinoma of ovary can be seen as fibroplasia (Hafner *et al*., 2013). The present study revealed both fibosarcoma and adenosarcoma as metastatic tumour of the intestine. Ovary with adenocarcinoma continues to ovulate from unaffected gland and eggs are passed out unless there is any hindrance. In a white leghorn in the present study enlargement of wall of ileum might have resulted in obstruction of oviduct resulting in internal laying. In a few birds, ALV identified from the tissues of ovary or tumour mass but not from the intestinal tissue affected with conditions like fibrosarcoma. Detecting DNA of ALV in the ovary but not in the intestinal tumour of the same bird could not be explained well. The cause of tumour on PCR screening found to be MDV in 47.22% (17/36) and ALV in 8.33% (3/36). Majority of lymphoma in the chicken are MD rather than lymphoid leucosis (LL) and this is in agreement with findings of Reece (1996). Studies in India also reported higher prevalence of MD in chicken rather than LL (Balachandran *et al*., 2009; Swathi *et al*., 2012). Diffuse infiltration of mixed cell including plasma cells, lymphocytes and lymphoblast in the MDV cases found in the study agrees with the finding of earlier studies of visceral MDV infection (Payne *et al*., 1976; Lobago and Woldemeskel, 2004). ALV can induce fibrosarcoma, haemangioma, myeloid leucosis and myelocytomatosis (Hafner *et al*., 2013). Similar lesions were identified with ALV in the present study.

Derangements of intestine like intussusception and volvulus are incidentally found in chicken on necropsy examination (Curtis, 1988; Katiyar *et al*., 1988; Ajayi *et al*., 2007). All the intestinal intussusception observed in the present study affected mid intestine especially distal jejunum or proximal ileum. When volvulus in one bird affected jejunum other two had affection at mid ileum. Blind end of the caecum involved in the volvulus observed in one bird.

Many earlier reports found coccidiosis with intussusception (Parihar and Shukla, 1964; Sharma, 1971, Palanivelu *et al*., 2014). On the other hand a few workers reported

intussusception without coccidiosis (Bandyopadhyay and Jain, 1967, Morrill, 1944 and Pandit and Tiwari, 1964). In the present study, coccidiosis was identified with the occurrence of volvulus in one bird and with intussusception in 2 birds. Rest of the cases of one intussusception and two volvulus cases were free of coccidia. This finding is similar to the observations of Williams (1986). CAV were identified in the intestine of two PD2 chicks with intussusception in the present study. Haridy *et al.* (2010), proposed immunosuppression by CAV, leading to bacterial infection of intestine and hyper-motility as a possible cause. The occurrence of intussusception in the present study with CAV and/ or coccidia indicates the possibility of development of the condition by the intestinal infestation or infection resultant reduced feed intake and intestinal hyper-motility.

Intestinal abnormalities are rare findings in chicken. A few of the previous reports on intestinal malformations were on duplication of ileum in babcock pullet (Gurdev *et al*., 1976), intestinal diverticulum in white leghorn (Balachandran *et al*, 1991), duplicated lower intestine (Dhoke *et al*., 1995) and ileal diverticulum (Wojnarowicz and Olkowski, 2005) in broiler chicks. Absence of normal intestinal mucosal and muscularis mucosal layer differentiates the diverticulum of the present case from the duplication of ileum or caecum as reported by some authors (Pizarro *et al*., 1994; Ahangaran *et al*., 2012). Keratinisation of the internal layer of the diverticulum may be because of persistent pressure developed in the diverticulum. Stunting of the bird may be an after effect of pressure development in the abdomen by the enlarging diverticulum. The actual cause for the development of the diverticulum is not known. Congenital diverticula like Meckel's diverticulum occur at anti-mesenteric surface, while acquired type of diverticula mostly occur in the mesenteric surface (Ablin *et al*., 1991). The mesenteric position of the diverticulum in the present case point out the possibility of development of the diverticulum as acquired type. Pre-existing weakness of structural wall of the intestine were attributed as a possible cause of development of such diverticulum by some workers (Wojnarowicz and Olkowski, 2005). Interestingly, middle of ileum can be seen as the common site of development of intestinal anomalies in chicken in the present study and most of the above-sited reports.

Heterakis gallinarum is a commonly found caecal worm of poultry (Lund *et al.*, 1970) which produce only subclinical infections. Importance of the worm is its role in the

transmission of *Histomonas meleagridis.* The adult worms were seen in large numbers in the caecum of adult white leghorn and PD3 birds during the study.

In an experimntal study by Schwarz *et al.* (2011), co-infection with *Heterakis* and *Histomonas meleagridis* resulted in caecal lesion in 14/15 birds and liver lesion in 6/15 birds at 2 weeks post inoculation. At 5th week PI , gross lesion was seen in caeca only in 1 out of 15 birds. No lesions were found in birds that were monoinoculated with *Heterakis* in the same study. In the present study, histomoniasis was identified in one bird from the PD3 flock. Caecal worms could not be observed in Aseel grower with histomoniasis. This may be due to the fact that the larvae of *Heterakis* is very minute (2-3mm) and the larval forms remains for about 3 weeks and the development of histomoniasis kills the environment for the development of the larvae (Hess and McDougald, 2013).

Summary and Conclusions

6 SUMMARY AND CONCLUSIONS

Gut diseases present serious health and welfare problems to the birds and are an economic challenge to the industry. Other than the impact on production, gut diseases and diarrhoea causes wetting litter materials and high rate of environmental contamination with many infectious agents. Despite the importance of the gut diseases, correct identification of cause of intestinal lesions are difficult, as it involve multiple infectious and non-infectious causes. Multiple viruses are attributed to cause intestinal disorders and runting-stunting syndrome. The present study was aimed to study the occurrence of patho-morphological changes of gut especially proventriculitis/proventricular dilatation in chickens. Suspected viruses were checked in the proventricular tissues for their presence using molecular technique and chicken embryo inoculation. Further, enteric viruses such as chicken astrovirus with suspected role in diarrhoea, avian nephritis virus in growth depression, avian rotavirus in caecal engorgement with watery content and gas, chicken parvovirus in runting-stunting syndrome were screened using PCR/ Rt-PCR in selected flocks for identifying their occurrence .

The study was conducted for one-year period from December 2015 to November 2016. Birds necropsied in routine post-mortem at ICAR- DPR and carcasses from commercial farms at Hyderabad were utilized for the study. Total of 17978 birds were necropsied during the period at ICAR- DPR and the birds belonged to 307 flocks of different age groups and breeds/lines. The breeds/lines of chicken were grouped into 5 categories such as layer, broiler, synthetic, single gene and native breeds. The layer lines include White leghorns of lines IWA, IWD, IWF, IWH, IWI and IWK. Broiler breeds include Punjab Broiler 1 and 2, Control

broiler, Crosses and Krishibro. Synthetic lines include PD1, PD2, PD3 and Gramapriya Male line (GML). The single gene breeds are Naked Neck and Dwarf while the native breeds include Aseel, Ghagus and Nicobari. Birds necropsied from the commercial farms were utilized for studying proventricular lesions. Selected samples from proventriculus were subjected to histological examination, embryo inoculation for isolating suspected viruses and PCR and Rt-PCR with specific primers for viruses like Chicken proventricular necrosis viruses, Fowl adenovirus1, Marek's disease, Avian leucosis virus, Reticulo endothelial virus, Chicken parvovirus, Avain nephritis virus, Chicken infectious anaemia virus, Infectious bronchitis virus, New castle disease virus and Chicken astrovirus. Selected intestinal samples were checked for presence of Fowl adenovirus1, Marek's disease, Avian leucosis virus, Reticulo endothelial virus, Chicken parvovirus, Avain nephritis virus, Avian rotavirus, Chicken infectious anaemia virus, and Chicken astrovirus.

The overall occurrence of gut lesions were 9.22% (1658/17978). A total of 329 (1.83%) birds out of 17,978 examined were exhibited proventricular lesions with highest (4.99%) occurrence in Aseel and the lowest (0.42%) occurrence in Dwarf line. While, 1405 (7.82%) out of 17,978 birds examined were showed intestinal lesions with highest (15.96%) occurrence in Nicobari and lowest (5.17%) in PB1 line. Age wise occurrence of proventricular lesions was 2.30% (148/6439), 2.26% (105/4647) and 1.10% (76/6892) in chicks, growers and adults respectively. The intestinal lesions were observed in 12.44% (801/6439), 5.85% (272/4647) and 4.82% (332/6892) in chicks, growers and adults respectively. In 120 commercial birds examined, proventricular lesions were observed in 17 out of 80 layers and 8 out of 40 broiler breeders.

Proventricular lesions were classified into 5 types based on gross lesions such as proventricular thickening, proventricular dilatation, proventricular haemorrhage, proventricular ulcers and miscellaneous proventricular lesions and their occurrence was 0.94% (168/17,978), 1.07% (191/17,978), 0.09% (16/17978), 0.07% (13/17,978) and 0.06% (10/17,978) respectively. In breed and age wise occurrence of proventricular thickening, highest occurrence was noticed in PD3 chicks (3.24%, 34/1051), Ghagus grower (8.20%, 5/81) and Naked neck adult (1.96%, 1/51) in the concerned age group.

Proventricular thickening was observed as sporadic occurrences, except in a few flocks that exhibited higher occurrence of the condition especially flocks belong to PD2, PD3 and Nicobari. Grossly the proventricular thickening was classified into mild, moderate and severe thickening. Severe thickening was mostly seen in adult birds and identified to be neoplastic in origin. Proventricular thickening due to neoplastic conditions showed ulceration in most of the cases. A few concurrent lesions observed with the proventricular thickening were catarrhal enteritis (45/168) especially in chicks and growers. In neoplastic thickening affections of other organs were splenomegaly (8/168), hepatic neoplasia (9/168) and tumors in flat bones and other parts of the body (2/168). Microscopical lesions in the proventricular thickening were observed in all the layers in varying degrees. The major lesions were duct cell hyperplasia, degeneration and necrosis of glandular epithelium, fibroplasia of muscularis mucosa and glandular septum and mononuclear cell infiltration in mucosa and submucosa. Neoplastic thickening were characterized by proliferation of either pleopmorphic lymphocytes and plasma cells or myeloid cells. Pleomorphic lymphoid proliferation observed in 17.7% (11/62) growers and 52.38% (11/21) adults with proventricular thickening. Myeloid cells were observed in 3.23% (2/62) growers and 9.52% (2/21) adults. Electron microscopic study revealed 100-120nm sized virus like particles in thickened proventriculus of a bird. Virus of concerned size was not detected in that bird by PCR examination. Proventricular thickening was reproduced only in one sample and in first passage. Oral administration of proventricular homogenate did not reproduce the disease. Enteric viruses like chicken parvovirus, avian nephritis virus, avian rotavirus, chicken astrovirus and fowl adenovirus1 were detected from the chicken with proventricular thickening, enteritis and RSS. Their role in development disease is needed to be further studied.

Proventricular dilatation was seen without marked microscopic lesions and free of suspected infectious viruses such as MDV and avian Borna virus. Proventricular mucosal haemorrhage found in the study was mainly due to MDV infection and a few due to ALV. Flaccid proventriculus and gizzard lesion with mild mucosal and muscular lesions were non-infectious cause suspected to be of vitamin E and selenium deficiency. Proventriculo-ventricular intussusception noticed in three birds and no direct relation with coccidiosis was observed with the occurrence.

