"STUDIES ON PANCREATIC DISORDERS IN CHABRO AND VANARAJA BREED OF CHICKEN WITH SPECIAL REFERENCE TO HISTOCHEMISTRY"

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By

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CERTIFICATE – I

This is to certify that the thesis entitled **"STUDIES ON PANCREATIC DISORDERS IN CHABRO AND VANARAJA BREED OF CHICKEN WITH SPECIAL REFERENCE TO HISTOCHEMISTRY"** submitted in partial fulfilment of the requirement for the degree of **Master of Veterinary Science** in the discipline of **VETERINARY PATHOLOGY** of the faculty of Post - Graduate studies, Bihar Animal Sciences University, Patna, Bihar is the bonafide research work carried out by **Dr. Arbind Kumar**, **Registration No-VM0040/2019-20**, under my guidance and supervision and that no part of this thesis has been submitted for any other degree.

It is further certified that the assistance and help received during the course of this investigation and preparation of the thesis have been fully acknowledged.

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CERTIFICATE – II

This is to certify that the thesis entitled "**STUDIES ON PANCREATIC DISORDERS IN CHABRO AND VANARAJA BREED OF CHICKEN WITH SPECIAL REFERENCE TO HISTOCHEMISTRY**" submitted by **Dr. Arbind Kumar, Registration No-VM0040/2019-20,** to the Bihar Animal Sciences University, Patna, Bihar in partial fulfillment of the requirement for the degree of **Master of Veterinary Science** in the discipline of **VETERINARY PATHOLOGY** has been approved by the Advisory Committee after an oral examination of the student's in collaboration with an External Examiner.

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CONTENTS

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ABSTRACT

The pancreas is a highly specialized multitasking organ which plays pivotal role in poultry production. The extent of pancreatic pathology in different poultry diseases of economic importance at different age group and breeds particularly in Chabro and Vanaraja breeds of poultry has been studied to evaluate the lesions of pancreas in different pathological conditions and its correlation with various poultry diseases.

An aggregate of 240 pancreas were gathered from chicken affected from various lesions like, viral, bacterial, protozoal, fungal, metabolic, and toxicological diseases. The diseases was conform by necropsy findings and histochemiatry with special staining. The gross pancreatic pathology exhibited bleached appearance, congestion, mottled appearance with multifocal necrotic lesions, pancreatic deformity and atrophied or hyperplastic changes in pancreas. The major

histopathological changes in pancreas in decreasing order of frequency were interstitial fibrosis, congestion, individualization of acinar cells, pancreatic fat necrosis in exocrine pancreas, periductular fibrosis and capsular thickening. However, no direct correlation was found between gross pathology and histopathological findings.

It has been observed that pancreatic disorders were more common in metabolic and chronic diseases as compared to other diseases. Significant pancreatic pathology and their dysfunctions are important contributory factor in chronic disease of known or idiopathic origin. Supplementation of pancreatic enzyme in poultry ration in case of disease outbreak/poor growth performance are suggestive for optimal functioning of pancreas and in turn assurance of healthy poultry flock.

Key words: Pancreas, Histopathology, Histochemistry, Vanaraja and Chabro.

Signature of Major Advisor **Signature of the Student**

ABBREVIATIONS

%	Per cent
>	More then
ANV	Avian nephritis virus
Approx.	Approximate
Art V	Avian rotavirus
C Ast V	Chicken Astrovirus
CRNA	Complementary Ribonucleic Acid
CCK	Cholecystokinin

CPCSEA	The Committee for the Purpose of Control and Supervision of Experiments on Animals
D.P.X.	mixture of Distyrene (a polystyrene), a plasticiser (tricresyl phosphate), and xylene
D. Red	Delhem Red
dl	Decilitre
DNA	Deoxyribonucleic Acid
DPI	Dots per inch
ECM	Extracellular matrix
EGF	Epidermal growth factor
FAdv-1	Fowl adenovirus type I
FGF	Fibroblast growth factor
Fig	Figure
FMD	Foot and Mouth Disease
GLAAV	Group I avian adenovirus
GLAAV	Gastrointestinal tract Adeno associated virus
GRP	Gastrin releasing peptides
H&E	Haematoxylin and Eosin
HCI	Hydrochloric acid
HIF-a	Hypoxia-inducible factor-alpha
hr	Hour

IB	Infectious Bronchitis
IBD	Infectious Bursal Diseases
IL-13	Interleukin-13
kg	Kilogram
LPS	Lipopolysaccharide
mg	Milligram
ml	Millilitre
MMPs	Matrix metalloproteinase
ND	New castle disease
P.A.S	Periodic acid
PB2	Punjab breed semi synthetic line
PDGF	Platelet derived growth factor
Ph	Power of hydrogen
PLA2	Phospholipase A2
PM	Post-mortem
PSC	Pancreatic stellate cell
PP-omas	Pancreatic polypeptide
PSHV-1	Psittacid herpes virus-I
RD	Ranikhet Disease
RSS	Runting stunting syndrome
BVCp	Bihar veterinary college
SIBO	Small intestinal bacterial overgrowth
TGF-a	Transforming growth factor-alpha
TGF-B	Transforming growth factor beta

USA	United State of America
VIP	Vasoactive intestinal peptide
Vs	Verses
BASU	Bihar Animal Science University

List of Tables

List of Figures

22	Photograph showing haemorrhagic & necrotic lesion on bursa (Gumboro Disease) in Vanaraja breeds of poultry.
23	Photograph showing shell less eggs (Infectious Bronchitis) Chabro birds.
24	Photograph showing Chicken Infectious Anaemia (Vanaraja).
25	Photograph showing irregularly shaped and pedunculated ovary in Pulloram Disease in Vanaraja.
26	Photograph showing greenish brown or bronze swollen liver (Fowl Typhoid) in Chabro poultry bird.
27	Photograph showing swollen face (Infectious Coryza) in Vanaraja bird.
28	Photograph showing haemorrhagic oophoritis and egg bound condition in Chabro breed of poultry.
29	Photograph showing typical lesion of Caecal Coccidiosis (engorged with clotted blood) in Vanaraja.
30	Photograph showing several immature round worm (*Ascaridia galli*) in intestinal lumen of Chabro bird.
31	Photograph showing Aavian Nephrosis in Vanaraja
32	Photograph showing Ascitis in poultry in Chebro breed of poultry.
33	Photograph showing fibrinous hepatitis in Vanaraja breed of poultry.
34	Photograph of kinked/deviated keel bone showing wasting of pectoral muscle in cachectic Chabro birds.
35	Photograph showing enlarged and pale liver in Vanaraja (Toxicosis).
36	Photograph showing *Fatty liver* haemorrhagic syndrome in Char.
37	Photograph showing marked shortening of pancreas due to atrophy, in Vanaraja Poultry Bird.
38	Photograph showing congested and acutely folded duodenum pancreatic loop in Chabro poultry bird.
39	Photograph showing bleached appearance of pancreas with mild end segmental deviation. (Vanaraja).
40	Photograph showing marked shortening of pancreas due to atrophy in Chabro poultry bird.
41	Photograph showing mid segmental curving of duodeno pancreatic complex with congested, in Chabro bird.
42	Photograph showing severely congested pancreas of Vanaraja bird.
43	Photograph showing hyperplasia of pancreas in Chabro poultry.
44	Photograph showing shrinked pancreas of Vanaraja birds.
45	Photograph showing of duodenopancreatic deformity. (Chabro bird).
46	Photograph showing folding and mottling of duodenopacreatic complex. (Vanaraja)
47	Photograph showing curving of duodenum with bleached appearance of pancreas. (Chabro).
48	Photograph showing mottling and coiling of pancreaticoduodenal loop (Vanaraja).

49	Photomicrograph showing normal acinar pattern of exocrine pancreatic gland of Vanaraja poultry bird (H&E Stain; 400X).
50	Photomicrograph showing acinar cells over distended with zymogen granules with nucleus situated in the basal zone and Islets of Langerhans (H&E Stain; 400X).
51	Photomicrograph showing Inter-lobular fibrosis in exocrine glands of pancreas in Chabro Poultry Birds (H&E Stain; 100X).
52	Photomicrograph showing atrophy of exocrine pancreatic glands due to Inter-lobular fibrosis in Chabro Poultry Birds (H&E Stain; 400X).
53	Photomicrograph showing massive fibrosis and proliferation of collagen fibre tissue surrounding the degenerating acinar cells (H&E Stain; 400X).
54	Photomicrograph showing massive sub capsular necrosis of acinar cells and infiltration of inflammatory cells surrounded by thick band of collagen fibre (H&E Stain; 100X).
55	Photomicrograph showing individualization of acinar cells of pancreatic glands (H&E Stain; 400X).
56	Photomicrograph showing congested blood vessels and dissociated acinar cells in pancreatic glands of Vanraja birds (H&E Stain; 400X).
57	Photomicrograph showing diffuse pancreatic fat necrosis and infiltration of inflammatory cells evident for acute pancreatitis in Chabro birds.
58	Photomicrograph showing marked interstitial fibrosis (H&E Stain; 400X).
59	photomicrograph showing alpha and beta cells of Islets of langerhans(H&E Stain; 1000X).
60	Photomicrograph showing *alpha* and beta cells of Islets of Langerhans (H&E Stain; 1000X).
61	Photomicrograph showing marked periductular fibrosis in pancreatic gland of Vanaraja chicks (H&E Stain; 100X).
62	Photomicrograph showing *normal pancreatic capsule of pancreas* in Chabro poultry bird. (H&E Stain; 100X).
63	Photomicrograph showing fibrous thickening of pancreatic capsule without infiltration of inflammatory cells in Chabro birds. (H&E Stain; 1000X).
64	Photomicrograph showing Islets Langerhans - Gomori's stain -100X
65	Photomicrograph showing Islets Langerhans surrounded by a connective tissue capsule and marked congested blood vessels- Gomori's stain -400X.
66	Photomicrograph showing Islets of Langerhans in pancraes (Heidenhain`s Iron Haematoxylin stain -100X) .
67	Photomicrograph of Heidenhain`s Iron Haematoxylin stained tissue section showing pancreatic Islets distinguished from the surrounding exocrine tissue by a continuous connective tissue capsule. Glucagone secreting alpha cells and insuline secreting beta cells -400X
68	Photomicrograph showing large duct with pancreatic secretions periductular fibrosis (Mallory trichome stain -40X) .
69	Photomicrograph showing large ductwith pancreatic secretionsand marked periductular fibrosis- Mallory trichome stain 100X.

1 INTRODUCTION

Pancreas is unique in its functions, both as an exocrine and endocrine organ as well. While the exocrine pancreas secretes digestive enzymes into the duodenum, the endocrine pancreas is composed of the islets of Langerhans, which contain specialized hormone-secreting cells. These cells include α cells, β-cells, δ-cells, and γ-cells, which secrete glucagon, insulin, somatostatin, and pancreatic polypeptide, respectively. The endocrine pancreas plays a critical role in regulating glucose homeostasis by secreting insulin and glucagon in response to high and low blood glucose concentrations, respectively. Birds displays the highest fasting blood glucose concentration among all vertebrates, with concentrations that are more than twice those in mammals (Braun and Sweazea, 2008; Scares and Braun, 2012).

The pancreas in poultry are collection of three elongated lobes viz dorsal, ventral and splenic, each of which empties the content of digestive enzymes through separate duct in the terminal part of ascending duodenal loop alongside bile duct and hepatic duct. It is relatively small in carnivores and granivores but larger in picivores and insectivores. In duck it is quite small extending up to 3/4th of duodenal loop. In mammals on the other hand, pancreas is flattened, lobulated and pink or grey tubuloalveolar gland, a large portion of which is located in the mesentery immediately adjacent to duodenum. However, the functional units and cellular arrangements of pancreatic parenchyma is quite similar in birds and mammals

The exocrine pancreas consists of compound tubule-alveolar glands which are divided into lobules. The exocrine portion consists of acini lined by secretory cells containing enzymes filled zymogen granules and basally oriented nuclei. The enzymes on proper stimulus are emptied into the duct system of pancreas connected to lumen of the acini. Duct finally opens in the lumen of the duodenum to carry out its digestive function.

Since the viscera of poultry is not compartmentalized it is easy for the infection to spread in the entire organ system of birds in conditions like mushy chick disease, chick edema disease, egg peritonitis, visceral gout, air sacculitis etc. to name a few. Curving of duodenal loop (J-like appearance) and pancreatic atrophy, has been reported during Postmortem examinations of broiler chicken infected with parvovirus (Nunez *et al.,* 2016) and zinc toxicosis in ostrich as well. It was also found that major histological lesions were acute pancreatic necrosis and their atrophy due to different strain of influenza viruses in duck (Brojer *et al.,* 2009), swan (*Cygnus cygnus*) (Teifke et *al.,* 2007) and chicks (Shinya et *al.,* 1995). Avirulent Newcastle Disease Virus was also shown to replicate in the pancreas of chickens causing acute pancreatitis. Nakamura et al., *(*2002) reported pancreatic multifocal necrosis in pancreatic acinar cells in adenovirus-associated hydro pericardium syndrome (Leechi Heart Diseases) of broiler chicken. Pancreatic Adenocarcinoma has been evidenced in guinea fowl and chicken by Okoye *et al., (*1993) and Abdul-Aziz (1995) respectively.