Haemorrhagic enteritis was noticed in 263 (1.46%) out of 17,978 birds examined. The occurrence of haemorrhagic enteritis in chicks, growers and adults was 1.49%, 1.61% and 1.33%, respectively. The highest occurrence of haemorrhagic enteritis observed in Naked Neck (4.67%) and nil in Krishibro. Occurrence of haemorrhagic enteritis among different segments was found to be high (31.94%, 84/263) in rectal region, followed by caecal region (26.24%, 69/263), and lowest in duodenal region (0.38, 5/263). Linear haemorrhage observed in the rectal mucosa of adult and a few grower birds were believed to be non-infectious and due to straining while handling or laying. Coccidiosis was identified in 166 cases of intestinal haemorrhage and mainly affected chicks and growers. *Eimeria tenella* and *Eimeria necatrix* were identified by PCR using specific primers.

A total of 24 (0.13%) out of 17,978 cases of necrotic enteritis involving jejunum, ileum, cecum and rectum, was observed during the study. Chicks exhibited 50% (2/4) of necrotic enteritis as necrotic typhlitis while that of growers were 84.62% (11/13) in the caecum. Necrotic typhlitis was found mainly due to coccidiosis. Ulceration of distal ileum, caecum and rectum were noticed in 5 birds in which protozoa identified in the liver of one bird (histomoniasis). *Clostridium perfringens* were identified by PCR in intestine with necrotic enteritis. *Clostridium colinum* detected in two cases of necrotic enteritis.

Occurrence of catarrhal enteritis was significantly high in chicks than the other age groups. Molecular screening for enteric virus in 16 flocks with catarrhal, necrotic, haemorrhagic enteritis and one without gross intestinal lesion, revealed multiple infection. RSS affected birds revealed 100% occurrence of ChPV (10/10). In two samples of stunted growth with catarrhal enteritis and proventricular enlargement, ChPV alone was detected. In the Krishibro flock with clinical signs of lameness and bone weakness ChPV detected in both proventriculus and intestine and co-infected with ANV and CAstV. The ChPV detected in native bird, Ghagus with stunted growth and co-infected with ANV. FAdV-1 detected in a stunted chicken co-infected with ChPV, ANV, CAstV, and AvRtV. Three cases of Avian Rota-D virus identified from the cases of catarrhal enteritis. Phylogenetic analysis of CAstV, revealed that the virus isolate has close similarity with nephro-pathogenic Indian strain. ChPV was identified for first time in India and the virus showed similarity with Ecuador strain. From a cases of RSS where

chicken parvovirus alone was detected, inoculated into 7 day old chicken embryos and the embryos exhibited runting from the first passage onwards and the virus detected by Rt-PCR in the affected embryonic tissues.

Intestinal neoplasm were observed in 36/17978 (0.2%) birds. The tumours were seen either as diffuse affection or as focal affection. All the intestinal tumours identified in adults only and were metastasized from other organs especially ovary and bone marrow. MDV (17 birds) and ALV (3 birds) were detected by PCR using specific primers. Other conditions observed in the intestine were intussusception (3, 0.017%), volvulus (3, 0.017%) and ileal diverticulum (1, 0.006%). Based on the results of this study the following conclusions were drawn.

- The overall occurrence of gut lesions was recorded as 9.22% that can be of greater economic impact.
- The overall occurrence of proventricular lesion was 1.83%, which include proventricular thickening, dilatation, haemorrhage, ulceration and miscellaneous conditions. Proventricular thickening in adult birds were mostly due to neoplastic conditions such as MDV and ALV.
- Proventricular thickening in the chicks and growers were principally characterised by microscopic changes such as thickening of muscularis mucosa by fibroplasia, enlarged gland ducts (sinuses), ductal cell hyperplasia. No primary cause for the thickening could be identified.
- Chicken parvovirus detected in proventriculus of chicks with runting –stunting syndrome. Further studies are required to establish its role in proventricular thickening.
- Chicken infectious anaemia virus alone or in combination with MD / ALV were detected in proventricular thickening.
- Proventricular ulcerations observed in the study was mainly due to neoplastic conditions
- The overall incidence of intestinal lesion was 7.82% which include haemorrhagic enteritis, necrotic enteritis, catarrhal enteritis, neoplastic enteritis and miscellaneous conditions
- Intestinal haemorrhage was found mainly due to coccidiosis

- Mid intestinal coccidia by *E. necatrix* and caecal by *E tenella* were the major cause
- Linear haemorrhages of the rectum seen in adult and grower birds need to be further explored.
- The chicken parvovirus isolated from the intestinal contents of chicks affected with runting-stunting syndrome induced runting of chick embryos.
- Chicken parvovirus, Chicken astrovirus, Avian nephritisvirus, Avian rotavirus and Fowl adenovirus1 were found to be associated with enteritis, proventricular thickening in birds affected with runting- stunting syndrome.
- The above viruses were also seen in bird died due to non-enteric conditions
- Majority of tumours found in the intestine were metastatic caused by MDV or ALV
- Finding of histomoniasis in chicken without severe liver lesions need to be looked into as otherwise confused with caecal coccidia
- Presence of *Clostridium colinum, Clostridium perfringens* detected in necrotic enteritis along with coccidiosis indicates that the coccidiosis is predisposing factor.
- Intestinal intussusception and volvulus were sporadic in nature not always associated with coccidiosis

Mini Abstract

7 MINI ABSTRACT

Gut disorders in chicken are high impact conditions that are difficult to diagnose due to multiple aetiology and environmental factors. Present study aimed to assess occurrence of patho-morphological changes in chicken gut with special emphasis on proventriculitis, proventricular dilatation and possible viral aetiology. The study was conducted during December 2015 to November 2016 at ICAR-DPR, Hyderabad (17,978 carcasses) and commercial farms (120 carcasses). The overall occurrence of gut lesions were 9.22%. A total 1.83% were exhibited proventricular lesions with highest occurrence in Aseel and the lowest occurrence in Dwarf line. While, 7.82% showed intestinal lesions with highest (15.96%) occurrence in Nicobari and lowest (5.17%) in PB1 line. Proventricular thickening observed in 0.94% birds were of neoplastic and non-neoplastic aetiology. Non-neoplastic proventricular thickening chiefly affecting chicks and growers were characterised by gross changes such as pale white serosa, excess mucus production with microscopic changes such as thickening of muscularis mucosa by fibroplasia, enlarged gland ducts, ductal cell hyperplasia and mild necrosis of glandular epithelium. Chicken parvovirus detected in thickened proventriculus with runting stunting syndrome. CAV alone or in combination with MDV/ ALV were detected in proventricular thickening. No primary cause for the thickening could be identified for non-neoplastic condition. Proventriculo-ventricular intussusception observed in 2 chicks and one grower bird. The overall occurrence of intestinal lesion was 7.82%. *Eimeria necatrix* and *E tenella* were the major cause of haemorrhagic enteritis. The chicken parvovirus isolated from the intestinal contents of stunted chicks, induced runting of chick embryos on intra-yolk inoculation. Chicken parvovirus, Chicken astrovirus, Avian nephritisvirus, Avian rotavirus and Fowl adenovirus1 were associated with enteritis, proventricular thickening in birds affected with runting- stunting syndrome and also in birds died due to non-enteric conditions. Histomoniasis found in the intestine with necrotic enteritis along with mild liver lesions. *Clostridium colinum* and *Cl perfringens* were detected in necrotic enteritis along with coccidiosis. Intestinal intussusception and volvulus were sporadic not always associated with coccidiosis.

लघु सारांश

8 लघु सारांश

मुर्गियों में पेट के विकार उच्च प्रभावकारी स्थितियाँ है, जिनका निदान करना मुश्किल होता है क्योंकि यह विभिन्न प्रकार के रोगजनकों तथा पर्यावरणिय कारकों से उत्पन्न होते हैं। वर्तमान अध्ययन का उद्देश्य मुर्गियों के पेट में विकृति–आकृतिगत परिवर्तनों का आंकलन करना है जिसमें मुख्यता प्रोवेन्ट्रीकुलाइटिस, प्रोवेन्ट्रीकुलर फैलाव और संभव विषाणुजनित कारकों का अध्ययन करना है। यह अध्ययन दिसम्बर 2015 से नवम्बर, 2016 तक आई.सी.ए.आर.–डी.पी.आर., हैदराबाद (17978 शव) और वाणिज्यिक कार्यों (120 शव) में किया गया। पेट के घावों का समग्र प्रसार 9.22 प्रतिशत पाया गया। कुल 1.83 प्रतिशत मुर्गियों में प्रोवेंट्रीकुलर घाव देखे गये, जिनमें सबसे ज्यादा असील में तथा सबसे कम डार्फ लाईन में देखे गये। हालांकि 7.82 प्रतिशत में आंतों के घाव देखे गये। जिनमें सबसे अधिक निकोबारी (15.96%) तथा सबसे कम पीवी1 लाईन (5.17%) में पाये गये। प्रोवेंन्ट्रीकुलर मोटाई 0.94 प्रतिशत में पाई गई, जोकि नियोप्लास्टिक और गैर नियोप्लास्टिक कारकों के कारण थी। गैर नियोप्लास्टिक मोटाई मुख्यतः मुर्गी के बच्चों तथा उत्पादकों में पाई गई, पीला–सफेद सिकोजा, ज्यादा नलगम उत्पादन देखा गया। सक्ष्म परिवर्तनोंमें मस्कुलेरिस मयूकोजा की सुजन देखी गई जोकि काब्रोप्लेजिया, ग्रंथियों का नदना, डक्टल सेल हाइपरप्लेसिया तथा हल्की गलेन्डुलर सेल नेक्रोसिस के कारण था। प्रोवेन्ट्रीकुल मोटाई में अकेला सी.ए.वी. अथवा अन्य संयोजकों एम.डी.वी./ए. एल.वी. के साथ पाया गया। गैर नियोप्लास्टिक मोटाई में कोई भी प्राथमिक कारण नहीं पहचाना जा सका। प्रोवेन्ट्रीकुलो–वेन्ट्रीकुलर इन्डसेस्पशन 2 मुर्गी के बच्चों तथा एक उत्पादक में देखी गई। आंत के घावों का कुल प्रसार 7.82 प्रतिशत था। रक्तस्रावी आंत्रशोध में मुख्यतः एमेरिया निक्याट्रिक्स तथा एमेरिया ठिनोला देखे गये। चिकन पारवो वाईरस को कमजोर मुर्गी के बच्चों के मल में निकाला गया तथा इससे चिक भ्रूंण की रंटिग देखी गई। चिकन पारवो वाइरस, चिकन एस्ट्रोवाइरस, एवियन नेफ्राइटिस वाइरस, एवियन रोटावाइरस तथा काउल एडिनोवाइरस–1 को आंत्रशोध, प्रोवेन्ट्रीकुलर मोटाई, रन्टिंग सिन्ड्रोम तथा और आंत्रशोध मामलों में पहचाना गया। हिस्टोमोनिएसिस को आंत में नेक्रोटिक आंत्रशोध तथा हल्के लिवर के घावों में पाया गया। क्लोट्रिडियम कोलाईनम और क्लोस्ट्रीडियम परफ्रीन्जन्स को नेक्रोटिक आंक्रशोध में कोक्सीडियोसिस के साथ्ज्ञ देखा गया। आंत की इन्टुस्सेयसन तथा वेलकुलस के छुटपुट मामले देखे गये तथा ये हमेशा कोक्सीडियोसिस से संबंधित नहीं होते।

References

9 REFERENCES

Abdul-Aziz, T. and Barnes, H.J. 2013. Miscellaneous and Sporadic Bacterial Infections. In Swayne, D.E., Glisson, J.R., McDougald L.R., Nolan, L.K., Suarez, D.L. and Nair, V., eds. Disease of poultry. 13th edition. Wiley-Blackwell, Iowoa. USA. pp.1017-1027.