Birds have high body temperature (42°C) and a high metabolic rate and anabolism. They are able to adapt to long catabolic states and to survive long periods without food.

Thus we observe that the pancreas is a highly specialized multitasking organ managed by neuro endocrine factors. Poultry industry is expanding very fast. India too has experienced exponential growth in the poultry industry. Demand for poultry meat and egg is growing every day. This has put the poultry industry under tremendous pressure to enhance production. Sheer pressure of enhancing the production became a breeding place for the genesis of various disease conditions due to failure of scientific management, lack of optimal balanced nutrition, biosecurity breeches leading to all kinds of infectious diseases and vaccine failure. There are numerous disease conditions which affect all age groups of birds. Since the viscera of poultry is not compartmentalised it is easy for the infection to spread in the entire organ system of birds in conditions like mushy chick disease, chick oedema disease, egg peritonitis, visceral gout, air sacculitis *etc t*o name a few.

Since the pancreas plays pivotal role in digestion of all types of food material as well as glucose metabolism for generation of energy, its malfunctioning either

primarily or secondarily will have significant impact on health and body weight gain of poultry.

Despite such vital and multifunctional nature of the organ pancreas, there is paucity of literature. Scanty information is available on pancreatic disorders and different pathological conditions. Also not much reports are available which could highlight the extent of pancreatic pathology in different disease condition of economic significance in poultry at different age group and breeds. Thus, it has been envisaged to investigate the pancreatic disorder in locally available breeds of poultry which are being maintained at our institutional poultry farm and we are receiving their carcass on daily basis.

The present study is, thus, proposed with following objectives:

OBJECTIVES

1. **Comparative study of pancreatic morphology of Chabro and Vanaraja breeds of Chickens.**

2. **To study the lesions of pancreas in different pathological conditions and its correlation with poultry diseases.**

2 REVIEW OF LITERATURE

2.1 Avian pancreatic Anatomy and Histology:

Nunez *et al.,* 2016 reported curving of duodenal loop (J-like appearance), pancreatic atrophy, and mesenteritis during Postmortem examinations of broiler chicken infected with parvovirus.

Carreira *et al.,* 2011 reported pancreatic atrophy due to zinc toxicosis in two African ostriches (*Struthio camelus*) due to exocrine pancreatic degeneration, necrosis, and atrophy. Grossly, one ostrich had a markedly diminished pancreatic mass. It was found that major histological lesions were acute pancreatic necrosis and their atrophy as well as multifocal necrotizing hepatitis due to different strain of influenza viruses through in-depth histological and immune histochemical evaluations. In duck (Brojer *et al.,* 2009), Whooper swan (*Cygnus cygnus*) (Teifke *et al.,* 2007), Broiler chicks (Shinya *et al.,* 1995)

Renu *et al* (2020) conducted histochemical Studies in Exocrine and Endocrine Part of Pancreas in Chabro Chicken and found that.All the cells of islets exhibited mild to moderate PAS positive reaction.

Meulemans *et al.,* 1998 found that non-virulent Newcastle disease virus (strain APMV-1 96/89 VB) was shown to replicate in the pancreas of chickens and the consistent histological lesions were observed in terms of acute pancreatitis.

Nakamura *et al., (*2002) reported pancreatic multifocal necrosis with intranuclear (I/N) inclusions body in pancreatic acinar cells & ventricular erosion

in adenovirus-associated hydro pericardium syndrome (Leechi Heart Diseases) of broiler chicken.

Pancreatic Adenocarcinoma has been evidenced in guinea fowl and chicken by Okoye *et al., (*1993) and Abdul-Aziz (1995) respectively.

Pancreas in poultry is an elongated, solid looking, pale yellow to pinkish organ nested characteristically between duodenal loop. The length of pancreas varies in different species. In poultry the pancreas extends upto the tip of duodenal loop, but in other species its length may vary. Pancreas is relatively small in carnivore, granivores and ducks while it is large in piscivores and insectivores. (Paik *et al*., 1974) Pancreas is generally divided into three lobes viz. dorsal, ventral and splenic. The spleenic lobe is the smallest of three, seen as small narrow strip that extends from head of the pancreas in the direction of spleen. Pancreas is a dual function organ, engaged in exocrine and endocrine functions. Exocrine pancreas constitutes important center for synthesis of digestive enzymes like amylase, lipase, elastase, trypsin, chymotrypsin etc, which are capable of digesting almost all types of nutrient material consumed by the birds. The exocrine pancreas is composed of tubulo acinar glands lined by acinar cells. The acinar cells generally appear pyramidal or triangular in shape and are arranged around a lumen in round, elliptical or irregular fashion. These enzymes of exocrine pancreas are stored in zymogen granules which are present in the acinar cells (Hodges, 1974).

The distribution of zymogen granules are more numerous in the apical part of acinar cells, while the nucleus is located at the base of the cell. Zymogen granules in routine Hematoxylin and Eosin (H&E) staining appear eosinophilic. Group of acini constitutes pancreatic lobule. Pancreatic lobes are separated by fibrous connective tissue. Their interlobular septa are distinct in mammalian pancreas and quite indistinct and non-discernible in avian pancreas (Aziz and Fletcher, 2016). This is the reason why the exocrine pancreas of poultry gives quite homogenous appearance. On the outer surface, pancreas is surrounded by thin connective tissue capsule. Each lobules though indistinguishable, receives blood from single arterial branch, however, much of the circulation to acinar parenchyma is supplied by portal system of capillaries that emerges from sinusoids of the islets. The exocrine tissue, distant froms islets are dicectly supplied by arterial branches (Henderson and

Daniel, 1979). The acinar cells release their enzyme into the lumen of acini which constitute the starting point for duct system of exocrine pancreas. The acinar duct merges with lobular duct and finally empties into main pancreatic duct. In poultry, there are normally three pancreatic ducts. Two pancreatic duct originates from ventral lobe and one duct from dorsal lobe. These ducts open into the distal end of the ascending limb of duodenum near the opening of bile ducts (Denbow, 2015).

Akao *et al*., 1986 described three dimensional pattern of ductuloacinar association in human being. The ductal system of pancreas can be divided into two main segments viz., the main collecting and interlobular ducts lined by columnar mucus secreting epithelium and the intra lobular duct to which the acinar and endocrine cells are related ontogenically. The intralobular duct tend to form anastamotic loops in which multiple acini may drain through the branches of the same ductule. There may also be connection between acinar cells of adjascent acini. Acinar cells which forms functional unit, crosses the boundaries of single acini, the significance of this arrangement is reflected in pathological processes of hyperplasia and atrophy in which the target unit may be a collection of acini (Klar E *et al.*, 1990).

The intralobular ducts of variable sizes may be seen histologically. Smaller ducts are lined with flattened epithelium while large duct has cuboidal lining epithelial cells. The main pancreatic ducts are lined by columnar epithelial cells with a thick sub epithelial layer consisting mainly of fibrous connective tissue and smooth muscles cells (Aziz and Fletehm, 2016).

2.2 Avian pancreatic physiology:

Endocrine pancreas (for different types of islets and their constituent cells). In poultry three types of islet occur viz. light (B-islet), dark (A-islets) and mixed islets are found. And these are releases glucagon, insulin, somatostatin and avian pancreatic polypeptide

The exocrine pancreas secretes number of enzyme that break down lipid, protein, carbohydrate, amino acids, elastic tissue etc. Out of the several enzymes secreted by pancreas. the proteolytic enzymes trypsin and chymotrypsin along with lipolytic enzyme phospholipase are secreted as precursor / inactive form and gets

activated in the lumen of intestine. This phenomenon helps prevention of autodigestion of pancreatic tissue by these powerful enzymes (Hodges, 1974).

Components of pancreas interdependency exists between the exocrine and endocrine through portal circulation, in which, afferent blood flows a glomeruloid path through the islets before supplying the adjacent acinar tissue which was observed by Henderson and Daniel, (1979) by using arterial and venous injections of Berlin blue, they also described that this circulatory arrangement helps in modulating the functions of islet cell types with each other as well as with the exocrine tissue.

Salvioli *et al.,* 2002 described neurology and neuropathology of pancreatic innervations. Innervations to pancreas are both from extrinsic sympathetic and parasympathetic nervous system and intrinsic neural component with presence of ganglia in pancreatic parenchyma. These ganglia plays pivotal role in both exocrine and endocrine pancreatic secretions. Several biologically active substances such as acetylcholine, norepinephrine, nitric oxide, vasoactive intestinal peptides, gastrin releasing peptides, galanin etc are synthesized by pancreatic neurons. In chronic pancreatitis and pancreatic atrophy. Alteration in neuronal supply develops as a compensatory mechanism.

Bockman *et al*. (1995), during his presentation delivered at the combined meeting of the American Pancreatic Association and the International Association of Pancreatology, which was held in Chicago, Illinois, Nov. 2-4, 1994, emphasized that stimulation of exocrine secretion of pancreas is mediated via vagus nerve supply and release of secretagogues by endocrine cells of duodenal mucosa. Entry of gastric acid and fatty acid in the duodenum brings about release of water and bicarbonate by pancreatic ductal epithelium and centro acinar cells located in the intralobular ductules. Bicarbonate mainly contributes in neutralizing the acidic environment of duodenum to optimal level. Cholecytokinin (CCK) is secreted by duodenal mucosal endocrine cells which plays pivotal role of secretagogue to stimulate relese of proenzymes and enzymes stored in zymogen granules. CCK has direct trophic effect acinar cells also. Trypsin has inhibitory effect on this pathway hence presence of any trypsin inhibitor particularly soyabean in the diet may lead to pancreatic hypertrophy due to unrestrained stimulation by CCK.

Mansouri (2012) has described the mechanism by which autodigestion of pancreas by its enzyme is prevented. Being carrier of highly reactive enzymes the pancreas protects itself from auto digestion by multiple mechanism. Firstly the pancreatic enzyme and proenzymes from early stages of its synthesis remains segregated in the lumen of rough endoplasmic reticulum of acinar cells. This sequestration is maintained in golgi apparatus also where packaging of enzymes in zymogen granules take place. The activation of proenzyme takes place in duodenal lumen where enzymatic cleavage of an activation peptide from trypsinogen is initiated by enterokinase of duodenal epithelial cells. The activated trypsin clears activation peptide from other digestive proenzymes. The regurgitation of pancreatic enzyme from duodenal loop is prevented by muscular sphincters of pancreatic duct opening. Lysosomal hydrolysis within acinar cells is also capable of digesting and degrading zymogen granules. Auto digestion has been claimed to be prevented by presence of protease inhibitors.

2.3 Exocrine Pancreas:

The exocrine pancreas is a labile organ. It synthesizes significantly higher protein on a weight for weight basis than does any other tissue and consumes a correspondingly large amount of precursor substrate. The response of the exocrine pancreas to changing nutrient intake is rapid Rantzer *et al*., (1997) studied the function of the exocrine pancreas by chronically fitting the pancreatic duct catheters and T-shaped cannula inserted into the duodenum for reintroduction of pancreatic juice and found that composition of pancreatic secretion takes drastic alteration to adapt to the composition of a new dict. Based on proportion of protein, carbohydrate or fat in the diet, variation in secretion of trypsin / chymotrypsin, amylase and lipase respectively is observed. Acinar cell hypertrophy and hyperplasia leading to organ enlargement occur in response to diet rich in protein and energy, when these substrates are withdrawn, reversal of organ and cells to its original size may be observed, in which autophagy and apoptotic changes play important role. On the other hand, pancreatic atrophy and atrophy of acinar cell is characteristically seen when the bird is subjected to dietary deficiency of protein and energy.

Age related involutionary changes have been described in pancreas by Majumdar *et al*., (1997). In senile conditions, pancreas undergoes atrophic changes

with development of intralobular fibrosis, degranulation and atrophy of acinar cells. This decreases the functional status of pancreas.

The acinar, ductal and islet cells carry regenerative capabilities. Acinar destruction is followed by rapid exocrine parenchymal regeneration. Minor cell loss are compensated well by mitotic activity of acinar cells and hypertrophic changes in the remaining cells. However persistent parenchymal injury usually provokes proliferation of ductular epithelium and connective tissues. The success of pancreatic response following injury has been reported to be influenced by activities of peptide growth factors, integrity of blood supply and excretory duct as well as degree of fibrogenesis. Ellenrieder *et al*., (2004) made it clear that pancreatic stellate cell (PSC) activation is regulated by a complex network of growth factors and cytokines and results in increased expression and release of collagens I and II, fibronectin and other components of extracellular matrix (ECM) proteins.

Ductular epithelium has been reported to carry the potential for differentiation towards acinar cells and islet cells. The main stimuli to initiate mitotic division of ductular epithelium are epidermal growth factor (EGF), keratinocyte growth factor and transforming growth factor-alpha (TGF-a) showed by Sanvito *et al*., (1994).