Ablin, L.W., Henney, H. S. and Berg. J. 1991. Intestinal diverticular malformations in dogs and cats. Compend. Contin. Educ. Pract. Vet. **13**:426-433.

Ahangaran, M.G., Azizi, S., Yadegari, M., Maghooli, M. and Hajimirzaei, R. 2012. Duplicated lower intestinal tract in a commercial broiler chicken with olypodia. Comp. Clin. Pathol. **21:**825–827.

Ajayi, O.L., Emikpe, B.O., Jagun, A. and Aina, S. 2007. Intestinal volvulus in domestic fowls. J. Anim. Vet. Adv. **6**(5): 606-607.

Ambali, A.G. 1992. Recent studies on the enterotropic strain of avian infectious bronchitis virus. Vet. Res. Commun. **16**:153–157.

Ambali, A.G. and Jones, R.C. 1990. Early pathogenesis in chicksof infection with an enterotropic strain of infectious bronchitis virus. Avian Dis. **34**:809–817.

Andral, B., Toquin, D., L'haridon, R., Jestin, A., Metz, M.H. and Rose, R. 1985. Les diarrhees du dindonneau: Unbilan des recherches virales effectuees (rotavirus, reovirus, adenovirus, pseudopicornavirus). Avian Pathol. **14**:147-162.

Arshad, S.S., Howes, K., Barron, G S., Smith, L.M., Russell, P.H. and Payne, L.N. 1997. Tissue tropism of the HPRS-103 strain of J subgroup avian leukosis virus and of a derivative acutely transforming virus. Vet. Pathol. **34:** 127–137.

Arshad, S.S., Smith, L.M., Howes, K., Russell, P.H., Venugopal, K. and Payne, L.N. 1999. Tropism of subgroup J avian leukosis virus as detected by *in situ* hybridization Avian Pathol. **28**: 163-169.

Balachandran, C., Murali Manohar, B., Venugopal, K. and Dorairajan, N. 1991. A case of intestinal diverticulum in a layer chicken. Indian Vet. J. **68:** 281.

Balachandran, C., Pazhanivel, N., Vairamuthu, S. and Murali Manohar. B. 2009. Marek's disease and Lymphoid leucosis in Chicken- A Histological Survey. Tamilnadu J. Vet. Anim. Sci. **5(4):** 167-170

Bancroft, J.D. and Gamble, M. 2008. Theory and Practice of Histological Techniques. 6th Ed., Churchill Livingstone, Elsevier, Philadelphia. pp. 657.

Bandyopadhyay, A.C. and Jain, S.K. 1967. A case of intussusception in a fowl. Indian Vet. J. **44**: 117-119.

Banerjee, P.S. and Yadav, C.L. 2001. Infectiousenterohepatitis in chickens in U.P. Indian J. Anim. Sci. **1**: 30-31.

Bano, L., Merialdi, G., Bonilauri, P., Dall'Anese, Capello, G.K., Comin, D., Cattoli, G., Sanguinetti, V., Hampson, D.J. and Agnoletti, F. 2008. Prevalence, disease associations and risk factors for colonization with intestinal spirochaetes (*Brachyspira* spp.) in flocks of laying hens in north-eastern Italy. Avian Pathol. **37**:281–286.

Barnes, H.J. 1987. Diseases of quail. Vet. Clin. North Am. Small Anim. Pract. **17**:1109–1144.

Baxendale, W. and Mebatsion, T. 2004. The isolation and characterisation of astroviruses from chickens. Avian Pathol. **33:** 364-370.

Bayyari, G.R., Huff, W.E., Balog, J.M., Rath, N.C. and Beasley, J.N. 1995. Experimental reproduction of proventriculitis using homogenates of proventricular tissue. Poult. Sci. **74**(11):1799-809.

Bayyari, G.R., Huff, W.E., Beasley, J.N., Balog, J.M. and Rath, N.C. 1996. The effect of dietary copper sulfate on infectious proventriculitis. Poult. Sci. **74**(12):1961-1969.

Behnke, E.L. and Fletcher, O.J. 2011. *Macrorhabdus ornithogaster* (Megabacterium) infection in adult hobby chickens in North America. Avian Dis. **55:**331–334.

Bezerra, D.A.M., da Silva, R.R., Kaiano, J.H.L., de Souza Oliveira, D., Gabbay, Y.B., Linhares, A.C. and Mascarenhas, J.D. 2014. Detection, epidemiology and characterization of VP6 and VP7 genes of group D rotavirus in broiler chickens. Avian Pathol. **43**: 238-243.

Bhattacharjee, P.S. and Jones, R.C. 1997. Susceptibility of organ cultures from chicken tissues for strains of infectious bronchitis virus isolated from the intestine. Avian Pathol. **26**(3): 553-563.

Bidin, M., Biđin, Z, Majnarić, D., Tisljar, M. and Lojkic, I. 2012 Circulation and phylogenetic relationship of chicken and turkey-origin astroviruses detected in domestic ducks (*Anas platyrhynchos domesticus*), Avian Pathol. **41:**555-562.

Bidin, M., Lojkic, I., Bidin, Z., Tiljar, M. and Majnaric, D. 2011. Identification and phylogenetic diversity of parvovirus circulatingin commercial chicken and turkey flocks in Croatia. Avian Dis. **55**:693–696.

Bierer, B.W., Carll, W.T., Eleazer, T.H. and Barnett, B.D. 1966. Gizzard erosion and lower intestinal congestion and ulceration due to feed and water deprivation in chickens of various ages. Poult. Sci. **45:** 1408-1411.

Biggs, P.M., Long, P.L., Kenzy, S.G. and Rootes, D.G.1968. Relationship between Marek's disease and coccidiosis. II. The effect of Marek's disease on the susceptibility of chickens to coccidial infection. Vet. Rec. **83**(12):284-289.

Brown, C.C., Baker, D.C. and Baker, I.K. 2007. Alimentary system.. In: Jubb, Kennedy & Palmer's Pathology of Domestic Animals, Vol. 2, 5th ed. (Maxie, M. G. ed.), Saunders, Edinburgh. pp. 94–100

Brugh, M. and Wilson, R.L. 1986. Effect of dietary histamine on broiler chickens infected with avian reovirus S1133. Avian Dis.**30:**199–203.

Buckles, E., Swayne, D. and Eaton, K. 1994. Cases of necrotising typhlitis associated with caecal spirochaete in common rheas (Rhea Americana). Vet. Pathol. **31**: 612.

Bulbule, N.R., Mandakhalikar, K.D., Kapgate, S.S., Deshmukh, V.V., Schat, K A. and Chawak, M.M. 2013. Role of chicken astrovirus as a causative agent of gout in commercial broilers in India. Avian Pathol. **42**(5): 464-473.

Burch, D.J.S., Strugnell, B.W., Steventon, A., Watson, E.N. and Harding, C. 2009. Survey of 222 flocks in Great Britain for the presence of Brachyspira species and their effect on production. In: Proceedings of the 5th International Conference on Colonic Spirochaetal Infections in Animals and Humans, Leon, Spain.

Campbell, J.G. 1969.Tumours of the Fowl. Lippincott, Philadelphia. pp. 1–292.

Campbell, J.G. and Appleby, E.C. 1966. Tumours in young chickens bred for rapid body growth (broiler chickens): A studyof 351 cases. J. Pathol. Bacteriol. **92:**77–90.

Carvallo, F.R., French, R.A., Gilbert-Marcheterre, K., Risatti, G., Dunn, J.R., Forster, F., Kiupel, M., Smyth, J.A. 2011. Mortality of one-week-old chickens during naturally occurring marek's disease virus infection. Vet. Pathol. **48**(5):993-998.

Chacon, J.L., Assayag, M.S., Revolledo, L., Astolfi-Ferreira, C.S., Vejarano, M.P., Jones, R. C. and Ferreira, A. J. P.2014. Pathogenicity and molecular characteristics of infectious bronchitis virus (IBV) strains isolated from broilers showing diarrhoea and respiratory disease. Br. Poult. Sci. **55**: 271–283.

Clench, M.H. and Mathias, J.R. 1995. The avian cecum: a review. Wilson Bull. **107**(l): 93-121.

Cogliani, C., Goossens, H. and Greko, C. 2011. Restricting Antimicrobial Use in Food Animals: Lessons from Europe. Microbe. **6**(6): 274-279.

Cooper, K.K. Songer, J.G. and Uzal, F.A. 2013. Diagnosing clostridial enteric disease in poultry. J. Vet. Diagnostic. Invest. **25(**3): 314–327

Coursodon, C.F., Trinh, H.T. and Mallozzi, M. 2010. *Clostridium perfringens* alpha toxin is produced in the intestines of broiler chicks inoculated with an alpha toxin mutant. Anaerobe. **16**:614–617.

Crespo, R. and Shivaprasad, H.L. 2013. Developmental, metabolic, and other non infectious disorders. In Swayne, D.E., Glisson, J.R., McDougald, L.R., Nolan, L.K., Suarez, D.L. and Nair, V., eds. Disease of poultry. 13th edition. Wiley-Blackwell, Iowoa. USA. p1254

Curtis, P.E. 1988. Volvulus in the fowl. Vet. Rec. **122**: 340.

Davis, J.F., Kulkarni, A. and Fletcher, O. 2012. Myocarditis in 9- and 11-day-old broiler breeder chicks associated with a reovirus infection. Avian Dis. **56**: 786–790.

Davis, R.B. 1973. Ulcerative enteritis in chickens: coccidiosis and stress as predisposing factors. Poult. Sci. **52**:1283–1287.

Day, J.M. 2013. Rota virus infection. In: Swayne, D.E., Glisson, J.R., McDougald,L.R., Nolan, L.K., Suarez, D.L. and Nair, V., eds. Disease of poultry. 13th edition. Wiley Black-Blackwell, Iowoa.USA.pp.381-391.

Day, J.M., Spackman, E. and Pantin-Jackwood, M. 2007. A multiplex RT-PCR test for the differential identification of turkey astrovirus type-1, turkey astrovirus type-2, chicken astrovirus, avian nephritis virus and avian rotavirus. Avian Dis. **51**: 681–684.

Day, M.J. and Zsak, L. 2010. Determination and analysis of the full-length chicken parvovirus genome. Virology. **399:** 59–64.

De Gussem, J., Swam, H., Lievens, K. and De Herdt, P.2010. Reovirus tenosynovitis in a flock of layer breeders. Avian pathol. **39:** 169–170.

De Gussem, M. 2007. Coccidiosis in poultry: review on diagnosis, control, prevention and interaction with overall gut health. In Proceedings of the XVI European Symposium on Poultry Nutrition Strasbourg, France. pp. 160-169.

Dekich, M. 1998. Broiler industry strategies for control of respiratory and enteric diseases. Poult. Sci. **77:** 1176–1180.

Devaney, R., Trudgett, J., Trudgett, A., Meharg, C. and Smyth, V.J. 2016. A metagenomic comparison of endemic viruses from broiler chickens with runting-stunting syndrome and from normal birds. Avian Pathol. **45**: 616–629.

deWit, J.J., Dam, G.B.T., van de Laar, J.M.A.M., Biermann, Y., Verstegen, I., Edens, F. andSchrier, C.C. 2011. Detection and characterization of a new astrovirus in chicken and turkeys with enteric and locomotion disorders. Avian Pathol. **40**:453–461.

Dhama, K., Saminathan, M., Karthik, K., Tiwari, R., Shabbir, M.Z., Kumar, N., Malik, S.Y. and Singh, K.R. 2015. Avian Rotavirus Enteritis - An Updated Review. Vet. Quarterly. **8:**39-84.

Dhoke, V.S., Thaware, M.V.J. and Bhagwat, S.S. 1995. Intestinal diverticulum in a broiler chick. Indian Vet J. **72**: 1096.

Dinev, I. 2010. Enzootic outbreak of necrotic gastritis associated with Clostridium perfringens in broiler chickens. Avian Pathol. **39**: 7-10.

Domańska-Blicharz, K. and Minta, Z. 2011. The role of parvoviruses in enteropathies of poultry. Polskie Drobiarstwo**. 3**(3):54–55.

Domanska-Blicharz, K., Tomczyk, G., Smietanka, K., Kozaczynski, W. and Minta, Z. 2011. Molecular characterization of fowl adenoviruses isolated from chickens with gizzard erosions. Poult. Sci. **90**(5): 983–989.

Dorner, J.W., Cole, R.J., Lomax, L.G., Gosser, H.S. and Diener, U.L. 1983. Cyclopiazonic acid production by Aspergillusflavus and its effects on broiler chickens. Appl. Environ. Microbiol.**46:** 698–703.

Dykstra, M.J., Charlton, B.R., Chin, R.P. and Barnes, H.J. 2013. Fungal infections. In Swayne, D.E., Glisson, J.R., McDougald,L.R., Nolan, L.K., Suarez, D.L. and Nair, V., eds. Disease of poultry. 13th edition. Wiley-Blackwell, Iowoa, USA. pp. 1088-1090.

Engström, B.E., Fermer, C., Lindberg, A., Saarinen, E., Båverud, V. and Gunnarsson, A. 2003. Molecular typing of isolates of Clostridium perfringens from healthy and diseased poultry. Vet. Microbiol. **94**(3): 225-235.

Escorcia, M., Fortoul, T.I., Petrone, V.M., Galindo, F., Lo´pez, C. and Te´llez, G. 2002.Gastric gross and microscopic lesions caused by the UNAM-97 variant strain of infectious bronchitis virus after the eighth passage in specific pathogen-free chicken embryos. Poult. Sci. **81**: 1647–1652.

Esquenet, C., De Herdt, P., De Bosschere, H., Ronsmans, S., Ducatelle, R. and Van Erum, J. 2003. An outbreak of histomoniasis in free-range layer hens. Avian Pathol. **32:** 305–308.

Estes, M.K. and Kapikian, A.Z. 2007. Rotaviruses. In: Knipe, D.M., Howley, P.M., Griffin, D.E., Lamb, R.A., Martin, M.A., Roizman, B. and Straus, S.E. (Eds), Fields Virology. 5th ed. Lippincott Williams and Wilkins, Philadelphia. pp. 1917-1974.

Eterradossi, N. and Saif, Y.M.2013. Infectious Bursal Disease.In: Swayne, D.E., Glisson, J.R., McDougald, L.R., Nolan, L.K., Suarez, D.L. and Nair, V., eds. Disease of poultry. 13th edition. Wiley-Blackwell, Iowoa.USA. pp. 219-246.

Fabri, T.H.F. 2000. Necrotic enteritis, Clostridial enteritis or dysbacteriosis? In Proceedings of the Elanco Symposium. Cork, Ireland.

Feberwee, A., Hampson, D.J., Phillips, N.D., La, T., vanderHeijen, H.M.J. F., Wellenberg, G.J., Dwars, R.M. and Landman,W.J.M. 2008. Identification of *Brachyspira hyodysenteriae* and other pathogenic *Brachyspira* species in chickens from laying flocks with diarrheaor reduced production or both. J. Clin. Microbiol. **46**: 593–600.

Feng, B., Xie, Z., Deng, X., Xie, L., Xie, Z., Huang, L., Fan, Q., Luo, S., Huang, J., Zhang, Y., Zeng, T., Wang, S. and Wang, L. 2016. Genetic and phylogenetic analysis of a novel parvovirus isolated from chickens in Guangxi, China. Arch. Virol. **161**(11): 3285-3289.

Fernandez, S., Pagotto, A.H., Furtado, M.M., Katsuyama, A.M., Madeira, A.M. and Gruber, A. 2003. A multiplex PCR assay for the simultaneous detection and discrimination of the seven *Eimeria* species that infect domestic fowl. Parasitol. **127:** 317–325

Finkler, F., Lima, D.A., Cerva, C., Moraes, L.B., Cibulski, S.P., Teixeira, T.F., Santos, H.F., Almeida, L.L., Roehe, P.M. and Franco, A.C. 2016. Chicken parvovirus and its associations with malabsorption syndrome. Res. Vet. Sci. **107**: 178-181.

Fitzgerald, S.D. 2013. Adeno virus Infection In: Swayne, D.E., Glisson, J.R., McDougald, L.R., Nolan, L.K., Suarez, D.L. and Nair, V., eds. Disease of poultry. 13th edition. Wiley-Blackwell, Iowoa.USA. pp. 289-290.

Fredrickson, T.N. 1987. Ovarian tumors of the hen. Environmental Health Perspectives. **73**: 35-51.

Ganapathy, K., Salamat, M.H., Lee, C.C. and Johara, M.Y. 2000 .Concurrent occurrence of salmonellosis, colibaccillosis and histomoniasis in a broiler flock fed with antibiotic-free commercial feed. Avian Pathol. **29**(6):639-642.

Ganapathy, K., Wilkins, M., Forrester, A., Lemiere, S., Cserep, T., McMullin, P. and Jones, R.C. 2012. QX-like infectious bronchitis virus isolated from cases of proventriculitis in commercial broilers in England. Vet. Rec. **171**(23): 597.

Gholamiandekhordi, A.R., Ducatelle, R., Heyndrickx, M., Haesebrouck, F. and Van Immerseel, F. 2006. Molecular and phenotypical characterization of *Clostridium perfringens* isolates from poultry flocks with different disease status. Vet. Microbiol. **113**(1): 143-152.

Glavits, R., Ivanics, E., Thuma, A., Kaszanyitzky, E., Samu, P., Ursu, K., Dencso, L. and Dan, A. 2011. Typhlocolitis associated with spirochaetes in duck flocks. Avian Pathol. **40**: 23–31.

Glisson, J.R., Hofacre, C.L. and Christensen, J.P. 2013. Fowl cholera In. Swayne, D.E., Glisson, J.R., McDougald, L.R., Nolan, L.K., Suarez, D.L. and Nair, V., eds. Disease of poultry. 13th edition. Wiley-Blackwell, Iowoa.USA. pp. 807-858.

Goodwin, M.A., Hafner, S., Bounous, D.I., Latimer, K.S., Player, E.C. Niagro, F.D. Campagnoli, R.P. and Brown, J. 1996. Viral proventriculitis in chickens. Avian Pathol. **25**:369–379.

Gough, R.E., Collins, M.S., Borland, E. and Keymer, L.F. 1984. Astrovirus-like particles associated with hepatitis in ducklings. Vet. Rec. **114:** 279.

Gouthaman, V., Singh, S.D., Dhama, K., Srinivasan, B., Saravanan, S., Gopalakrishnamurthy, T.R., Deb, R., Mathapati, B. and Ramakrishnan, M.A. 2015. Detection and partial genetic characterisation of a novel variant of avian nephritis virus in Indian poultry flocks showing diverse clinical signs. Acta Veterinaria Hungarica. **63**(4): 499–507.

Graubmann, H.D. and Grafner, G. 1971. Ulcerative enteritis inthe chicken and quail disease. Occurrence and pathology. Monatsh Vet. Med. **26:** 903–907.

Guilford, W.G. 1996. Diseases of swallowing. In: Guilford WG, Centre SA, Strombeck DR. Editors. Strombeck's Small Animal Gastroenterology. 3rd ed. WB Saunders, Philadelphia. pp. 211-235.

Gurdev, S.G., Balwant, S. and Sahota, P.S.1976.Congenital anomalies of domestic fowl: seven cases. Avian Dis. **20**: 581-586.

Guy J.S., West, A.M. and Fuller, F.J. 2011. Physical and genomic characteristics identify chicken proventricular necrosis virus (R11/3 virus) as a novel birnavirus. Avian Dis. **55**(1):2-7.

Guy, J. S. 1998. Virus infections of the gastrointestinal tract of poultry. Poult. Sci. **77**(8):1166–1175.

Guy, J. S., Barnes, H. J., Smith, L., Owen, R. and Fuller, F. J. 2005. Partial characterization of an adenovirus-like virus isolated from broiler chickens with transmissible viral proventriculitis. Avian Dis. **49:** 344–351.

Guy, J. S., Smith, L.G., Evans, M.E. and Barnes, H.J. 2007. Experimental reproduction of transmissible viral proventriculitis by infection of chickens with a novel adenovirus-like virus (isolate R11/3). Avian Dis. **51**:58–65.

Hafner, S. and Guy, J.S. 2013. Proventriculitis and proventricular dilatation of broiler chickens. In: Swayne, D.E., Glisson, J.R., McDougald,L.R., Nolan, L.K., Suarez, D.L. and Nair, V., eds. Disease of poultry. 13th edition. Wiley-Blackwell, Iowoa, USA. pp. 1328-1423.

Hafner, S., Reece, R.L. and Williams, S.M. 2013. Other tumours In: Swayne, D.E., Glisson, J.R., McDougald,L.R., Nolan, L.K., Suarez, D.L. and Nair, V., eds. Disease of poultry. 13th edition. Wiley-Blackwell, Iowoa, USA. pp.604-674

Hampson, D.J. 2013. Avian Intestinal Spirochetosis. In: Swayne, D.E., Glisson, J.R., McDougald,L.R., Nolan, L.K., Suarez, D.L. and Nair, V., eds. Disease of poultry. 13th edition. Wiley-Blackwell, Iowoa, USA. pp. 995-1007.

Haridy, M., Goryo, M.,Sasaki, J and Okada, K. 2010. Intestinal volvulus with coagulative hepatic necrosis in a chicken. J. Vet. Med. Sci.**72**(4): 489–492.

Harrach B., Benkö, M., Both, G.W., Brown, M., Davison, A.J., Echavarria, M., Hess, M., Jones, M.S., Kajon, A., Lehmkuhl, H.D., Mautner, V., Mittal, S.K. and Wadell, G. 2012. FamilyAdenoviridae. In: A.M.Q. King, M.J. Adams, E.B. Carstens, andE.J. Lefkowitz, eds. Virus Taxonomy: Ninth Report of the International Committee on Taxonomy of Viruses, 9th ed. ElsevierAcademic Press, New York. pp. 125–141.

Hauck, R., Gallardo, R.A., Woolcock, P.R. and Shivaprasad, H. L. 2016. A coronavirus associated with runting stunting syndrome in broiler chickens. Avian Dis. **60:** 528–534.