Bechem, (1998), Apte *et al*., (1999), Haber, (1999) and Ellenrieder *et al*., (2004) gave a description of pancreatic stellate cells and role it plays in pancreatic fibrogenesis. In mammals, stellate cells, similar to that found in liver has been reported. They appear to be a major contributory factor in pancreatic fibrogenesis. The stellate cell in latent stage expresses desmin. In cases of injury the stellate cell shows major transformation to myofibroblastic type of cells with capability to synthesize fibriller collagen including collagen 1. The functional change in stellate cell is brought about in response to platelet derived growth factor (PDGF) and transforming growth factor beta (TGF- B) which induces proliferative capabilities in stellate cells and fibrogenesis respectively (Shek *et al*., 2002). The growth factors are released by macrophage and peracrine secretion by adjacent cells as well as by activated stellate cells. Stellate cells also express matrix metalloproteinases (MMPs), which are capable of degrading extracellular matrix components and their tissue inhibitors. As TGF B, reduces the expression of MMP-3 and MMP-9.

Pancreatic fibrosis may be an outcome of both increased collagen synthesis and reduced collagen degradation.

The PH of pancreatic secretion is alkaline in nature. In chicken it varies between 6.4 and 6.8 (Hudan *et al*., 1972), Pancreatic secretion come in two phase, the aquatic phase (contain water and bicarbonate) and enzymatic phase in which enzymes are released. Pubols, (1991) determined the proportion of different pancreatic enzymes in the secretion. The highest percentage of enzyme in pancreatic secretion is made of procarboxypeptidase A and B (29.8%) followed by amylase (28.9%), chymotrypsin A, B. and C (20%) trypsin inhibitor (11.3%) and trypsinogen (10%). However, the proportion of different enzymes in pancreatic secretion changes with nature of diet i.e. the secretion of enzymes specific for particular diet will show enhancement.

2.4 Endocrine pancreas:

The endocrine pancreas constitutes about 1-2 percent of pancreatic parenchyma. It is made up of collection of roughly rounded or irregular shaped bunch of secretory cells found scattered in pancreatic parenchyma. These endocrine glandular tissues are called islets of langerhans. The distribution of islets in avian pancreas does not follows any specific pattern. Its number is highest in the splenic lobe, (Rideau, 1988), presence of islets are relatively higher in anterior third of pancreas than the other parts.

The acinar portal circulation permits the hormones synthesized by islet cells to exert trophic inhibitory effect on acinar cells. Insulin and pancreatic polypeptides are trophic for acinar tissue, while somatostatin and glucagon are inhibitory.

The islet of langerhans though functionally are same in avian species as we see in mammalian species, however, its cellular composition and distribution are distinctly different (Hodges, 1974). There are three main cell types viz alpha, beta and delta which constitutes islet of Langerhans. Alpha cells which are columnar in shape and is largest amongst the three cell types, secretes glucagon. The main function of glucagon is to bring about hyperglycaemia by converting hepatic glycogen into glucose. Beta cells are smaller than alpha cells. It secretes all important hormone insulin, which plays central role in glucose metabolism and its utilization from the body by the cells. Its deficiency or reduced uptake by cells leads to diabetes mellitus. Insulin is primarily responsible for bringing about

hypoglycaemia. The shape of beta cells varies between rounds to polygonal. Delta cells are round and smallest in size. It secretes somatostatin. The main function of somatostatin is to inhibit secretion of both insulin and glucagon as per requirement in the process of glucose metabolism.

In poultry islets are mostly aggregation of either alpha or beta cells with minor presence of delta cells. Based on this fact the islets are called alpha, beta and mixed islets. Alpha islets with alpha cells as main constituent are large in size with irregular outline, while beta islets are small, round and shows presence of darker beta cells mainly. Mixed islets, which are not frequently seen, carries both alpha and beta cells. Delta cells are mostly distributed in the periphery of the islets (Aziz and Fletcher, 2016).

Sturkie, (2014), based on the staining characteristics described avian pancreatic islet into three types viz., light, dark and mixed islet. Light islets consist of beta and delta cells Dark islet comprise of alpha and delta cells. They are larger in size than light "B" islets. mixed islet the beta cells predominate while small proportion comprise of alpha and del cells, which is contrary to the description of Schmidt, (2003), who opined that light islet. Comprises of A and D cells; dark comprises of B and D cells while mixed islet contained all three cells viz. A, B, and D.

A significant feature of avian pancreas is predominance of glucagon producing cells over the insulin producing cells in a ratio of about 2:1 (Manakova and Titlbach, 2007). Higher number of a cells in avian pancreas as compared to mammalian pancreas has also been reported (Rideau, 1988). In an exhaustive study on blood and nerve supply to pancreas, Rideau, (1988) further reported that islet tissue is richly vascularized and irrigated by pancreaticoduodenal artery and drained by pancreatico duodenal vein. He carried out electron microscopy of islets to report that main endocrine cell types of islet of langerhans are associated with nerve terminals. He further observed that nerve supply to islets is less marked to A-islets than to the B islets however delta cells are richly innervated. Birds have high blood glucose level (180mg %). High level of exogenous or endogenous insulin is required to induce hypoglycemia, thus chicken features high blood glucose and low insulin sensitivity. Glucagon on the other hand extert a potent hyperglycemic effect and is the lipolytic hormone in chicken. In his study on insulin -glucagon ratio in

the control of glucose metabolism, Dupont *et al*. (2008) strongly suggested that, in normally fed chicken the control of plasma glucose level relies more on insulin than glucagon. Presence of glucagon however was stated to be essential. Davis and Vasilatos-younken, (1995) workedon pathological model of "hypoglycemia-spiking mortality syndrome", where pancreatic glucagon content was extensively depleted following a viral infection. Chicken became anorectic and finally die from starvation in a profound hypoglycemic state. To inhibit pancreatic glucagon release, glucose require presence of insulin (Dupont *et al*., 2008). Insulin stimulates glucose and amino acid transport in various cells. However its effect on glucose transport is mostly limited to muscles. Insulin also greatly stimulates liver lipogenesis and expression of lipogenic enzymes. Glucagon on the other hand counteracts insulin stimulation of lipogenesis in chicken liver. It was propounded that birds with high body temperature (about 42°C) and high metabolic rate are able to adapt to prolonged catabolic states and can survive long periods without food.

Insulin plays important role in the regulation of food intake. Experiment with intracerebro ventricular injection of insulin inhibited food intake in young leghom chicken (Honda *et al*., 2007; Sharaishi *et al*., 2011b). In another study Dupont *et al*. (2008) demonstrated that insulin immune neutralization in fat growing chicken decreased food intake, most likely by inducing very high plasma glucose level.

2.5 Pancreatic Pathology:

The exocrine component of pancreas is highly labile in nature, which makes it susceptible to many adverse influences. Involvement of individual acinar cells in the process of degeneration and necrosis is quite common in various local and systemic disorder including febrile state, viral infections, intoxications, septicaemias, state of shock, nutritional deficiencies etc. In advanced inflammatory, necrotizing or neoplastic processes the degenerative changes become more extensive and lead to fibrosis or atrophy. Diseases of the exocrine pancreas in pet, exotic and wild birds have extensively been reviewed by Graham and Heyer, (1992).

Often the autolytic change in the pancreas may create confusion with pathological processes. During autolytic condition the colour of the organ may grossly deepen to a dark red brown or green. Histologically there is patchy

disposition with autolysed area giving bleached looks which tend to resist eosin and gives slate grey colour under the influence of hematoxylin (Charles *et al*., 2007)

The characteristic microscopic feature of degeneration in acinar cell has been described as reduction in the size of the cell, nucleus moving to center of cell from basal position and significant loss of rymogen granules. The necrotic cells often shrink and are often surrounded by narrow clear halos. The cytoplasm may develop vacuolation and nucleus undergoes pyknosis and karyorhexis (Walker et al., 1993 and Laine *et al*., 1996), a toxicological study Walker (1993) reported that experimental administration of ethionine/arginine/methionine or phenylalanine resulted into multifocal acinar degeneration and pancreatic atrophy in rodents.

Obstruction of the pancreatic ducts has been reported to be the result of space occupying lesion, as chronic inflammation associated fibrosis or due to luminal obstruction by parasites, inflammatory exudates, scar tissue or pancreoliths. Pound and Walker, (1981) studied the effect of experimental ligation of the pancreatic duct and reported that pancreas exhibited ductal ectasia with ruptured intercellular junction, rapid atrophy, degeneration and loss of acinar cells by apoptosis and necrosis, mild interstitial edema, inflammation and periductular fibrosis. Later proliferation/hyperplasia of ductular epithelium develop. It was also observed that due to ductal obstruction discharge of digestive enzyme stops despite its continuous synthesis in acinar cells, thus acinar cells appear overdistended with zymogen granules. In such circumstances the organelles (golgi complex) carrying enzymes become

Susceptible to rupture. It disrupts both zymogen enzyme exocytosis and transport of lysosomal enzyme from golgi complex into secretory pathway instead of going into lysosome. This local leakage of co-localized pancreatic and lysosomal enzymes leads to pancreatic necrosis and degeneration. It was stated that in spontaneous cases of ductal obstruction the lesions are much more severe / advanced due to complication arising as a result of ischemia and pressure generated by scarring in which case islet cells are also prone to suffer injuries.

2.6 Non-infectious causes of pancreatic pathology:

Most of the non-infectious causes of pancreatic pathology have been described to be due to nutritional deficiencies or excesses. Selenium deficiency has been held responsible for lesions such as cytoplasmic vacuolation of acinar cells

with presence of central eosinophilic amorphous mass 1 many of vacuolar lesions. This is followed by atrophy of acinar cells with depleted zymogen granules and interstitial fibrosis. In advanced cases, marked fibrosis developed into distinct atrophy of acinar cells and resultant dilatation of ducts.

Pancreas has been found to be quite susceptible to develop degenerative change due to zinc toxicosis in several species of birds and animals (Gabrielson, (1996); Graham, (1988); Kazekos and van vleet, (1989); Smith and Embeing (1993). It has been observed that pancreas constitute the major target organ for zinc toxicosis. The major pancreatic lesion in zinc toxicity was found to be degeneration and necrosis of exocrine elements followed by atrophy and fibrosis, as the condition become chronic. It was described that atrophic acinar cells without discernible zymogen granules may form dilated like structure embedded in scar tissue. These structures which are named "tubular complex" often is confused with hyperplastic ductules. Acinar cells were also reported to be targeted in duckling but were deleted by apoptosis.

Apoptosis can be often seen amongst the acinar cells. It has been reported to be more commonly seen during embryonic development, normal involution, pathological strophy and during regression from hyperplastic changes Kazakos and Van vleet, (1989) observed that apoptosis is the major mean of acinar cell loss due to zine toxicity in duckling.

De Lisle *et al*. (1996) opined that pancreas plays important role in maintaining zine homoestasis, accumulating zinc absorbed from intestine and secreting it into pancreatic juice. There is afinity of zinc for acinar epithelium because of the presence of zinc binding protein, metallothionein. Incidence of pancreatitis in wild water fowl due to zinc toxicosis in mining district of Oklahoma, Kansas and Missouri, USA has been reported by Sileo *et al*. (2003). they found toxic level of zinc in pancreatic tumor of affected birds. The major lesions reported were mild to severe degenerative changes in exocrine pancreas, generalized atrophy of acinar cells with vacuolar changes and relative lack of zymogen granules. The severe cases revealed distended. Lumen, exocrine pancreatic hyperplasia with complete lack of zymogen granules in acinar cells. The acini were usually separated by immature fibrous tissue.

Braselton and Fitgerald, (2011) reported pancreatic atrophy in two African ostriches due to zinc toxicosis. They detected high zine level in the pancreatic tissue of diseased ostriches which on Post mortem revealed diminished pancreatic mass, while histopathological examination showed massive pancreatic acinar atrophy, marked intestinal fibrosis, acinar necrosis and tubular complex formation. In the ostrich with normal pancreas, the serum zinc level was reported to be within normal range.

Zinc toxicity has been specifically reported to be involved in pancreatic pathology. Carreira *et al*., 2011, reported pancreatic atrophy due to zinc toxicosis in two african ostriches (Struthio camelus) Though not pathognomonic or specific in nature, pancreas under zinc toxicosis shows apoptosis, disrupted acinar architecture and fibrosis. Simultaneous regenerative changes may also be observed in ductular epithelium which otherwise were disorganized and misshapen. Zine toxicosis with pancreatic pathology in a free flying trumpeter swan (Cygnus buccinator) has also been reported by Carpenter *et al*., 2004.

In his study Pang (1986) observed that mycotoxins like T-2 toxin, vomitoxin and diacetoxy scirpenol produced frequent incidence of multifocal pancreatic necrosis. These mycotoxin has also been reported to bring about pancreatic interstitial edema, Hyperplasia of ductular epithelium and necrosis of islet cells.

Graham, (1994) proposed defective fat metabolism in obese birds kept on high fat dict leads to pancreatic pathology characterized by diffined coagulative necrosis of acinar cells along with granular cellular debries in psittacine birds specifically Quaker parekets Sileo *et al.* (2003) reported pancreatitis in wild zine poisoned waterfowl. Activation of trypsin from trypsinogen within the pancreatic tissue was stated to be the main underlying cause for such pathology.

Defective glucose metabolism, followed by development of diabetes mellitus has been reported in several species of birds such as budgerigars, toucans, African grey parrot, red tailed hawkes, emperor penguins, cockatiels, macaws etc (Candeletta *et al.*, 1993; Pilny and Luong, 2005)

In a spontaneous case of diabetes mellitus in red-tailed hawk (Buteo jamaicensis), Wallner-Pendleton et al. (1993) observed mottled pancreas with white spots and microscopically there was markedly vacuolated islet cells which were histochemically proven to be beta cells.