Helmboldt, C.F. and Bryant, E.S. 1971. The pathology of necroticenteritis in domestic fowl. Avian Dis. **15:** 775–780.

Hess, M and McDougald, L.R. 2013. Histomoniasis (Blackhead) and other protozoan diseases of the intestinal tract. In Swayne, D.E., Glisson, J.R., McDougald L.R., Nolan, L.K., Suarez, D.L. and Nair, V., eds. Disease of poultry. 13th edition. Wiley-Blackwell, Iowoa. USA. pp 1172-1182.

Hess, M. 2013. Aviadenovirus infection. In: Swayne, D.E., Glisson, J.R., McDougald, L.R., Nolan, L.K., Suarez, D.L. and Nair, V., eds. Disease of poultry. 13th edition. Wiley-Blackwell, Iowoa. USA. pp. 290-300

Hess, M., Grabensteiner, E. and Liebhart, D. 2006. Rapid transmission of the protozoan parasite *Histomonas meleagridis* in turkeys and specific pathogen free chickens following cloacal infection with a mono-eukaryotic culture. Avian Pathol. **35**(4): 280–285.

Hewson, K.A., Denise O'Rourke, and Noormohammadi, A. H. 2010.Detection of Avian Nephritis Virus in Australian Chicken Flocks. Avian Dis. **54**(3): 990-993.

Hitchner, S.B. 1970. Infectivity of Infectious Bursal Disease Virus for Embryonating Eggs. Poult. Sci. **49**(2):511–516.

Hoerr, F.J. 2013. Mycotoxicoses .In: Swayne, D.E., Glisson, J.R., McDougald, L.R., Nolan, L.K., Suarez, D.L. and Nair, V., eds. Disease of poultry. 13th edition. Wiley-Blackwell, Iowoa, USA. pp. 1271- 1286.

Hoerr, F.J.1998. Pathogenesis of enteric diseases. Poult. Sci. **77:**1150–1155

Honkavuori, K.S., Shivaprasad, H.L., Williams, B.L., Quan, P.L., Hornig, M. and Street, C. 2008. Novel bornavirus in psittacine birds with proventricular dilatation disease. Emerg. Infect. Dis. **14:**1883–1886

Horrox, N.E. 1980. Some observations and comments on rotaviruses in turkey poults. 29th West. Poult. Dis. Conf. pp. 162-164.

Hu, J., Fuller, L., Armstrong, P. L. and McDougald, L. R. 2006. *Histomonas meleagridis* in chickens: attempted transmission in the absence of vectors. Avian Dis. **50**(2): 277-279.

Huff, G.R., Zheng, Q., Newberry, L.A., Huff, W.E., Balog, J.M., Rath, N.C., Kim, K.S., Martin, E.M., Goeke, S.C. and Skeeles, J.K. 2001. Viral and bacterial agents associated with experimental transmission of infectious proventriculitis of broiler chickens. Avian Dis. **45**(4):828-43.

Imada, T., Yamaguchi, S., Mase, M., Tsukamoto, K., Kubo, M. and Morooka, A. 2000. Avian nephritis virus (ANV) as a new member of the family Astroviridae and construction of infectious ANVcDNA. J. Virol. **74:** 8487-8493.

Jansson, D.S., Fellström, C., Rasback, T., Vagsholm, I., Gunnarsson, A., Ingermaa, F. and Johansson, K.E. 2008. Phenotypicand molecular characterization of *Brachyspira* sp. isolated from laying hens in different housing systems. Vet. Microbiol. **130:** 348–362.

Jensen, L.S., Dunn, P. A. and Dobson, K. N. 1991. Induction of oral lesions in broiler chicks by supplementing the diet with copper. Avian Dis. **35:** 969–973.

Jeurissen, S.H., Lewis, F., van der Klis, J.D., Mroz, Z., Rebel, J. M. and ter Huurne, A.A. 2002. Parameters and techniques to determine intestinal health of poultry as constituted by immunity, integrity, and functionality. Curr. Issues Intest. Microbiol. **3:** 1–14.

Johnson, P.A. and Giles, J.R. 2006. Use of genetic strains of chickens in studies of ovarian cancer. Poult. Sci. **85:** 246-250.

Jones, R.C. 2000. Avian reovirus infections. Rev. Sci. Tech. Off. Int. Epiz. **19**(2): 614-625

Jones, R.C., Hughes, C.S. and Hentry, R.R. 1979. Rotavirus infection in commercial laying hens. Vet. Rec. **104:** 22.

Kalia, M.L., 1958. Histomoniasis (Enterohepatitis) in indigenous fowls. Indian Vet. J. **35:** 105-107.

Kamalavenkatesh, P., Vairamuthu, S., Balachandran, C., Manohar, B.M. and Raj, G.D. 2005. Immunopathological effect of the mycotoxins cyclopiazonic acid and T-2 toxin on broiler chicken. Mycopathologia. **159:** 273-279.

Katiyar, A. K., Shrivastava, A. B., Awadhiya, R. P. and Vegad, J. L. 1988. Intestinal volvulus in a domestic fowl. Vet. Rec.122: 284.

Kattoor, J.J., Malik, Y.P.S., Sharma, K., Kumar, N. and Batra, M. 2013b. Molecular evidences of group D rotavirus in commercial broiler chicks, India. Avian Biol. Res. **6:** 313-316.

Kattoor, J.J., Sharma, K., Kumar, N., Batra, M., Jindal, N., Yadav, A.S. and Malik, Y.S. 2013a. Distribution frequency of avian rotaviruses in India, 2011-2013. Adv. Anim. Vet. Sci. **1:** 148-150.

Kemp, R. L., and Reid. W. M. 1966. Studies on the etiology of blackhead disease: the roles of Histomonas meleagridis and Candida albicans in the Unites States. Poult. Sci. **45:**1296–1302.

Keyburn, A.L., Boyce, J.D., Vaz, P., Bennam, T.L., Ford, M.E., Parker, D., Rubbo, A.D., Rood, J.I. and Moore, R.J. 2008. NetB, a new toxin that is associated with avian necrotic enteritis caused by *Clostridium perfringens*. PLoS Pathog.**4:** e26.

Keyburn, A.L., Sheedy, S.A., Ford, M.E., Williamson, M.W., Awad, M.M., Rood, J.I. and Moore,R .J. 2006. Alpha-toxin of *Clostridium perfringens* is not an essential virulence factor in necrotic enteritis in chickens. Infect. Immun.**74:** 6496–6500.

Kim, H.R., Yoon, S.J., Lee, H.S. and Kwon, Y.K. 2015. Identification of a picornavirus from chickens with transmissible viral proventriculitis using metagenomic analysis. Archives of virology, **160**(3): 701-709.

Kisary, J. 1985. Experimental infection of chicken embryos andday-old chickens with parvovirus of chicken origin. Avian Pathol. **14:** 1–7.

Kisary, J., Nagy, B. and Betay, Z. 1984. Presence of parvovirus in the intestine of chickens showing stunting syndrome. Avian Pathol.**13:** 339-343.

Koo B.S., Lee H.R., Jeon E.O., Han M.S., Min K.C., Lee S.B. and Mo I.P.. 2013a. Molecular survey of enteric viruses in commercial chicken farms in Korea with a history of enteritis. Poult. Sci. **92**(11):2876-2885.

Koo, B.S., Lee, H.R., Jeon, E.O., Jang, H.S., Han, M.S. and Mo, I.P. 2013b. An unusual case of concomitant infection with chickens astrovirus and group A rotavirus in broilers with a history of severe clinical signs. J. Vet. Sci. **14:** 231–233.

Kouwenhoven, B., Davelaar, E G. and Van Walsum, J. 1978b. Infectious proventriculitis causing runting in broilers. Avian Pathol. **7:**183–187.

Kouwenhoven, B., Vertommen, M. and van Eck, J. H. H. 1978a.Runting and leg weakness in broilers; Involvement of infectious factors. Vet. Sci. Commun. **2**(1): 253–259.

Kumar, S., Garg, R. , Moftah, A., Clark, E.L., Macdonald, S.E., Chaudhry, A.S., Olivier Sparagano, Banerjee, P.S., Kundu, K., Tomley, F.M. and Blake, D.P. 2013. An optimised protocol for molecular identification of *Eimeria* from chickens. Vet. Parasitol. **199**(1-2): 24-31.

Kusumakar, A.L., Savita, Malik, Y.P.S. and Minakshiprasad, G. 2010. Genomic diversity among group A rotaviruses from diarrheic children, piglets, buffalo and cow calves of Madhya Pradesh. Indian J. Microbiol. **50:** 83-88

Kwatra, M.S. and Choudhary, B. 1976. A presumptive diagnosis in the fowl (*Gallusgallus domesticus*) from Assam, India. Avian Dis. **20:** 401-406.

Kyung-Il Kang, El-Gazzar, M., Sellers, H.S., Dorea, F., Williams, S.M., Kim, T., Collett, S. and Mundt, E. 2012. Investigation into the aetiology of runting and stunting syndrome in chickens. Avian Pathol. **41**(1): 41-50.

Langhout, D.J., Schutte, J.B., Van Leeuwen, P., Wiebenga, J. and Tamminga, S. 1999. Effect of dietary high- and low-methylated citrus pectin on the activity of the ileal microflora and morphology of the small intestinal wall of broiler chicks. British Poult. Sci. **40:** 340-347.

Lenz, S.D., Hoerr, F.J., Ellis, A.C., Toivio-Kinnucan, A and Yu, M. 1998. Gastrointestinal pathogenicity of adenoviruses and reoviruses isolated from broiler chickens in Alabama. J. Vet. Diagn. Invest. **10:** 145–151.

LeRoy, C.I., Mappley,L.J., La Ragione,R.M., Woodward, M.J. and Claus, S.P.2015. *Brachyspirapilosicoli*-induced avian intestinal spirochaetosis. Microb. Ecol. Hlth. Dis. **26:** 28853.

Lew, A.E., Anderson, G.R., Minchin, C.M., Jeston, P.J. and Jorgensen, W.K. 2003. Inter- and intra-strain variation and PCR detection of the internal transcribed spacer 1 (ITS-1) sequences of Australian isolates of *Eimeria* species from chickens. Vet. Parasitol. **112 :** 33–50.

Lobago, F. and Woldemeskel. 2004. An Outbreak of Marek's Disease in Chicken in Central Ethiopia. Trop. Anim. Hlth. Prod. **36:** 397-406.

Lobago, F., Worku, N., and Wossene,A. 2005. Study of coccidiosis in Kombolcha poultry farm, Ethopia. Trop. Anim. Hlth. Prod. **37**(3):245-251.

Lovland, A., Kaldhusdal, M. and Reitan, L.J. 2003. Diagnosing *Clostridium perfringens*-associated necrotic enteritis in broiler flocks by an immunoglobulin G anti-alpha-toxin enzyme linked immunosorbent assay. Avian Pathol. **32:** 527–534.

Lozano, L.F., Hammami, S., Castro, A.E. and Osburn, B. 1992. Comparison of electron microscopy and polyacrylamide gel electrophoresis in the diagnosis of avian reovirus and rotavirus infections. Avian Dis. **36**(2):183–188.

Lublin, A., Mechani, S. and Bumbarov, V. 2004. Involvement of rotavirus in intestinal infections of poultry and pet birds. Abstract presented at the 28th annual Israel veterinary symposium in memory of Dr. Ora Egozi, 2004. Isr. J. Vet. Med. **59:** 3.

Lund, E.E., Chute, A.M. and Myers, S.L. 1970. Performance in chickens and turkeys of chicken-adapted *Heterakis gallinarum*. J. Helminthol. **44:** 97–106.

Maas, H.J. and Van De Venne. 1985. Hyperplasia of proventricular duct epithelium in broilers. Avian Pathol. 14(1):115- 125.

Maeda, M., Imada, T., Taniguchi, T. and Horiuchi, T. 1979. Pathologicalchanges in chicks inoculated with the picornavirus "aviannephritis virus". Avian Dis. **23:** 588-596.

Manarolla, G., Pisoni, G., Moroni, P., Gallazzi, D., Sironi, G. and Rampin, T. 2009. Adenoviral gizzard erosions in Italian chicken flocks. Vet. Rec. **164**(24): 754–756.

Mappley, L.J., La Ragione, R.M. and Woodward, M.J. 2014. Brachyspira and its role in avian intestinal spirochaetosis.Vet. Microbiol. **168** (3-4): 245-260.

Marguerie, J., Leon,O.,Albaric,O., Guy,J.S.and Guerin, J.L. 2011.Birnavirus-associated proventriculitis in French broiler chickens. Vet. Rec. **169:** 394–396.

Martinez-Costas, J., Grande, A., Varela, R., Garcia-Martinez, C. and Benavente, J. 1997. Protein architecture of avian reovirus S1133 and identification of the cell attachment protein. J. Virol. **71:** 59–64.

Martins, R.S., Horta, A.C., Siqueira, A.M., Lopes, S.Q., Resende, J.S., Jorge, M.A., Assis, R.A., Martins, N.E., Fernandes, A.A., Barrios, P.R.., Costa, T.J.R. and Guimaraes, L.M.C. 2006. *Macrorhabdus ornithogaster* in ostrich, rhea, canary, zebrafinch, free range chicken, turkey, guinea-fowl, columbinapigeon, toucan, chuckar partridge and experimental infection inchicken, Japanese quail and mice. Arq. Bras. Med. Vet. Zootec. **58:** 291–298.

Marusak, R. A., West, M. A., Davis, J.F., Fletcher, O.J., and Guy, J.S. 2012. Transmissible viral proventriculitis identified inbroiler breeder and layer hens. Avian Dis. **56:** 757–759.

Matthijnssens, J., Otto, P., Ciarlet, M., Desselberger, U., Van Ranst, M. and Johne, R. 2011. VP6 sequence-based cut-off values as a criterion for rotavirus species demarcation. Arch. Virol. **157:** 1177-1182.

McDougald, L.R. 2005. Blackhead disease (histomoniasis) in poultry: a critical review. Avian Dis. **49:** 462-476.

McDougald, L.R. 2013. Internal Parasites. In: Swayne, D.E., Glisson, J.R., McDougald, L.R., Nolan, L.K., Suarez, D.L. and Nair, V., eds. Disease of poultry. 13th edition. Wiley-Blackwell, Iowoa, USA. pp. 1117-1146.

McDougald, L.R. and Fitz-coy, S.H. 2013. Coccidiosis. In. Swayne, D.E., Glisson, J.R., McDougald, L.R., Nolan, L.K., Suarez, D.L. and Nair, V., eds. Disease of poultry. 13th edition. Wiley-Blackwell, Iowoa, USA. 1148-1172.

McDougald, L.R. and Hu, J. 2001. Blackhead disease (*Histomonas meleagridis*) aggravated in broiler chickens by concurrent infection with cecal coccidiosis (*Eimeria tenella*) Avian Dis. **45:** 307–312

McGill, S.E., Lenard, Z.M., See, A.M. and Irwin, P.J. 2009. Nonsurgical treatment of gastroesophageal intussusception in a puppy. J. Am. Anim. Hosp. Assoc. **45(4):**185-90.

McNeilly, F., Connor, T.J., Calvert, V.M., Smyth, J.A., Curran, W.L., Morley, A.J., Thompson, D., Singh, S., McFerran, J.B., Adair, B.M. and McNulty, S. 1994. Studies on a new enterovirus-like virus isolated from chickens. Avian Pathol. **23:** 313–327.

McNulty, M.S., Allan, G.M. and McCracken, R.M. 1983. Experimental infection of chickens with rotaviruses: clinical and virological findings. Avian Pathol. **12:** 45–54.

McNulty, M.S., Curran, W.L. and McFerran, J.B. 1980. Detection ofastroviruses in turkey faeces by direct electron microscopy. Vet. Rec. **10:** 561.

Mettifogo, E., Nuñez, L.F., Chacón, J.L., Santander, Parra, S.H., Astolfi-Ferreira, C.S., Jerez J.A., Jones, R.C. and Piantino Ferreira, A.J. 2014. Emergence of enteric viruses in production chickens is a concern for avian health. Scientific World Journal. 450423.

Miller, P.J. and Koch, G. 2013. Newcastle Disease.In: Swayne, D.E., Glisson, J.R., McDougald,L.R., Nolan, L.K., Suarez, D.L. and Nair, V., eds. Disease of poultry. 13th edition. Wiley-Blackwell, Iowoa, USA. pp. 89-107.

Minakshi Prasad, G., Verma, S. and Dahiya, S. 2004. Detection of avian rotaviruses from diarrhoeic poultry in India. Ind. J. Microbiol. **44:** 205-206.

Montgomery, R. D., Boyle, C. R., Maslin, W. R. and Magee, D. L. 1997. Attempts to reproduce a runting/stunting-type syndrome using infectious agents isolated from affected Mississippi broilers. Avian Dis. **41:** 80–92.

Moraes, J.C., Marcie, A., Amelia, F.A., Sartor, A.,Bellato, V., de Moura,A.,Magalhaes, M.L.B., Pereira, B.A., de Souza,A and Miletti, L.C.2015.Prevalence of Eimeria spp. in Broilers by Multiplex PCR in the Southern Region ofBrazil on Two Hundred and Fifty Farms. Avian Dis. **59**:277-281.

Mori, Y., Borgan, M.A., Ito, N., Sugiyama, M. and Minamoto, N. 2002. Diarrhea-inducing activity of avian rotavirus NSP4 glycoproteins, which differ greatly from mammalian rotavirus NSP4 glycoproteins in deduced amino acid sequence, in suckling mice. J. Virol. **76:** 5829-5834

Morrill, D.R. 1944. Intussusception of the cecum of a chicken. J. Am. Vet. Med. Assoc. **105:** 289.

Morrow, C. 2001. Solving the problems of necrotic enteritis. British Poult. Sci. **42:** 64-68.

Morse, G.B. 1907. Quail disease in the United States. United States Department of Agriculture, BAI Circ. 109.

Mortimer, I. 2002. The detection of dysbacteriosis. In Proceedings of the Elanco Global Enteritis Symposium. Cambridge, UK.

Myers, S.E., Dunn, P.A., Phillips, N.D. La, T. and Hampson, D.J. 2009. *Brachyspira intermedia* and *Brachyspira pilosicoli* are commonly found in older laying flocks in Pennsylvania. Avian Dis. **53:** 533–537.

Nakamura, K., Tanaka, H., Mase, M., Imada, T. and Yamada, M. 2002. Pancreatic necrosis and ventricular erosion in adenovirus associated hydropericardium syndrome of broilers. Vet. Pathol. **39**(3): 403–406.

Newberne, P. M., Muhrer, M. E. Craghead, R. and O'Dell B. L. 1956.An abnormality of the proventriculus of the chick. J. Am. Vet. Med. Assoc. **128:** 553–555.

Niture, G.S., Karpe, A.G., Miniakshi Prasad, G., Bhonsle, A.V. and Patil.S.V. 2010. Detection of group D avian rotavirus among layer poultry from western India. Int. J. Poult. Sci. **9**(1): 72-76.

Noiva, R., Guy, J.S., Hauck, R. and Shivaprasad, H.L. 2015. Runting stunting syndrome associated with transmissible viral proventriculitis in broiler chickens. Avian Dis. **59**(3): 384-7.

Nolan, L.K., Barnes, H.J., Vaillancourt, J., Abdul-Aziz, T. and Logue, C.M. 2013. Colibacillosis.In: Swayne, D.E., Glisson, J.R., McDougald,L.R., Nolan, L.K., Suarez, D.L. and Nair, V., eds. Disease of poultry. 13th edition. Wiley-Blackwell, Iowoa, USA. pp. 776.

Nunez, L. F. N., Sa, L. R. M., Parra, S. H. S., Astolfi-Ferreira, C. S., Carranza, C. and Ferreira, A. J. P. 2016. Molecular detection of chicken parvovirus in broilers with enteric disorders presenting curving of duodenal loop, pancreatic atrophy, and mesenteritis. Poult. Sci. **95**(4): 802-810.

Nunez, L.F.N., Santander Parra, S.H., Mettifogo, E., Astolfi-Ferreira,C.S. and Piantino Ferreira, A.J. 2015. Isolation and molecular characterisation of chicken parvovirus from Brazilian flocks with enteric disorders. British Poult. Sci. **56**(1): 39-47.

Okuda, Y., Ono, M, Shibata, I., Sato, S. and Akashi, H. 2006. Comparison of the polymerase chain reaction-restriction fragment length polymorphism pattern of the fiber gene and pathogenicity of serotype-1 fowl adenovirus isolates from gizzard erosions and from feces of clinically healthy chickens in Japan. J. Vet. Diagn. Invest.**18:**162–167.

Okuda, Y., Ono, M., Shibata, I. and Sato, S. 2004. Pathogenicity of serotype 8 fowl adenovirus isolated from gizzard erosions of slaughtered broiler chickens. J. Vet. Med. Sci.**66** (12): 1561–1566.

Ono, M., Okuda, Y., Shibata, I., Sato, S. and Okada, K. 2007. Reproduction of adenoviral gizzard erosion by the horizontal transmission of fowl adenovirus serotype 1. J. Vet. Med. Sci. **69**:1005–1008.

Ono, M., Okuda, Y., Shibata, I., Sato, S. and Okada. 2004. Pathogenicity by parenteral injection of fowl adenovirus isolated from gizzard erosion and resistance to reinfection in adenoviral gizzard erosion in chickens. Vet. Pathol. **41**(5): 483–489.

Otto, P., Liebler-Tenorio, E.M., Elschner, M., Reetz, J., Lohren, U. and Diller, R. 2006. Detection of rotaviruses and intestinal lesions in broiler chicks from flocks with runting and stunting syndrome (RSS). Avian Dis. **50**: 411-418.

Page, R.K., Fletcher, O.J., Rowland, G.N., Gaudry, D and Villegas, P. 1982. Malabsorption syndrome in broiler chickens. Avian Dis. **26:** 618–624.

Palade, E.A., Kisary, J., Benyeda, Z., Mándoki, M., Balka, G., Jakab, C., Végh. B, Demeter, Z., Rusvai, M. 2011. Naturally occurring parvoviral infection in Hungarian broiler flocks. Avian Pathol. **40:** 191-197.

Palanivelu, M., Asok Kumar, M., Singh, S. D., Kumar, P., Barathidasan, R., Prabhu, S.N. and Pragya, B. 2014. Intussusception of jejunum in pullet naturally infected with coccidiosis: A case report. Indian J.Vet.Pathol. **38**(2): 135-136.