Saluja (1989) attributed the main cause of vacuolations in acinar cells to accumulation of substrates in lysosomes which can be seen in storage disorders like alpha and beta mannosidosis and galactosidosis. Vacuolation of ductal epithelium was reported to be due to glycogen. Vacuolation of ductal epithelium and galactoglycogen accumulation has been also reported to be a feature of diabetes mellitus.

Graham and Heyer (1992) described amyloidosis as another important non-infectious pathological condition which produces characteristic pathology in pancreas. There is formation of homogenous or fibrillar eosinophilic material that effaces and replaces exocrine and endocrine cells in cases of amyloidosis. In severe cases, only individual or small groups of disorganized acini separated by amyloid remain present. Pancreatic islets also become undistinguished due to amyloidosis.

2.7 Infectious causes of pancreatic pathology:

2.7.1 Viral causes:

One of the important histopathological finding of diseased pancreas has been reported to be multifocal pancreatic degeneration and necrosis. In such cases associated inflammation is minimal. Adeno virus has been associated with this lesion in several species of animals and poultry. In mammals, viruses such as canine distemper virus and canine parvo virus, felid herpes virus, FMD virus, classical swine fever virus, etc has been associated with multifocal pancreatic necrosis. (Van Pelt and Crauded, 1987; lovanna, 1996)

Capua (1994) observed scattered foci of necrotic area with large deeply basophilic intranuclear inclusion bodies in acinar cells were lesions seen in some cases of aviadenoviral infection Le inclusion body hepatitis in chicks. In guinea fowl adenovirus infection can cause severe pancreatitis. In these cases necrotic lesions of pancreas show infiltration of heterophils and macrophages (Chaslton and Bickford, 1995). Goodwin *et al*. (1996) in their study demonstrated avian adenovirus through DNA in situ hybridization as a tool for rapid diagnosis in birds which exhibited massive necrotizing adenovirus hepatitis and pancreatitis In chicks infected with avian encephalomyelitis virus, small foci of mononuclear cells / lymphoid cells has also been reported. There was also formation of encapsulated follicles of large lymphoblast like cells.

Barton *et al*. (1992) observed characteristic multifocal areas of necrosis and acinar cell degeneration with infiltration of lymphoid cells characteristically seen in pancreas of pigeon suffering from paramyxovirus type 1. In advanced cases pancreas manifested marked fibrosis, Simpson, (1993) had also proposed association of Paramyxovirus -3 infection with pancreatitis in Neophema parakeet.

Hooper *et al.,* (1995) studied the relationship in chicken between the virulence of

some avian influenza virus and their pathogenicity for various organ. It was observed that pancreatic parenchyma showed necrosis of varying extent and severity in birds suffering from highly pathogenic avian influenza viruses. Birds dying due to West-Nile virus too had shown areas of multifocal necrosis in exocrine pancreas and infiltration of inflammatory cells of mixed nature.

Karyomegaly of acinar cells along with presence of intranuclear inclusion bodies have been evidenced in polyoma virus infection, however in these infection infiltration of inflammatory cells have not been reported. Turkey viral hepatitis has also been associated with pancreatic pathology. Multifocal necrosis, degeneration and infiltration of mononuclear cells and heterophils are the important histomorphological features described. Development of syncitial cell has also been recorded.

Pancreatic pathology often leads to pancreatic deformity. Nunez *et al*., (2006) detected chicken parvovirus as sole pathogen in broiler birds which exhibited curving of duodenal loop and exocrine pancreatic atrophy. The intestine was also tested by molecular method for detection of avian nephritis virus (ANV), chicken astrovirus (C Ast V), avian rotavirus (Art V), avian reo virus, Infection bronchitis virus, fowl adenovirus (FAdv-1). They propounded that enteritis, pancreatitis and mesenteritis could disrupt the normal function of digestive system, consequently leading to reduced weight gain. In another study psittacid herpes virus DNA was detected in the pancreas of a macaw suffering from pancreatic duct carcinoma (Mundhenk *et al*., 2009). The virus was not detected in any other tissue. Authors suggested an etiological involvement of herpes virus in the development of carcinoma. Though not proven conclusively. Multifocal haemorrhagic necrosis in the pancreas has also been reported to be an important histopathological and gross lesion in birds suffering from highly pathogenic avian influenza virus (H5N1) in

mute (Cygnus colour) and whooper (Cygnus cygnus) swan. Teifke *et al*., 2007 also immunohistochemically demonstrated the HPAW nucleoprotein in pancreas. Bro jer *et al.* (2009) also described the pathology of avian influenza H5N1 infection in wild tufted duck (Aythyafuligula). They reported the main histological lesions associated with the presence of avian influenza antigen were found in the brain, pancreas, and upper respiratory tract.

Psittacid herpes virus-I (PsHV-I) has also been reported to affect the endocrine pancreas in cockatiel (Nymphicus hollandicus), which showed classical signs of diabetes mellitus such as weight loss, polydipsia and polyuria with marked hyperglycemia (Phalen *et al*., 2007). Histologically herpes viral inclusions were found in many acinar and ductal cells. They concluded that genotype I variant of PsHV I can cause localized pathology in pancreas. Detection of PsHV-I in pancreas of Amazon parrot has also been reported by Legler *et al.*, (2008).

Meulemans *et al*., (1998) reported non-virulent strain of New castle disease virus (APMV-I 96/89 VB) as an infectious agent capable of causing acute pancreatitis in one day old SPF chicken when administered orally. The non-virulent ND virus strain was isolated from backyard female broiler birds.

Nakamura *et al.,* extensively explored the pancreatic pathology caused by virulent ND virus which had infected grower broiler birds (22 to 46 day old) where the affected birds showed neurological and respiratory clinical signs, grossly manifested multiple white spotted mottling in pancreas. The spots mainly represented extensive degeneration, necrosis and depletion of acinar cells in the pancreas. ND virus antigen was also detected in pancreas by immunohistochemical reaction.

Qamar *et al.*, (2013) studied the histopathological changes in various organ of broiler suffering from Runting Stunting Syndrome (RSS) including pancreatic pathology. They reported bleached appearance of pancreas as most common pathology in RSS. Other important pathology observed were degeneration of acini, vacuolar degeneration in acinar cells which also showed loss of zymogen granules. Ruff (1982) had also reported bleached appearance of pancreas as major finding (47%) in stunted broilers.

Shinya *et al*. (2007) attributed pancreatic pathology in avian influenza virus infected SPF chicks to be the main underlying factor in uneven growth and body

weight gain exhibited by infected chicks. They demonstrated significant affinity of type A avian influenza virus for pancreatic tissue where it persisted for longer period of time as compared to other visual organs. According to them pancreatic lesions were caused by direct lytic effect of virus on acinar cells.

Caricchioli *et al*., (2015) extensively studied the exocrine and endocrine pancreatic lesions in avian influenza A experimentally infected turkeys. The infective virus were low pathogenicity H7N3 or H7N1 avian influenza viruses. They observed initial pathology of acute necrotizing pancreatitis (4-7 DPI), followed by regenerative ductular proliferation (8 17 DPI). Significant number of birds (11/28) on 4-10 DPI also developed loss of pancreatic islets these were categorized as most severe lesions. Ultimately at 39 DPI, pancreas revealed chronic fibrosing pancreatitis. They also demonstrated capability of avian influenza virus for damaging effect on both exocrine and endocrine part of pancreas.

2.7.2 Bacterial infection:

Relatively lesser number of bacteria has been reported to produce pancreatic pathology Chlamydia psittaci may bring about multifocal to diffuse necrosis and infiltration of macrophages, finally it may lead to development of interstitial fibrosis. Wiberg *et al*., (2003) has proposed small intestinal bacterial overgrowth (SIBO) as an important basis for development of pancreatic pathology.

Laine *et al*. (1996) proposed that bacterial lipopolysaccharide (LPS) participated in the pathogenesis of pancreatic inflammatory disease. His study investigated the role of endotoxaemia in the pathogenesis of pancreatic acinar cell injury. Escherichia coli LPS (5. mg/kg) was injected into the peritoneal cavity of the rats. The concentration of pancreatic phospholipase A2 (PLA2) in plasma was measured and pancreatic tissue were examined by histology. The concentration of pancreatic acinar cells and fragmentation of DNA typical of apoptosis in pancreatic tissue was seen 24 hours after an LPS injection. Pancreatic acinar atrophy was seen 72 hours after the LPS injection. These data showed that LPS causes release of pancreatic Pl.A2 into blood plasma, activation of PLA2 in pancreatic tissue, and

apoptosis of acinar cells. Few cases of granulomatous lesion due to Mycobacterium avium has also been reported in birds.

2.8 Parasitic and Protozoal causes of pancreatic pathology:

Kumar et al., 1974 reported Euamphimerus walterberghi n. sp. (Opisthorchiidae: Trematoda) parasitizing the pancreatic duct of the Congo peacock (Afropavocongensis). Jardine and Verwoerd (1996) detected cryptoporidium species from epithelium of an ostrich during histopathological examination. It primarily caused pancreatic atrophy.

2.9 Neoplasia:

Cancerous changes of pancreas are rare. Mostly pancreas suffers from adenocarcinoma where neoplastic cells are arranged as tubular or glandular structures. Such birds were reported to suffer from ascites and nodular growth on pancreas. These lesions in the pancreas have been described in guinea fowl by Okoye and Ilochi (1993).Adenovirus associated pancreatic pathology in cases of hydro pericardium syndrome has been earlier reported by Nakamura *et al.* (2002). They observed significant histopathological lesions characterized by multifocal necrosis of acinar cells, with intranuclear inclusion bodies which were reported to be group I avian adenovirus (GI AAV) antigen found after immunohistochemical reactions.

Metastatic lesion of ovarian and oviductal carcinoma has been reported in pancreas, Islet cell carcinoma in a parakeet was reported by Ryan *et al.*, (1982) with resulted diabetes mellitus, though malignancies of endocrine pancreas are rare in nature. In poultry development of neoplastic lesion of lymphoid leukosis and Marek's disease has been reported in pancreas. Incidence of pancreatic carcinoma has also been reported in a cockatiel by Swartout and Wyman (1987)

Gersell (1979) first found tumer producing pancreatic polypeptide (PP-omas)

Incidentally during the extraction of insulin from chicken pancreas in 1968 and was found later to be present in the pancreases of many species of animal including dogs and man Metastatic growth of cholangiocarcinoma and nnal adenocarcinoma in the pancreas of a golden eagle (Aquila chrysectus) was described by Mikaelian *et al*. (1998). Abdul-Ariz TA. (1995) also reported poorly differentiated pancreatic adenocarcinoma in chicken. Exocrine pancreatic

insufficiency due to pancreatic adenocarcinoma in a Yellow Naped Amazon (*Amazona ochrocepohela*) has also been observed by Ritchey *et al.* (1997).

Styles, describe bile and pancreatic duct hyperplasia and papilloma formation in some birds with internal papillomatosis (PsHV 3). Hillyer et al. (1991) has also reported bile duct carcinoma in two out of ten Amazon parrots with cloacalpacillomas.

3 MATERIALS AND METHODS

3.1. Experimental Design and Methodology:

Duration of study: - Three Months.

Location of study:-Department of Veterinary Pathology, BVC, Patna.

Research specimens: -

- All the dead birds of any age, sex of Chabro and Vanaraja poultry breed received for post-mortem examination from PR&TC and LFC, BASU, Patna and local poultry farm in and around Patna.

- Clinically sick birds brought to Department of Veterinary, Pathology for disease investigation were sacrificed by employing humane method such as cervical dislocation technique as approved by CPCSEA, New Delhi.

WORK PLAN

Experimental groups

Age Group	**No. of Birds (Chabro & Vanaraja)**	**Specimen**	**Research Parameters**	
0-2 weeks	40 birds (from each breed)	Pancreas (all 3 lobes)	Gross, Routine Microscopic changes and Histo chemistry whenever needed	Amyloid deposits in pancreases of birds suffering from Chronic disease
2-4 Weeks				
Adult Birds				

In the present study, records pertaining to pancreatic disorders of total no.240 dead birds (Chabro and Vanaraja) during the post-mortem examinations conducted at the Department of Veterinary Pathology, Bihar Veterinary College Patna, Bihar Animal Science University, Patna, were examined and tabulated. The information with regard to age, Breeds weight, types of feed, vaccination, clinical signs etc. were collected as per requisition form submitted to the Department. All precautionary measures were taken to protect our self and environment from infective diseases, if any. The post-mortem examination were conducted as early as possible to minimize the putrefaction or autolysis. Cause of death were determined on the basis of necropsy findings. Using these data, age and cause specific mortality rate were calculated. Mortality rate were calculated on the basis of number of birds from a specific group died out of total carcasses received during three months.

3.2 Parameters to be studied:

The details of birds received for PM examination or disease investigation in terms of age, sex, breed, body weight, body condition, history (if any) were recorded. The extent of pancreatic Pathology in different disease condition of Chabro & Vanaraja breeds of chicken were done on the basis of following examinations.

I. Gross pathology examination: -

The dead bird were subjected to thorough necropsy as early as possible. All the three lobes of pancreas were examined carefully of any gross pathological

lesions. In addition, the pathological lesions of significance if present in any of the organ system were recorded for the sake of tentative diagnosis of the disease condition where ever required bacteriological or serological or pathological examination were carried out for conformation of the disease conditions.

Irrespective of age, sex and breed chronic disease conditions are characterized by significant loss of sternal and thigh muscles volume, low relative bodyweight and atrophic organs of the body specially heart and liver.

II. Histopathological examination: -

Tissue pieces of 3-5 mm thickness were collected in cross section from dorsal ventral and third lobe while cross section from splenic pancreas were collected from terminal part of pancreas near spleen. These tissues were preserved in 10% buffered neutral formalin for further section cutting and staining processes for at least 2 days and tissue processing were done as per the standard protocol (Luna 1968) employed for preparation of histopathological slide and routinely stained with H&E stain.

Following protocol was employed for preparation of permanent histopathological slide and staining.

Washing

The fixed tissues were washed in hanging position, overnight under running tap water to remove the traces of fixatives.

Dehydration

Dehydration was carried out by passing the tissue through ascending grade of acetone viz. 50%, 70%, 90% and two change in 100% acetone for one hour each.

Clearing

It was achieved by passing the tissue through two changes of para benzene for 30 minutes each.

Paraffin Embeding

Three changes in molten paraffin (merck) having congealing point 60° to 62°C was given for paraffin impregnation of the tissue.

Block making

L moulds were used to prepare paraffin blocks of the collected tissue. For the purpose fresh unused paraffin was taken care was taken to avoid trapping of air under the tissue while preparing the blocks.

Sectioning

Semi-Automatic Microtome was used to cut the tissue blocks into fine sections of 5 to 6 micron thickness. The section so obtained was heat fixed and subjected to routine hematoxylin and eosin stain as well as for different histochemical studies

❖ **EHRLICH'S HEMATOXYLIN AND EOSIN STAINING (L. G. LUNA, 1968)**

Staining procedure for routine H&E staining:

Removal of paraffin wax

1) Section was placed in two changes of xylol for 2 minutes to dissolve the wax

Hydration

2) The section was taken out of xylol and was transferred to absolute alcohol for 1 minutes, to make it opaque.
3) The section was removed from the absolute alcohol, drained, and placed in descending grade of alcohol viz. 90%, 70% and 50% for 1 minute each.
4) The slide rinsed in distilled water and the routine method from stage 4 was continued.

Staining

4) The slide was transferred to a coupling jar containing Ehrlich's haematoxylin for 12 minutes.
6) Rinsed in tap water.
7) Differentiated in acid alcohol, three quick dips.
8) Washed in tap water very briefly.
9) Dipped in ammonia water until sections was bright blue, (five dips).
10) Washed in running tap water for 10 minutes.
11) Stained with eosin for 2 minutes.

Dehydration

12) After draining, sections were transferred to absolute alcohol 1, where they were agitated for 10-15 seconds.
13) They were then taken from absolute alcohol I to absolute alcohol 11 for 30 seconds.,

Clearing

14) Sections were transferred from absolute alcohol II to xylol I and left until completely clear.

15) Section when clear was transferred to xylol Il from which they were mounted.

Mounting

16) A sufficient number of coverslips of appropriate size for the section to be mounted was wiped with a soft, fluffless. Such coverslips were conveniently laid rows on a piece of filter paper folded like a concertina, which allowed them to be picked up by the edges.

17) A coverslip was laid on the blotting paper, the section was removed from the xylol, the surplus xylol was removed by wiping the back of the slide and around the section, leaving a margin of about one-eighth of an inch.

18) Two drops of D.P.X. was placed on the section; being laid along the middle of the section to reduce the likelihood of trapping air bubbles.

19) The slide was quickly inverted over the coverslip, one end was placed on the blotting paper and the other end slowly lowered until the D.P.X. touches the coverslip. The D.P.X. quickly spreads under the coverslip, and the coverslip guided into place with a dissecting needle. This whole operation was completed in 5-10 seconds.

Result

Nuclei: Blue with some metachromasia.

Cytoplasm: Various shades of pink-identifying different tissue components

III. Histo-Chemical Examination: -

Histochemical examination will be carried out to study the details of exocrine pancreas (for zymogen granules) and endocrine pancreas (for different types of islets and their constituent cells) in all the affected birds whereas amyloid level will be studied to detect any chronic inflammatory conditions in birds suffering from cachexia *i.e*. chronic disease conditions.

The histochemical parameters which will be conducted to study pancreatic Pathology are as follows: -

1) Heidenhain's Iron Haematoxylin Stain (C.F.A. Culling, (1974)

Heidenhain's iron haematoxylin is a regressive cytological stain which stains tissue jet black, and by careful selective differentiation, many tissue and cell components can be revealed in shades of black and grey. This makes it useful for photomicrography. In this technique, iron alum is also used as a differentiating agent and as an oxidising agent, which oxidises haematoxylin to haematin the active staining component. Being a cytological stain, tissue sections must be very thin to enables demonstration of cell constituents. Staining time is 30-45 minutes at 56°C. Heidenhain's iron haematoxylin will demonstrate Islet of Langerhans.

The use of iron hematoxylin according to Haidenhain's prescription, with the subsequent passage of stained and differentiated histological sections through the ammonia solution for detection of A-cells in the pancreatic islets. The A-cells cytoplasm stained dark blue intensively; as to the nuclear structures--they acquired bluish-black tints. The method can be used for the study of morphology, topography and the number of A-cells in the islets (Zharkov VP, Boĭko RT.1978).Staining time:- Thirty to forty-five minutes at 56°C. Gave adequate results with formal saline.

Principle

This method stains tissue jet black and, by selective differention, may be used to demonstrate a great variety of tissue components in shades of black and grey, it is, therefore, ideal for photography. It is permanent, provided that the alum is properly removed, and is applicable after any fixative. It is only used regressively (over-stained and then differentiated), and experience is required to obtain the best results. The technique of Heidenhain's iron differs from the other haematoxylin techniques in two respects: the mordant is employed separately from the

haematoxylin; and the mordant is employed as a differentiating agent. Iron alum, in addition to being a mordant, is a powerful oxidizing agent, and it oxidizes the haematoxylin into a colourless soluble compound which diffuses into the alum solution.

Staining Technique:

Removal of paraffin wax

1) Section was placed in xylol for 2 minutes to dissolve the wax.

Hydration

2) The section was taken out of xylol and was transferred to absolute alcohol for 1 minutes, to make it opaque.
3) The section was removed from the absolute alcohol, drained, and placed in 90 per cent alcohol for 1 minute.
    a. The section was taken from 90 per cent alcohol, drained, and placed in Lugol's iodine for 3 minutes.
    b. The slide, after draining and rinsing in water, was transferred to 3 per cent sodium thiosulphate, and left for 3 minutes, following which it was placed in the slide-washing tray for a few minutes, rinsed in distilled water and the routine method from stage 4 was continued.

Staining

4) Transferred to a couplin jar containing iron alum solution for 45 minutes at 56°C, in paraffin oven.

5) Rinsed rapidly in water.

6) Transfered to a coplin jar containing the haematoxylin solution and left for the same

time and at the same temperature as in iron alum.

7) Rinsed rapidly in water.

8) Differentiated in iron alum solution, 2 per cent solution which gave a slower and

more easily controlled differentiation.

9) The slide was washed in running water for 5 minutes to remove the iron alum.

10) Dehydrated, cleared and mounted in D.P.X.

Result

Islets appear light grey in black background of acinar cells.

1) P.A.S. (Periodic Acid Schiff) Procedure for zymogen granules (C.F.A. Culling, 1974**)**

PAS stain is used to detect glycogen, this is normally present in Pancreas and other muscles. Periodic acid-Schiff (PAS) stain, periodic acid reacts with aldehyde group of the carbohydrate and afterwards reaction with the Schiff's reagent produced red or purple red colour

Which has a very long history in histo-chemistry, is frequently used to demonstrate glycogen in muscle. It is worth bearing in mind, however, that not only glycogen but also other polysaccharides, as well as neutral mucopolysaccharides, muco- and glycoproteins, glycolipids and some unsaturated lipids and phospholipids, are stained with this reaction. The zymogen granules are acidophil in nature and are P.A.S. positive. As per the recommended techniques of Culling, 1974 the P.A.S. Positive substances/granules appear Bright red whereas nuclei appear Blue in colour. Other tissue constituents remain yellow.

The zymogen granules are acidophilic in nature and P.A.S. positive.

Method of Staining

1. The sections were deparaffinised and hydrated to distilled water.
2. Oxidized for 5-10 min in 1 per cent aqueous periodic acid.
3. Washed in running water for 5 minutes, and rinsed in distilled water.
4. Treated with Schiff reagent for 10-30 minutes.
5. Washed for 10 minutes in running water.
6. Counterstain with haematoxylin.
7. Dehydrated, cleared and mounted in D.P.X.

Results

P.A.S. positive substances…….. Bright red

Nuclei……...……. Blue

Other tissue constituents……..…….. Yellow

3 Gomori's method for pancreatic islet cells (Gomori, G.:1941**)**

Gomori's chrome alum haematoxylin phloxin staining technique [Gomori, 1941] is a satisfactory method of differential staining for the alpha and beta cells of Pancrease. With Gomori's methods, the alpha and beta cells could be reliably recognized in freshly collected pancreatic tissue sample. But the tissue sample must be fixed in Bouin's or l0% buffered neutral formalin when the sections are thin the granules of the alpha cell are seen individually. They are stained in deep fuchsin, fuchsin-brown or brown-lilac. These three varieties of granular staining of the alpha cell may coexist in the same islet. Mitochondria cannot be differentiated from alpha cell granules. The golgi apparatus appears as a juxtanuclear vacuole. A cyanophilic macular structure of irregular shape and position is clearly demonstrated.

Beta cell granules stain salmon-pink. Mitochondria stain red-brown or fuchsin-red when these structures are segregated, the color difference is striking. When the cytoplasm can be seen between granules, it has a pale grayish tint. The Golgi net of the beta cells appears as a series of branched clear channels. Occasionally beta cells show, on one edge a cuticula that is brilliantly stained red-orange.

The delta cells do not possess granules but have a glassy sky-blue cytoplasm with small rod-shaped red mitochondria. The Golgi net of the delta cell appears as a clear canal that is moderately branched. A cyanophilic macular structure is also present, and it can be distinguished from the rest of the blue cytoplasm by its higher degree of refractility.

Fixation: 10% buffered neutral formalin. Since formalin-fixed, the paraffin sections were mordanted in Bouin's solution for 16 hours before staining.

Technique.

Paraffin sections cut at 6 microns.

Staining procedure:

1. The sections were deparaffinised and hydrated to distilled water.
2. Mordanted in Bouin's solution for 16 hours.
3. Washed in tap water to remove picric acid, for 15 minutes.
4. Potassium permanganate solution for I minute.
5. Differentiated in sodium bisulfite solution.
6. Washed well in tap water.
7. Chromium hematoxylin solution for 10 minutes.

8. Differentiated in acid alcohol solution for 1 minute.

9. Washed in tap water until the section was a clear blue.

10. Phloxine B solution for 5 minutes.

11. Rinsed in distilled water.

12. Phosphotungstic acid solution for I minute.

13. Washed in tap water for 5 minutes till the section regained its red color..

14. Differentiated in 95% alcohol.

15. Dehydrated in absolute alcohol, then cleared in xylene, two changes each.

16. Mounted with D.P.X.

RESULTS

Alpha cells – red

Beta cells-blue

Delta cells-from pink to red and indistinguishable from the alpha cells.

4) MALLORY'S TRICHROME METHOD (Crooke-Russell Modification). (C.F.A.Culling, 1974)

Method

1) The sections were deparaffinised and hydrated to distilled water.

2) Sections were treated with iodine-sodium thiosulphate sequence.

3) Washed well in water.

4) Nuclei was stained in Ehrlich's haematoxylin.

5) Rinsed off excess stain in water.

6) Differentiated in acid alcohol solution for I minute.

7) Washed in tap water until the section was a clear blue.

8) Sections then stained for 5 minutes in 1 per cent aqueous acid fuchsin.

9) Washed in tap-water for a brief time then rinse in distilled water.

10) Transferred to aniline blue-orange G mixture for 20 minutes.

11) Washed in running tap-water for 5 minutes.

12) Dehydrated and differenciated in 95 per cent alcohol, followed by absolute alcohol.

13) Cleared in xylol and mounted in D.P.X.

Results

Collagen, reticulin fibers and basophil granules..... Deep blue

Cartilage, mucin, amyloid…… Lighter blue

Fibrin, glia fibres, and acidophil granules……. Red

Red blood cells and myelin ……Orange vermilion

Nuclei…… Blue-purple

Details of histo-chemical staining for evaluation of pancreatic pathology will be as follows: -

Tissue	Fixative	Technique	Reference
Routine Histo-Pathology	10% buffeered neutral formalin	Haematoxylin & Eosin Stain	L.G.Luna (1968)
Islets of Langerhans	Formal Saline	Heidenhain"s Iron Haematoxylin	C.F.A.Culling (1974)
Zymogen Granules	Formal Saline	P.A.S. (Periodic Acid Schiff Procedure	C.F.A.Culling (1974)
Alpha (α), Beta (β) and Delta(δ) cells	Bouin's or 10% buffered neutral formalin	Gomori's Method for Pancreatic Islet Cells	Gomori,G. (1941)
Collagen	10% buffered neutral formalin	Mallory's trichrome method	C.F.A.Culling (1974)

3.3 Photomicrography:

Photomicrograohy was done to exhibit the various histopathological and histochemical changes observed during study of pancreatic pathology.