Pandit, C.N. and Tiwari, A.K. 1964. A case of intussusception in a hen. J. Vet. Anim. Husb. Res. **8:** 126-127.

Panneman, H. 2000. Clostridial enteritis/dysbacteriosis, fast diagnosis by T-RFLP, a novel diagnostic tool. In Proceedings of the Elanco Global Enteritis Symposium. Cork, Ireland.

Pantin-Jackwood, M.J., Day, J.M., Jackwood, M.W. and Spackman, E. 2008. Enteric viruses detected by molecular methods in commercial chicken and turkey flocks in the United States between 2005 and 2006. Avian Dis. **52**(2):235-44.

Pantin-Jackwood, M. J. 2013. Multicausal enteric diseases. In: Swayne, D.E., Glisson, J.R., McDougald,L.R., Nolan, L.K., Suarez, D.L. and Nair, V., eds. Disease of poultry. 13th edition. Wiley-Blackwell, Iowoa, USA. pp. 1322-1325.

Pantin-Jackwood, M. J., Brown, T. P. and Huff, G. R. 2005. Reproduction of proventriculitis in commercial and specificpathogen-free broiler chickens. Avian Dis. **49:** 352–360.

Pantin-Jackwood, M.J., Brown, T.P., Kim, Y. and Huff, G.R.G. 2004. Proventriculitis in broiler chickens: effects of immunosuppression. Avian Dis. **48:** 300–316.

Pantin-Jackwood, M.J., Day, J.M., Jackwood, M.W. and Spackman, E. 2008. Enteric viruses detected by molecular methods in commercial chicken and turkey flocks in the United States between 2005 and 2006. Avian Dis. **52:**235-244.

Pantin-Jackwood, M.J., Spackman, E. And Woolcock, P.R. 2006. Molecular characterization and typing of chicken and turkey astroviruses circulating in the United States: implications for diagnostics. Avian Dis. **50:** 397-404.

Parihar, N.S. and Shukla, V. 1964.Intussusception in poultry. Indian Vet. J. **41**: 576-578.

Pattison, M. 2002. Some clinical and pathological features of enteritis in broilers- observations on treatment in the UK. In Proceedings of the Elanco Global Enteritis Symposium, Cambridge, UK. pp. 1-7.

Payne, L. N., Frazier, J. A. and Powell, P. C. 1976. Pathogenesis of Marek's Disease. Int. Rev. Exp. Pathol. **16:** 59-153.

Pennycott, T.W., Duncan, G and Venugopal, K. 2003. Marek'sdisease, candidiasis and megabacteriosis in a flock of chickens (*Gallus gallusdomesticus*) and Japanese quail (*Coturnix japonica*). Vet. Rec. **153:** 293–297.

Peroulis-Kourtis, I., O'Riley, K., Grix, D., Condron, R.J. and Ainsworth, C. 2002. Molecular characterisation of Victorian Newcastle disease virus isolates from 1976 to 1999. Aust. Vet. J. **80:** 422-424.

Pinard, R., de Winter, A., Sarkis, G. J., Gerstein, M. B., Tartaro, K. R., Plant, R. N., Egholm, M., Rothberg, J. M. and Leamon, J. H. 2006. Assessment of whole genome amplification-induced bias through high-throughput, massively parallel whole genome sequencing. BMC Genomics. **7:** 216 – 237.

Pizarro, M., Thomas, A., Brown, P., Villegas, P. and Rowland, G.N.1994. Intestinal diverticulum in a turkey. Avian Dis. **38:**184-187.

Porter, R.E. Jr. 1998. Bacterial enteritides of poultry. Poult. Sci.**77**:1159–1165.

Poupoulis, C. and Jensen, L. S. 1976. Effect of high dietary copperon gizzard integrity of the chick. Poult. Sci. **55:** 113–121.

Prescott, J. F. 1979. The prevention of experimentally induced necrotic enteritis in chickens by avoparcin. Avian Dis. **23:** 1072–1074.

Raman, M., Raman, U., Banu, S.S., Raj, D and Raj, G.D. 2011. Lesion scoring technique for assessing the virulence and pathogenicity of Indian field isolates of avian *Eimeria* species. Vet. Arhiv. **81**(2): 259-271.

Randall, C.J., Macdonald, J.W., Mills, C.P.J., Kirkpatrick, K.S. and Pattison, M. 1985. Proventricular haemorrhages in broilers. Vet. Rec. **117**(11): 273-274

Raue, R. and Hess, M. 1998. Hexon based PCRs combined with restriction enzyme analysis for rapid detection and differentiation of fowl adenoviruses and egg drop syndrome virus. J. Virol. Methods. **73**(2):211-7.

Reece, R. 2002. Transmissible (infectious) proventriculitis of Australian broiler chickens. Proceedings of the AVPA Conference, Surfer's Paradise, Australia. 35–38.

Reece, R. L. 1996. Some observations on naturally occurring neoplasms of domestic fowls in the State of Victoria Australia (1977–87). Avian Pathol. **25**(3): 407-447.

Reis Júnior, J. L., Beckstead, R. B., Brown, C. C. and Gerhold, R. W. 2009. *Histomonas meleagridis* and capillarid infection in a captive chukar (*Alectoris chukar*). Avian Dis. **53**(4): 45-46.

Riddell, C and Kong, X.M. 1992. The influence of diet on necrotic enteritis in broiler chickens. Avian Dis. **36**:499–503.

Riddell, C. 1976. The influence of fiber in the diet on dilation (hypertrophy) of the proventriculus in chickens. Avian Dis. **20:** 442–445.

Roussan, D. A., Shaheen, I.A., Totanji, W.S., Khawaldeh, G.Y. and Al Rifai, R.H. 2012a. Simultaneous detection of *Clostridium perfringens* and *Clostridium colinum* by duplex-polymerase chain reaction. Poult. Sci. **91**: 3080–3085

Roussan, D.A., Shaheen, I.A., Khawaldeh, G.Y., Totanji, W.S. and Al-Rifai, R.H. 2012b. Simultaneous detection of astrovirus, rotavirus, reovirus and adenovirus type I in broiler chicken flocks. Pol. J. Vet. Sci. **15**(2):337-344

Ruff, M.D. and Rosenberger, J.K. 1985. Concurrent infections with reoviruses and coccidia in broilers. Avian Dis. **29**: 465–478.

Sagartz, J.E., Swayne, D.E., Eaton, K.A., Hayes, J.R., Amass, K.D., Wack, R. and Kramer, L. 1992. Necrotizing typhlocolitis associated with a spirochete in rheas (*Rhea Americana*). Avian Dis. **36**: 282–289.

Saif, L.J., Saif, Y.M. and Theil, K.W. 1985. Enteric viruses in diarrhoeic turkey poults. Avian Dis. **29:** 798-811.

Sajewicz-Krukowska,J., Krzysztof Pać, Lisowska, A., Minta, Z. Króliczewska, B. and Domańska-Blicharz, K. 2016. Astrovirus induced 'white chicks' condition – field observation, virus detection and preliminary characterization. Avian Pathol. **45**(1): 2-12.

Savita, Kusumakar, A.L., Malik, Y.P.S. and Prasad, M. 2008a. Detection and characterization of group A and D avian rotaviruses in India. Indian J. Biotechnol. **7**:554- 556.

Savita, Kusumakar, A.L., Malik, Y.P.S. and Prasad, M. 2008b. Co-occurrence of avian rotavirus and bacterial pathogens in diarrheic poultry. Indian J. Anim. Sci. **78**:478-479.

Sawale, G.K., Mane, D.V., Gupta, S.C., Srivastava, P.K., Sabale, S.S., Bharkad, G.P. and More, B.K. 2011. Occurrence of histomoniasis in free range village chicken. Indian J. Vet. Pathol. **35**(2): 223-224

Schat, K.A. and van Santen, V.L. 2013. Chicken infectious anaemia. In: Swayne, D.E., Glisson, J.R., McDougald,L.R., Nolan, L.K., Suarez, D.L. and Nair, V. eds. Disease of poultry. 13th edition. Wiley-Blackwell, Iowoa, USA. pp. 248-264.

Schat, K.A. and Venugopal Nair 2013. Marek's Disease. In: Swayne, D.E., Glisson, J.R., McDougald,L.R., Nolan, L.K., Suarez, D.L. and Nair, V. eds. Disease of poultry. 13th edition. Wiley-Blackwell, Iowoa, USA. pp. 515-553.

Schultz-Cherry, S.L. 2013. Astrovirus infection.In: Swayne, D.E., Glisson, J.R., McDougald, L.R., Nolan, L.K., Suarez, D.L. and Nair, V., eds. Disease of poultry. 13th edition. Wiley-Blackwell, Iowoa, USA. pp 391- 405.

Schwarz, A., Gauly,M, Abel,H., Daş,G., Humburg, J., Weiss, A., Breves, G. and Rautenschlein,S. 2011. Pathobiology of Heterakis gallinarum mono-infection and co-infection with Histomonas meleagridis in layer chickens. Avian Pathol. **40** (3): 277-287.

Shah, J.D., Desai, P.T., Zhang, Y., Scharber, S.K., Baller, J., Xing Z.S. and Cardona, C.J. 2016. Development of the intestinal RNA virus community of healthy broiler chickens. PLoS ONE. **11**(2): e0150094

Shapiro, F., Nir, I. and Heller, D. 1998. Stunting syndrome in broilers: effect of stunting syndrome inoculum obtained from stunting syndrome affected broilers, on broilers, leghorns and Turkey poults. Poult. Sci. **77**(2): 230–236.

Sharma, U.K. 1971. Intussusception in poultry. Indian Vet. J. **48**: 337-341.

Sharma, U.K. 1972. Intussusception of the proventriculus ofchickens. Avian Dis. **16**: 453–457.

Shifrine, M., Adler, H.E. and Ousterhout, L.E. 1960. The pathology of chicks fed histamine. Avian Dis. **4**: 12–21.

Shillinger, J.E. and Morley, L.C. 1932. Studies on ulcerative enteritis in quail. J. Am. Vet. Med. Assoc. **84**: 25–35.

Shirai, J., Tanimura, N., Uramoto, K., Narita, M., Nakamura, K. and Kawamura, H. 1992. Pathologically and serologically different avian nephritis virus isolates implicated in etiology of baby chick nephropathy. Avian Dis. **36**: 369–377.

Shivaprasad, H.L. and Barrow, P.A. 2013. Pullorum disease and fowl typhoid. In: Swayne, D.E., Glisson, J.R., McDougald, L.R., Nolan, L.K., Suarez, D.L. and Nair, V. eds. Disease of poultry. 13th edition. Wiley-Blackwell, Iowoa, USA. pp 678-692.

Shrivastava, A.B., Katiyar, A.K., Awadhiya. R,P. and Vegad, J.L. 1989. Ventricular intussusception in a domestic fowl. Avian Pathol. **18**: 547-550.

Smit, H.F., Dwars, R.M., Davelaar, F.G. and Wijtten, G.A. 1998. Observations on the influence of intestinal spirochaetosis in broiler breeders on the performance of their progeny and on egg production. Avian Pathol. **27**: 33–141.

Smith, L.M., Brown, S.R. Howes, K., McLeod, S., Arshad, S.S., Barron, G.S., Venugopal, K., McKay, J.M. and Payne, L.N. 1998. Development and application of polymerase chain reaction (PCR) tests for the detection of subgroup J avian leukosis virus. Virus Res. **54**:87-98

Smyth, J.A., Connor, T.J., McNeilly, F., Moffet, D.A., Calvert, V.M. and McNulty, M.S. 2007. Studies on the pathogenicity of enterovirus-like viruses in chickens. Avian Pathol. **36:** 119–126.