3.4 Statistical analysis:

Data so collected was critically analyzed for determining the role of pancreas as a contributing factor in affecting health of the birds. The relationship between both gross and microscopic pancreatic pathology with age, sex, breed of the birds as well as weight of dead birds was statistically analyzed. The statistical analysis and test for significance

were carried out by calculating X^2 Statistics for 2x2 contingency table. Whenever and wherever required, Yates Correction was employed as per standard protocol.

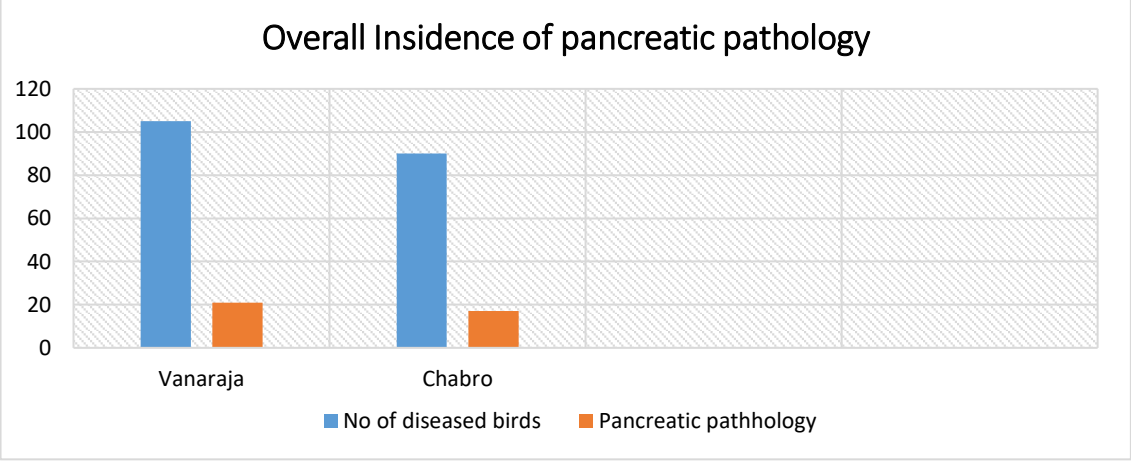
4 RESULTS

Present study was taken up to evaluate the pancreatic pathology of Vanaraja and Chabro poultry birds suffering from different disease conditions, irrespective of their age, sex, breed, body weight and body condition history (if any). During the period of study, post mortem examination was performed on 240 birds which were brought to Department of Veterinary Pathology for disease diagnosis. The results obtained during the study are as following.

4.1 Overall disease incidence and pancreatic pathology

In our study 21% and17% (21Chabro birds out of 120 and 26 Vanaraja birds out of 120) birds examined showed definite pancreatic pathology (Fig. 01).

Figure- 01: Bar graph showing overall incidence of pancreatic pathology in chicken during three months of studies.



In the present studies, incidences of altogether twenty one different diseases were recorded. These diseases have been grouped under viral, bacterial, fungal, parasitic / protozoal, metabolic and toxicological conditions. Different diseases that were detected during the study are presented in (Table. 01).

4.2 Incidence of pancreatic pathology in different group of diseases

The breakup of pancreatic pathology under different group of diseases is presented in (Table-01). It was observed that maximum incidence of pancreatic pathology was observed in fungal (66.66%, Vanaraja and 20% Chabro) and metabolic (66. 66% Chabro and 66.66% Vanaraja) diseases. Least pancreatic pathology was registered in viral diseases (11.42% Chabro and 14.28% Vanaraja) though the number of diseased birds was highest in this group. Pancreatic pathology due to bacterial diseases (28.12%Vanaraja and 25% Chabro) and suspected mycotoxicosis (14.28% Chabro and 28. 57% Vanaraja) was significantly higher than viral diseases.

Statistical analysis revealed that the difference in pancreatic pathology between different major group of etiological agents were significant except between viral vs. parasitic, bacterial vs. suspected mycotoxicosis and fungal vs. metabolic conditions.
The variation in the incidence of pancreatic pathology with disease group is graphically depicted in (Fig. 02).

Table- 01: Incidence of pancreatic pathology in different group of diseases in Vanaraja and Chabro breed of poultry.

Types of Diseases	**No of Birds suffering from Diseases**		**Incidence of Pancreatic Pathology**			
			Numbers of affected Birds		**Percentage of affected birds**	
	Vanaraja	Chabro	Vanaraja	Chabro	Vanaraja	Chabro
Viral	70.00	70.00	10.00	8.00	14.28	11.42
Bacterial	32.00	32.00	9.00	8.00	28.12	25.00
Fungal	3.00	5.00	2.00	1.00	66.66	20.00

Parasitic/Protozoal	5.00	3.00	1.00	1.00	20.00	33.30
Metabolic	3.00	3.00	2.00	2.00	66.66	66.66
Toxicological	7.00	7.00	2.00	1.00	28.57	14.28
Total	120	120	26.00	21.00	21.00	17.00

Figure - 02: Bar graph showing incidence of pancreatic pathology in different group of diseases in Vanaraja and Chabro breed of poultry.

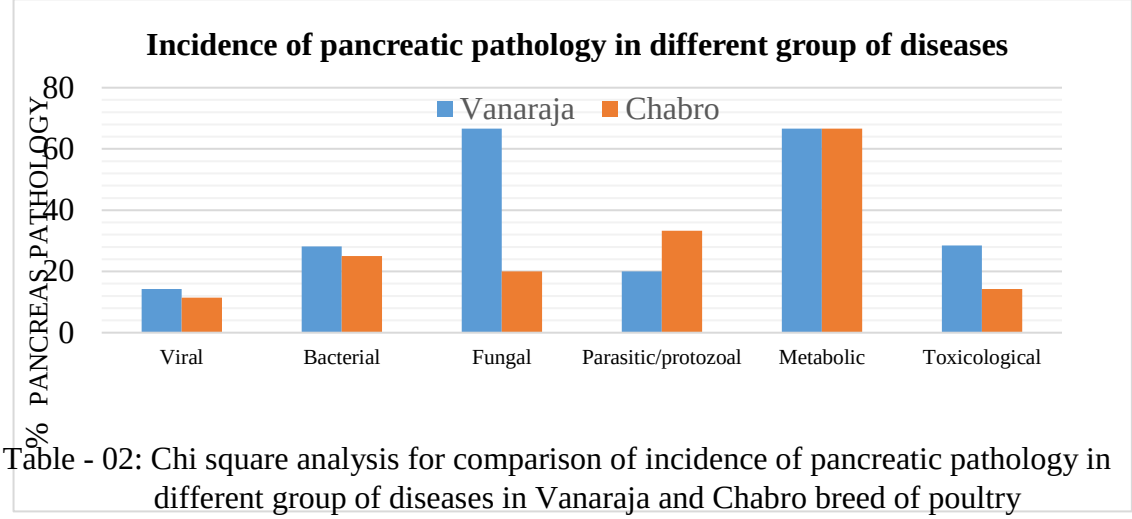


Table - 02: Chi square analysis for comparison of incidence of pancreatic pathology in different group of diseases in Vanaraja and Chabro breed of poultry

Comparison	**X^2 Statistic**		**Comparison**	**X^2 Statistic**	
	Vanaraja	Chabro		Vanaraja	Chabro
Viral vs Bacterial	45.5000**	84.0000**	Bacterial vs Toxicological	63.0000**	0.5090ns
Viral vs Fungal	36.7500**	68.2500**	Fungal vs Protozoal	60.9000**	84.0000**
Viral vs Protozoal	63.0000**	68.2500**	Fungal vs Metabolic	54.6000**	0.2500 NS
Viral vs Metabolic	56.0000**	84.0000**	Fungal vs Toxicological	55.1250**	78.0000**
Viral vs Toxicological	49.8750**	43.401**	Protozoal vs Metabolic	105.0000**	152.2500**
Bacterial vs Fungal	63.0000**	89.2500**	Protozoal vs Toxicological	98.4375**	6.0000*
Bacterial vs Protozoal	65.8000**	3.2500*	Metabolic vs Toxicological	84.8750**	111.0000**
Bacterial vs Metabolic	65.3333**	105.0000**			

NS : - Non – significant, P>0.05

** :- Highly significant p < 0.01

* :- Significant, p<0.05

4.3 Incidence of pancreatic pathology in different diseases:

The incidence of pancreatic pathology within individual group of diseases is presented in decreasing order of frequency in table 02. It is evident from the table that incidence of pancreatic involvement is significantly higher in all the listed viral diseases with highest incidence being observed in cases of IBD. (75% Vanaraja and 66.7 % Chabro) by IB (50 % Vanaraja and 10.52% Chabro) and others. Contrary to rest of the viral diseases the pancreatic pathology was extremely low (2.3% Vanaraja and 2.7% Chabro) due to RD though mortality of birds registered was highest. The overall incidence of pancreatic pathology in viral disease other than RD had been observed to be 55.% Viral diseases IBD and RD had the distinction of registering over all highest (75% vanaraja and 66.7% Chabro) and lowest (2.30 Vanaraja and 2.7%) disease wise incidence of pancreatic pathology respectively.

Amongst the bacterial diseases, pasteurellosis showed highest incidence of pancreatic pathology (50% Vanaraja and 66.7% Chabro) followed by cases of Oophoritis (50%Vanaraja and 50% Chabro). Least cases of pancreatic pathology was seen in birds suffering from Yolk sac infections of bacterial origin (12.8 Chabro and 10% Vanaraja). High incidence of pancreatic pathology was also observed in birds suffering from brooder's pneumonia (66.7% Vanaraja and 20% Chabro), Chick Oedema disease (50% Chabro and 50% Vanaraja) and suspected cases of mycotoxicosis (33.4% Vanaraja and 25% Chabro). The graphical presentation of diseases with incidence of pancreatic pathology is groups of diseases as well as within a particular group presented in (Table 03)

Figure-03: Bar graph showing incidence of pancreatic pathology in different disease Conditions in Vanaraja and Chabro breed of poultry.

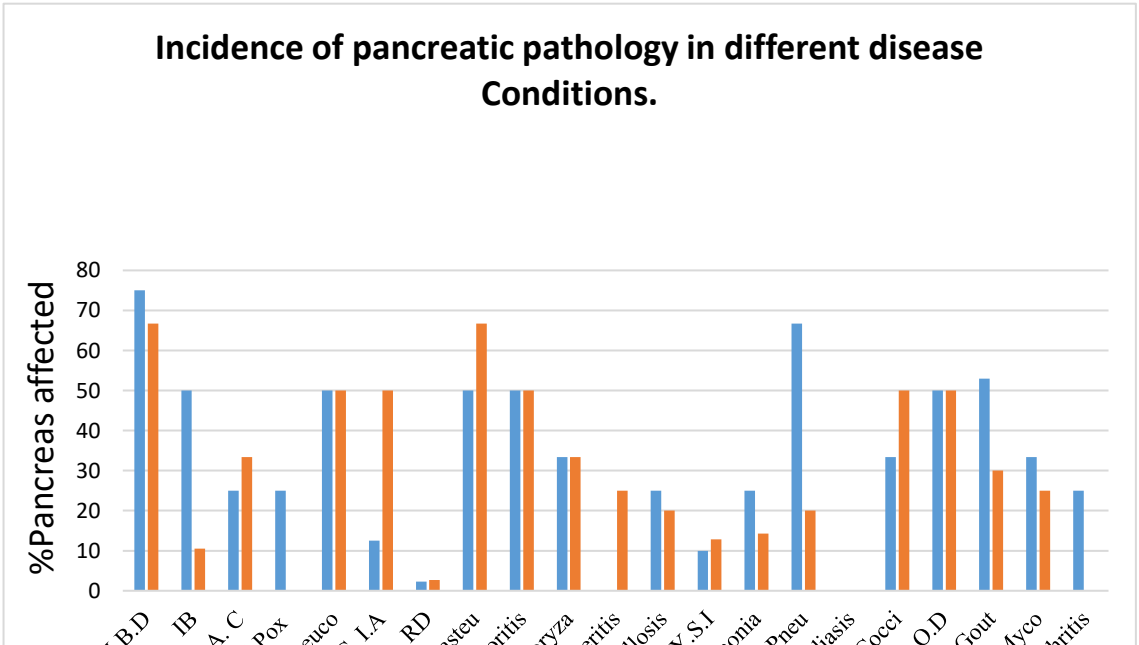


Table: - 03: Incidence of pancreatic pathology in different disease Conditions in Vanaraja and Chabro breeds of poultry.