Smyth, V., Jewhurst, H., Wilkinson, D., Adair, B., Gordon, A. and Todd. D. 2010. Development and evaluation of real-time TaqManR RT-PCR assays for the detection of avian nephritis virus and chicken astrovirus in chickens. Avian Pathol. **39:**467–474.

Smyth, V.J. 2017. A Review of the Strain Diversity and Pathogenesis of Chicken Astrovirus. Viruses. **9**(2): 29.

Smyth, V.J., Jewhurst, H.L., Wilkinson, D.S., Adair, B.M., Gordon, A.W. and Todd, D. 2010. Development and evaluation of real-time TaqMan® RT-PCR assays for the detection of avian nephritis virus and chicken astrovirus in chickens. Avian Pathol. **39**: 467–474.

Smyth, V.J., Jewhurst, H.L., Adair, B.M. and Todd, D. 2009. Detection of chicken astrovirus by reverse transcription polymerase chain reaction. Avian Pathol. **38:** 293-301.

Smyth, V.J., Todd, D., Trudgett, J., Lee, A. and Welsh, M.D. 2012. Capsid protein sequence diversity of chicken astrovirus. Avian Path. **39:** 151–159.

Songer, J.G. and Uzal, F. 2013. Ulcerative enteritis. In. Swayne, D.E. (Ed.), Diseases of poultry. Iowa, IA: John Willey and sons, Inc. pp. 944-948

Spackman, D., Gough, R.E., Collins, M.S. and Lanning, D. 1984. Isolation of an enterovirus-like agent from the meconium of dead-in-shell chicken embryos. Vet Rec. **114**: 216–218

Spandidos, D.A. and Graham, A.F. 1976. Physical and chemical characterization of an avian reovirus. J. Virol. **19**: 968–976.

Stanton, T.B. 2006. The genus *Brachyspira*. In: Dworkin, M., Falkow, S., Rosenberg, E., Schleifer, K.H., Stackebrandt, E. (Eds.). The Prokaryotes. 3rd ed. Springer, New York.pp. 330–356.

Stephens, C.P. and Hampson.D.J. 1999. Prevalence and diseaseassociation of intestinal spirochaetes in chickens in eastern Australia. Avian Pathol. **28**:447–454.

Suarez, D.L. 2013. Avian Encephalomyelitis. In: Swayne, D.E., Glisson, J.R., McDougald, L.R., Nolan, L.K., Suarez, D.L. and Nair, V. eds. Disease of poultry. 13th edition. Wiley-Blackwell, Iowoa, USA. pp. 486-493

Svensson, L., Uhnoo, I., Garndien, M. and Wadell, G. 1986. Molecular epidemiology of rota virus infection in Upsala, Sweden, 1981: Disappearance of a predominated electropherotype. J. Clin. Microbiol. **18**:101-111.

Swathi, B., Kumar, A. A. and Reddy, M. R. 2012. Histological and molecular diagnosis of poultry tumours. Indian J. Vet. Pathol. **36**(1): 41-48.

Takase, K., Shinohara, K., Tsuneyoshi, M., Yamamoto, M. and Yamada, S. 1989. Isolation and characterisation of cytopathic avian enteroviruses from broiler chicks. Avian Pathol. **18:** 631–642.

Tarasiuk K, Wozniakowski G, Samorek-Salamonowicz E. 2012. Occurrence of chicken parvovirus infection in poland. Open Virol J. **6**: 7-11.

Taylor, R. D. and Jones, G. P. D. 2004. The influence of whole graininclusion in pelleted broiler diets on proventricular dilatation and ascitesmortality. Brit. Poult. Sci. **45**: 247–254.

Teirlynck, E., Gussem, M. D. E., Dewulf, J. Haesebrouck, F., Ducatelle, R. and Van Immerseel, F. 2011. Morphometric evaluation of "dysbacteriosis" in broilers. Avian Pathol. **40**(2): 139-144.

Ter Veen, C., de Bruijn, N.D. Dijkman, R. and de Wit, J.J. 2017. Prevalence of histopathological intestinal lesions and enteric pathogens in Dutch commercial broilers with time, Avian Pathol. **46**: 95-105.

Thayer, S. G. and Walsh, J. L. 1993. Evaluation of cross-contamination on automatic viscera removal equipment. Poult. Sci. **72**: 741–746.

Todd D, Trudgett J, McNeilly F, McBride N, Donnelly B, Smyth VJ, Jewhurst HL, Adair BM. 2010. Development and application of an RT-PCR test for detecting avian nephritis virus. Avian Pathol. **39**(3):207-13.

Toffan, A., Bonci, M., Bano, L., Valastro, V., Vascellari, M., Capua, I. and Terregino, C. 2013. Diagnostic and clinical observation on the infectious bronchitis virus strain Q1 in Italy. Veterinaria Italiana. **49**(4): 347-355.

Van der Heijden, H. M. J. F and Landman, W. J. M. 2011. High seroprevalence of *Histomonas meleagridis* in Dutch layer chickens. Avian Dis. **55**(2): 324-327.

Van Immerseel, F., Buck, J.D., Pasmans, F., Huyghebaert, G., Haesebrouck, F. and Ducatelle, R. 2004. *Clostridium perfringens* in poultry: An emerging threat for animal and public health. Avian Pathol. **33:** 537–549.

Villarreal, L.Y.B., Brandão, P.E., and Chacón, J.L. 2007. Molecular characterization of infectious bronchitis virus strains isolated from the enteric contents of Brazilian laying hens and broilers. Avian Dis. **51(**4): 974–978.

Wani, S.A., Bhat, M.A., Ishaq, S.M., Ashrafi, M.A., Buchh, A.S. and Haq, M. 2003. Detection of a mammalian-like group A rotavirus in diarrhoeic chicken. Vet. Microbiol. **94:** 13-18.

Weissenbock, H., Bakonyi, T., Sekulin, K., Ehrensperger, F., Doneley, R.J. and Durrwald, R.,I. 2009. Avian bornaviruses in psittacine birds from Europe and Australia with proventricular dilatation disease. Emerg. Infect. Dis. **15:** 1453–1459

Williams, R. B. 1986. On the etiology of intestinal intussusceptionin the fowl. Avian Pathol.**15**: 301–304.

Williams, R. B. 2005. Intercurrentcoccidiosis and necrotic enteritis of chickens: Rational, integrated disease management by maintenance of gut integrity. Avian Pathol. **34:**159–180.

Wilson, J., Tice, G., Brash, M.L. and St Hilaire, S. 2005. Manifestations of *Clostridium perfringens* and related bacterial enteritides in broiler chickens. Worlds Poult. Sci. J. **61**: 435-449

Wojnarowicz, C. and Olkowski, A.A. 2005. Ileal diverticulumin a broiler chicken. Vet. Rec. **156**: 613-615.

Yamaguchi, S., Imada, T. and Kawamura, H. 1979. Characterization of a picornavirus isolated from broiler chicks. Avian Dis. **23**: 571–581.

Yegani, M. and Korver, D. R. 2008. Factors affecting intestinal health in poultry. Poult. Sci. **87:**2052–2063.

Yu, L., Jiang, Y., Low, S., Wang, Z., Nam, S.J., Liu, W. and Kwang, J. 2001. Characterization of three infectious bronchitis virus isolates from China associated with proventriculitis in vaccinated chickens. Avian Dis. **45**: 416–424.

Zahoor, M.A., Liebhart, D. and Hess, M. 2011. Progression of histomonosis in commercial chickens following experimental infection with an *in vitro* propagated clonal culture of *Histomonas meleagridis*. Avian Dis. **55**:29–34.

Zsak L, Strother, K.O. and Day, J.M. 2009. Development of a polymerase chain reaction procedure for detection of chicken and turkey parvoviruses. Avian Dis. **53**(1):83-8.

Zsak, L. and Day, J.M. 2010. The pathogenicity of parvovirus in chickens. Proceedings of the American Association of Avian Pathologists Annual Meeting, Atlanta, GA. abstract 9293.

Zsak, L., Cha, R.M. and Day, J.M . 2013. Chicken Parvovirus–Induced Runting-Stunting Syndrome in Young Broiler. Avian Dis. **57**(1):123-127.

Zsak, L., Day, J.M. and Strother, K.O. 2009. Enteric disease inbroiler chickens following experimental infection with chicken parvovirus. Proceedings of the American Association of Avian Pathologists Annual Meeting, Seattle, WA. abstract 7598.

Zsak, L., Strother, K.O. and Kisary, J. 2008. Partial genome sequence analysis of parvoviruses associated with enteric disease in poultry. Avian Pathol. **37**: 435-441.

Zsak, L. 2013. Enteric parvovirus infections of chickens and turkeys. In: Swayne, D.E., Glisson, J.R., McDougald, L.R., Nolan, L.K., Suarez, D.L. and Nair, V., eds. Disease of poultry. 13th edition. Wiley-Blackwell, Iowoa, USA. pp. 399-403.

Appendix

APPENDIX

Antibiotic solution (100 ml)

Benzyl Penicillin sodium	10^7 I.U.
Streptomycin sulphate	10 g
PBS To make	100 ml

Filter sterilize and store in aliquots at -20° C

Phosphate buffered saline (PBS) (1000 ml)

NaCl	8.0 g
KCl	0.2 g
Na2HPO4 x 12 H_2O	2.37 g
KH_2PO_4	0.2 g

Aqua bidest. To make 1000 ml

Autoclave at 15 psi at 121o C for 15 min. Store at room temperature

DEPC H_2O (1000 ml)

DEPC	500 µl
Aqua bidest.	1000 m

Proteinase K stock (10 mg/ml)

Proteinase K	10 mg
Aqua bidest.	1 ml

Store at -20° C

Tris-Borate EDTA (TBE) Buffer (5X)

Tris base	54.00 g
Boric acid	27.50 g
0.5M EDTA (pH 8.0)	20.00 mL
Distilled water (upto)	1000 mL

Tris and EDTA were mixed in 800 mL of autoclaved water, stirred until dissolved completely. Then boric acid was added and stirred, final volume was adjusted to 1000mL with autoclaved distilled water. Stored at room temperature

0.5X Working Solution of TBE (0.045 M Tris-borate, 0.001 M EDTA)

TBE Buffer (5X)	100 mL
Double distilled water	900 mL

Sterilized by autoclaving at 15 lb pressure, 120^{0}C for 20 min.

Stored at room temperature.

Ethidium Bromide stock solution (10 mg/mL)

Ethidium Bromide	1 g
Double distilled water	100 mL

Solution stirred on magnetic stirrer for several hours to ensure that the dye dissolved properly.

The solution was transferred to aluminium foil wrapped amber colored bottle and stored at 4°C.

6 x Gel loading buffer (10 ml)

Bromophenol blue	250 mg
Xylene cyanol FF	250 mg
Sucrose	4.0 g
Aqua bidest. To make 10 ml	

Ethidium bromide staining solution for gels (1000µl)

Ethidium bromide	500 mg
Double distilled water	100 ml

Potassium dichromate 2.5 % (1000 ml)

Potassium dichromate –	25g
Aqua bidest. To make	1000ml
Stored at room temperature	

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