Disease	**No of Birds affected**		**Pancreas affected**		**%Pancreas affected**	
	Vanaraja	Chabro	Vanaraja	Chabro	Vanaraja	Chabro
IBD	4.00	3.00	3.00	2.00	75.00	66.7
IB	2.00	19.00	1.00	2.00	50.00	10.52
Cyst adeno Carcinoma	4.00	3.00	1.00	1.00	25.00	33.4
Fowl Pox	4.00	4.00	1.00	00	25.00	00
Lymphoid Leucosis	4.00	2.00	2.00	1.00	50.00	50.00
Chicken Infectious anaemia	8.00	2.00	1.00	1.00	12.50	50.00
Ranikhet disease	44.00	37	1.00	1.00	2.30	2.70
Pasteurellosis	4.00	3.00	2.00	2.00	50.00	66.70
Oophoritis	2.00	2.00	1.00	1.00	50.00	50.00
Coryza	3.00	3.00	1.00	1.00	33.40	33.40
Enteritis	5.00	4.00	2.00	1.00	40,00	25.00
Collibacillosis	4.00	5.00	1.00	1.00	25.00	20.00
Yolk sac Infection	10.00	8.00	1.00	1.00	10.00	12.80
Pneumonia	4.00	7.00	1.00	1.00	25.00	14.28
Brooder Pneumonia	3.00	5.00	2.00	1.00	66.70	20.00
Ascaridiasis	2.00	1.00	00	00	00	00
Coccidiosis	3.00	2.00	1.00	1.00	33.40	50.00
Chick Oedema Disease	2.00	2.00	1,00	1.00	50.00	50.00
Gout	1.00	1.00	1.00	1,00	100	100
Suspected Mycotoxicosis	3.00	4.00	1.00	1.00	33.40	25.00
Nephritis	4.00	3.00	1.00	00	25.00	00
Overall Total	120	120	26.00	21.00	21.00	17.00

Table - 04: Chi square analysis for comparison of incidence of pancreatic pathology in different group of diseases in Vanaraja and Chabro breed of poultry.

Comparison – in among viral diseases	**X^2 Statistic**		**Comparison – in among viral diseases vs bacterial diseases**	**X^2 Statistic**	
	Vanaraja	**Chabro**		**Vanaraja**	**Chabro**
IBD vs IB.	0.431^{NS}	0.621^{NS}	IBD vs Pasteurellosis.	0.0082^{NS}	0.0028^{NS}
IBD vs Cyst adeno Carcinoma.	0.0664^{NS}	0.0862^{NS}	IBD vs Oophoritis.	6.7457*	4.4351*
IBD vs Fowl Pox.	0.0664**	0.0987**	IBD vs Coryza.	14.O143**	24.O131**
IBD vs Lymphoid Leucosis.	7.0763**	8.0463**	IBD vs Enteritis.	16.0396**	11.0232**
IBD vs Chicken Infectious anaemia.	8.5972**	6.4272**	IBD vs Collibacillosis.	18.9304**	11.303**
IBD vs RD.	84.4880**	72.4660**	IBD vs Yolk sac Infection.	38.4662**	48.3602**
IB vs Cyst adeno Carcinoma.	0.021^{NS}	0.041^{NS}	IBD vs Pneumonia.	82.2121**	74.0242**
IB vs Fowl Pox.	0.21^{NS}	0.11^{NS}	IB vs Pasteurellosis.	0.0635^{NS}	0.0453^{NS}
IB vs Lymphoid Leucosis.	4.324^{*}	4.324^{*}	IB vs Oophoritis.	4.046*	8.006*
IB vs Chicken Infectious anaemia.	6.0432^{**}	9.0232^{**}	IB vs Coryza.	9.8124**	6.0126**
IB vs RD.	67.3376**	72.3276**	IB vs Enteritis.	12.8358**	21.6353**
Cyst adeno Carcinoma vs Fowl Pox.	0.6576^{NS}	0.8476^{NS}	IB vs Collibacillosis.	11.8506**	9.7508**
Cyst adeno Carcinoma vs Lymphoid Leucosis.	0.0063^{NS}	0.0043^{NS}	IB vs Yolk sac Infection.	24.809**	42.2106**

Comparison – in among viral diseases	**X^2 Statistic**		**Comparison – in among viral diseases vs bacterial diseases**	**X^2 Statistic**	
	Vanaraja	**Chabro**		**Vanaraja**	**Chabro**
Cyst adeno Carcinoma vs Chicken Infectious anaemia.	0.1782^{NS}	0.2344^{NS}	IB vs Pneumonia.	48.1574**	34.1473**
Cyst adeno Carcinoma vs RD.	6.0541|**	7.0321|**	Cyst adeno Carcinoma vs Pasteurellosis.	0.0011^{NS}	0.0009^{NS}
Fowl Pox vs Lymphoid Leucosis.	0.0073^{NS}	0.0063^{NS}	Cyst adeno Carcinoma vs Oophoritis.	0.0007^{NS}	0.0015^{NS}
Lymphoid Leucosis vs Chicken Infectious anaemia	0.17872^{NS}	0.2072^{NS}	Cyst adeno Carcinoma vs Coryza.	0.4343^{NS}	0.6432^{NS}
Lymphoid Leucosis vs RD.	62.0541**	81.0241**	Cyst adeno Carcinoma vs Enteritis.	0.6666^{NS}	0.6463^{NS}
Chicken Infectious anaemia vs RD.	33.5707**	23.6707**	Cyst adeno Carcinoma vs Collibacillosis.	1.3057^{NS}	0.8054^{NS}
Comparison – in among viral diseases vs bacterial diseases	**X^2 Statistic**		Cyst adeno Carcinoma vs Yolk sac Infection.	2.5821^{NS}	2.5821^{NS}
	Vanaraja	**Chabro**	Fowl Pox vs Pasteurellosis.	0.0011^{NS}	0.0009^{NS}
Cyst adeno Carcinoma vs Pneumonia	4.824*	0.54300*	Fowl Pox vs Oophoritis.	0.0009^{NS}	0.0011^{NS}
Fowl Pox vs Coryza.	0.6421^{NS}	0.9120^{NS}	**Comparison – in among Viral vs Funga.**	**X^2 Statistic**	
Fowl Pox vs Enteritis.	0.0421^{NS}	0.4444^{NS}		**Vanaraja**	**Chabro**
Fowl Pox vs Collibacillosis.	1.3057^{NS}	2.6012^{NS}	IBD vs Brooder Pneumonia.	0.0012^{NS}	0.0034^{NS}

Comparison – in among viral diseases vs bacterial diseases	X^2 Statistic		Comparison – in among Viral vs Fungal	X^2 Statistic	
	Vanaraja	**Chabro**		**Vanaraja**	**Chabro**
Fowl Pox vs Yolk sac Infection	2.4731^{NS}	2.2721^{NS}	IB vs Brooder Pneumonia	0.0138^{NS}	0.0108^{NS}
Fowl Pox vs Pneumonia	5.925*	5.021*	Cyst adeno Carcinoma vs Brooder Pneumonia	0.2322^{NS}	0.2044^{NS}
Lymphoid Leucosis vs Pasteurellosis	4.864*	4.8691*	Fowl Pox vs Brooder Pneumonia	0.2344^{NS}	0.2433^{NS}
Lymphoid Leucosis vs Oophoritis	0.0216^{NS}	0.0169^{NS}	Lymphoid Leucosis vs Brooder Pneumonia	0.4706^{NS}	0.4708^{NS}
Lymphoid Leucosis vs Coryza	1.8367^{NS}	0.0216^{NS}	Chicken Infectious anaemia vs Brooder Pneumonia	1.1988^{NS}	1.1978^{NS}
RD vs Brooder Pneumonia	18.9399**	18.9388**	**Comparison – in among Viral Vs parasitic/ protozoal**	**X^2 Statistic**	
Lymphoid Leucosis vs Enteritis	2.6953^{NS}	1.9991^{NS}		**Vanaraja**	**Chabro**
Lymphoid Leucosis vs Collibacillosis	5.6212*	5.9213*	R.D Vs Ascaridiasis	2.7658^{NS}	2.8567^{NS}
Lymphoid Leucosis vs Yolk sac Infection	15.5319**	14.527**	Chicken Infectious anaemia Vs Coccidiosis	13.4849**	13.3749**
Lymphoid Leucosis vs Pneumonia	8.226**	9.203**	I.B.D Vs Ascaridiasis	15.543**	15.204**
Chicken Infectious anaemia vs Pasteurellosis	8.226**	7.826**	I.B.D Vs Coccidiosis	39.8318**	38.8418**
Chicken Infectious anaemia vs Oophoritis	0.5185^{NS}	0.6175^{NS}	I.B Vs Ascaridiasis	12.3557**	12.0552**

Comparison – in among viral diseases vs bacterial diseases	X^2 Statistic		Comparison – in among Viral Vs parasitic/ protozoal	X^2 Statistic	
	Vanaraja	**Chabro**		**Vanaraja**	**Chabro**
Chicken Infectious anaemia vs Coryza	0.3878^{NS}	0.472^{NS}	I.B Vs Coccidiosis	31.2313**	28.3135**
Chicken Infectious anaemia vs Enteritis	0.5696^{NS}	0.8686^{NS}	Cyst adeno Carcinoma Vs Ascaridiasis	1.4514^{NS}	1.4588^{NS}
Chicken Infectious anaemia vs Collibacillosis	3.0116^{NS}	4.0223^{NS}	Cyst adeno Carcinoma Vs Coccidiosis	3.8586^{NS}	3.7658^{NS}
Chicken Infectious anaemia vs Yolk sac Infection	10.015**	13.021**	Fowl Pox Vs Ascaridiasis	1.4514^{NS}	1.0014^{NS}
Chicken Infectious anaemia vs Pneumonia	26.4213**	19.2133**	Fowl Pox Vs Coccidiosis	3.7658^{NS}	3.0485^{NS}
RD vs Pasteurellosis	83.7668**	93.7448**	Lymphoid Leucosis Vs Ascaridiasis	3.6301^{NS}	3.8201^{NS}
RD vs Oophoritis	66.5552**	68.2500**	Lymphoid Leucosis Vs Coccidiosis	19.1415**	19.0015**
RD vs Coryza	89.2500**	109.1744**	Chicken Infectious anaemia Vs Ascaridiasis	1.8229^{NS}	1.5292^{NS}
RD vs Enteritis	89.2500**	87.2400**	R.D Vs Coccidiosis	5.8859*	5.0019*
RD vs Collibacillosis	11.6779**	12.7997**	**Comparison – in among Viral Vs Toxicological**	X^2 Statistic	
RD vs Yolk sac Infection	36.1700**	31.7221**		**Vanaraja**	**Chabro**
RD vs Pneumonia	2.2913^{NS}	0.2913^{NS}	R.D Vs Suspected Mycotoxicosis	98.8996**	95.8571**
I.B.D Vs Chick oedema Disease	0.0301^{NS}	0.0401^{NS}	R.D Vs Nephrosis	35.9571**	31.6781**

Comparison – in among Viral diseses Vs Metabolic Diseases	X^2 Statistic		Comparison – in among Viral Vs Toxicological	X^2 Statistic	
	Vanaraja	Chabro		Vanaraja	Chabro
I.B.D Vs Gout	1.9716^{NS}	1.0713^{NS}	Chicken Infectious anaemia Vs Nephrosis	9.2119**	8.3761**
I.B Vs Chick oedema Disease	0.0016^{NS}	0.0012^{NS}	Lymphoid Leucosis Vs Suspected Mycotoxicosis	2.6135^{NS}	2.5721^{NS}
I.B Vs Gout	1.28^{NS}	1.02^{NS}	Lymphoid Leucosis Vs Nephrosis	13.4341**	11.6381**
Cyst adeno Carcinoma Vs Chick oedema Disease	0.0028^{NS}	0.0028^{NS}	Chicken Infectious anaemia Vs Suspected Mycotoxicosis	5.4403*	6.8204*
Cyst adeno Carcinoma Vs Gout	0.1229^{NS}	0.1129^{NS}	I.B.D Vs Suspected Mycotoxicosis	0.7533^{NS}	31.6223**
Fowl Pox Vs Chick oedema Disease	0.0028^{NS}	0.0026^{NS}	I.B.D Vs Nephrosis	35.9773**	26.6213**
Fowl Pox Vs Gout	0.1229^{NS}	0.0128^{NS}	I.B Vs Suspected Mycotoxicosis	0.35^{NS}	0.45^{NS}
Lymphoid Leucosis Vs Chick oedema Disease	4.6678^{NS}	4.9978^{NS}	I.B Vs Nephrosis	26.9231**	21.7621**
Lymphoid Leucosis Vs Gout	0.002^{NS}	0.006^{NS}	Cyst adeno Carcinoma Vs Suspected Mycotoxicosis	0.1383^{NS}	0.85^{NS}
Chicken Infectious anaemia Vs Chick oedema Disease	8.7761**	5.9761**	Cyst adeno Carcinoma Vs Nephrosis	2.512^{NS}	0.913^{NS}
Chicken Infectious anaemia Vs Gout	11.2132**	9.05431**	Fowl Pox Vs Suspected Mycotoxicosis	0.1293^{NS}	0.2873^{NS}

Comparison – in among Viral diseases Vs Metabolic Diseases	**X^2 Statistic**		**Comparison - in among Bacterial Diseases.**	**X^2 Statistic**	
	Vanaraja	**Chabro**		**Vanaraja**	**Chabro**
R.D Vs Chick oedema Disease	110.0580**	98.0989**	Pasteurellosis Vs Oophoritis	4.5448*	4.948*
R.D Vs Gout	15.4328**	16.9431**	Pasteurellosis Vs Coryza	11.7324**	14.7527**
Oophoritis vs Gout	0.0016^{NS}	0.0019^{NS}	Pasteurellosis Vs Yolk sac Infection	35.2612**	34.2212**
Pasteurellosis vs Gout	1.1429^{NS}	1.1220^{NS}	Pasteurellosis Vs Pneumonia	62.8672**	62.9671**
Comparison – in among Viral Vs Toxicological	**X^2 Statistic**		Oophoritis Vs Coryza	2.6574^{NS}	2.3647^{NS}
	Vanaraja	**Chabro**	Oophoritis Vs Enteritis	2.992^{NS}	2.612^{NS}
Fowl Pox Vs Nephrosis	2.1542^{NS}	1.4514^{NS}	Oophoritis Vs Pneumonia	40.5458**	39.5456**
Oophoritis vs Chick Oedema Disease	4.2533*	4.1677*	Coryza Vs Enteritis	0.0003^{NS}	0.0003^{NS}
Comparison – among Bacterial Vs Metabolic Diseases	**X^2 Statistic**		Coryza Vs Pneumonia	21.8213**	21.6212**
	Vanaraja	**Chabro**	Pasteurellosis Vs Enteritis	12.9482**	11.7568**
Coryza vs Chick Oedema Disease	13.782**	13.827**	Pasteurellosis Vs Collibacillosis	16.6373**	16.2103**
Pasteurellosis vs Chick Oedema Disease	0.094^{NS}	0.064^{NS}	Enteritis Vs Collibacillosis	2.0504^{NS}	2.0605^{NS}
Coryza vs Gout	0.8523^{NS}	0.8573^{NS}	Enteritis Vs Yolk sac Infection	16.2983**	16.8362**
Enteritis vs Chick Oedema Disease	16.6784**	16.5000**	Enteritis Vs Pneumonia	45.726**	45.826**
Enteritis vs Gout	1.0404^{NS}	1.0016^{NS}	Collibacillosis Vs Yolk sac Infection	0.6227^{NS}	0.6115^{NS}

Comparison – in among Bacterial Vs parasitic/protozoal	**X^2 Statistic**		**Comparison – among Bacterial Vs Toxicological**	**X^2 Statistic**	
	Vanaraja	**Chabro**		**Vanaraja**	**Chabro**
Pasteurellosis vs Ascaridiasis	14.2306**	15.2306**	Collibacillosis vs Gout	2.5503^{NS}	2.5598^{NS}
Pasteurellosis vs Coccidiosis	39.4966**	39.0012**	Yolk sac Infection vs Chick Oedema Disease.	40.4866**	40.0978**
Oophoritis vs Ascaridiasis	4.3214*	4.9213*	Yolk sac Infection vs Gout	5.2328*	5.0328*
Oophoritis vs Coccidiosis	22.3462**	22.3872**	Pneumonia vs Chick Oedema Disease	70.5857**	70.5742**
Oophoritis vs Coccidiosis	22.3462**	22.3872**	Pneumonia vs Gout	11.8507**	11.0095**
Comparison – In between parasitic / protozoal	**X^2 Statistic**		**Comparison – among Bacterial Vs parasitic/protozoal**	**X^2 Statistic**	
				Vanaraja	**Vanaraja**
			Enteritis vs Ascaridiasis	1.1604^{NS}	1.2621^{NS}
	Vanaraja	**Vanaraja**	Enteritis vs Coccidiosis	17.5641**	21.3342**
			Coryza vs Ascaridiasis	0.8819^{NS}	0.8901^{NS}
Ascaridiasis Vs Coccidiosis	0.246^{NS}	0.84^{NS}	Coryza vs Coccidiosis	10.103**	10.113**

Comparison – among Bacterial Vs Fungal diseases	**X^2 Statistic**	
	Vanaraja	**Chabro**
Pasteurellosis Vs Brooder Pneumonia	0.0603^{NS}	0.0401^{NS}
Oophoritis Vs Brooder Pneumonia	0.3866^{NS}	0.3977^{NS}
Coryza Vs Brooder Pneumonia	2.2864^{NS}	2.8642^{NS}
Enteritis Vs Brooder Pneumonia	3.9987*	2.9963*
Collibacillosis Vs Brooder Pneumonia	3.9305*	3.8789*
Yolk sac Infection Vs Brooder Pneumonia	6.9305**	6.8302**
Pneumonia Vs Brooder Pneumonia	13.8018**	11.9017**

Comparison – among Bacterial Vs parasitic/protozoal	**X^2 Statistic**	
	Vanaraja	**Chabro**
Coryza vs Ascaridiasis	0.8819^{NS}	0.8901^{NS}
Pasteurellosis vs Ascaridiasis	14.2306**	15.2306**
Pasteurellosis vs Coccidiosis	39.4966**	39.0012**

Comparison – among Bacterial Vs Metabolic Diseases	**X^2 Statistic**	
	Vanaraja	**Chabro**
Collibacillosis vs Gout	2.5503NS	2.5598NS
Yolk sac Infection vs Chick Oedema Disease	40.4866**	40.0978**
Yolk sac Infection vs Gout	5.2328*	5.0328*
Pneumonia vs Chick Oedema Disease	70.5857**	70.5742**
Pneumonia vs Gout	11.8507**	11.0095**
Collibacillosis vs Chick Oedema Disease	19.2406**	19.9603**

Comparison – among Bacterial Vs parasitic/protozoal	**X^2 Statistic**	
	Vanaraja	**Chabro**
Coryza vs Coccidiosis	10.103**	10.113**
Enteritis vs Ascaridiasis	1.1604^{NS}	1.2621^{NS}
Enteritis vs Coccidiosis	17.5641**	21.3342**
Oophoritis vs Ascaridiasis	4.3214*	4.9213*

Comparison – in among Bacterial Vs Toxicological	X^2 Statistic		Comparison – in Fungal Diseases Vs Toxicological Diseases	X^2 Statistic	
	Vanaraja	**Chabro**		**Vanaraja**	**Chabro**
Pasteurellosis Vs Suspected Mycotoxicosis	0.325^{NS}	0.212^{NS}	Brooder Pneumonia Vs Suspected Mycotoxicosis	0.2292^{NS}	0.2282^{NS}
Pasteurellosis Vs Nephrosis	42.5401**	31.7503**	Brooder Pneumonia vs Nephrosis	6.6289*	6.5079*
Oophoritis Vs Suspected Mycotoxicosis	2.2431^{NS}	2.1395**	**Comparison – among parasitic / protozoal Vs Metabolic Diseases**	**X^2 Statistic**	
Oophoritis Vs Nephrosis	14.6399**	11.3799**		**Vanaraja**	**Chabro**
Coryza Vs Suspected Mycotoxicosis	8.0211**	9.0522**	Ascaridiasis vs Chick Oedema Disease	16.1583**	11.2183**
Coryza Vs Nephrosis	5.0876*	6.0961*	Ascaridiasis vs Gout	2.3626^{NS}	3.2009^{NS}
Enteritis Vs Suspected Mycotoxicosis	11.0366**	13.0276**	Coccidiosis vs Chick Oedema Disease	52.4301**	32.4201**
Enteritis Vs Nephrosis	13.4302**	11.0399**	Coccidiosis vs Gout	8.3422**	6.0978**
Collibacillosis Vs Suspected Mycotoxicosis	12.6243**	14.7281**	**Comparison – among parasitic / protozoal Vs Toxicological**	**X^2 Statistic**	
Collibacillosis Vs Nephrosis	38.5601**	31.8501**		**Vanaraja**	**Vanaraja**
Yolk sac Infection Vs Suspected Mycotoxicosis	35.6601**	41.4301**	Ascaridiasis Vs Suspected Mycotoxicosis	12.6154**	10.9471**
Yolk sac Infection Vs Nephrosis	0.00964^{NS}	0.0298^{NS}	Ascaridiasis vs vs Nephrosis	0.0102^{NS}	0.0341^{NS}
Pneumonia Vs Suspected Mycotoxicosis	62.9942**	42.5341**	Pneumonia Vs Nephrosis	12.5962**	9.8621**

Comparison – among Fungal Vs parasitic/ protozoal	**X^2 Statistic**		**Comparison – among parasitic / protozoal Vs Toxicological**	**X^2 Statistic**	
	Vanaraja	**Chabro**		**Vanaraja**	**Chabro**
Brooder Pneumonia vs Ascaridiasis	4.6503^{NS}	1.5601^{NS}	Coccidiosis vs Suspected Mycotoxicosis	42.7721**	53.9431**
Brooder Pneumonia vs Coccidiosis	7.967**	11.920**	Coccidiosis vs Nephrosis	1.12176^{NS}	1.02121^{NS}
Fungal Vs Metabolic Diseases	**X^2 Statistic**		**Comparison – In between Metabolism Diseases**	**X^2 Statistic**	
	Vanaraja	**Chabro**		**Vanaraja**	**Chabro**
Brooder Pneumonia Vs Chick Oedema Disease	0.1182^{NS}	0.1372^{NS}			
Brooder Pneumonia vs Gout	0.0241^{NS}	0.0631^{NS}	Chick Oedema Disease vs Gout	0.7542^{NS}	0.6947^{NS}

NS : - Non – significant, P>_ 0.05

** :- Highly significant p < 0.01

* :- Significant, p<0.05

4.4 Effect of age on pancreatic pathology.

The overall and disease wise variation in the incidence of pancreatic pathology in different Age group of birds is depicted in table number 03, 04 and fig. 04, 05 respectively. It was observed that maximum pancreatic pathology was followed by Age group 0-2 weeks (20%Vanaraja and 15% Chabro) and adult birds (30% Vanaraja and 10% Chabro). A significant variation was also observed in susceptibility to increased pancreatic pathology under different disease registered in Age group 2-4 Weeks Poultry birds (30.00% Vanaraja and 27.5% Chabro) conditions between the three age groups. 2-4 Weeks showed increased susceptibility to develop pancreatic pathology while suffering from IB, fowl pox, pasteurellosis, enteritis, colibacillosis, chick oedema disease and suspected mycotoxicosis. The diseases which produced greater percentage of pancreatic pathology in chicks were IBD, IB, brooder pneumonia, chick oedema disease, gout and suspected mycotoxicosis. In adult birds, diseases like IB, cystadenocarcinoma, pasteurellosis, visceral gout and suspected mycotoxicosis produced greater incidence of pancreatic abnormalities. Few diseases like IB, enteritis, ascaridiasis and suspected mycotoxicosis showed similar trend with respect to percent involvement of pancreatic abnormalities. The variation in pancreatic pathology between Age group 0-2 Weeks and adult poultry birds was statistically non-significant (Table 07). The statistical analysis to certain variation in incidence of pancreatic pathology in different age group of birds is shown in Table 07.

Table- 05: Incidence of pancreatic pathology in different age group of poultry in Vanaraja and Chabro breed of poultry.

Age Group	0-2 weeks		2-4 weeks		Adults	
	Vanaraja	Chabro	Vanaraja	Chabro	Vanaraja	Chabro
Number of Birds with pancreatic pathology	8.00	6.00	12.00	11.00	6.00	4.00
Number of Birds affected	40	40	40	40	40	40
Incidence of pancreatic pathology in percentage	20.00	15.00	30.00	27.50	15	10.00

Figure - 04: Bar graph showing incidence of pancreatic pathology in different age group of poultry in Vanaraja and Chabro breed of poultry.

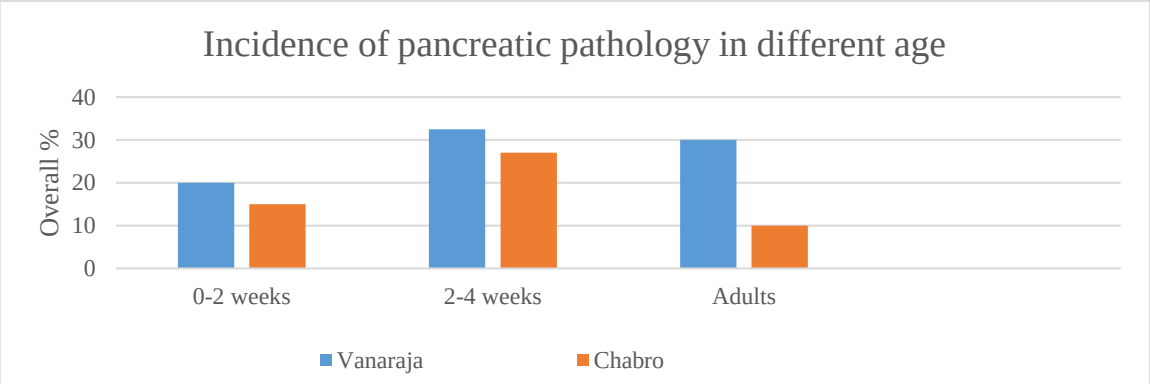


Table - 06: Incidence of pancreatic pathology in different age group of poultry during different diseases in Vanaraja and Chabro breed of poultry.

Poultry Diseases	Incidence of pancreatic pathology in percentage (%)					
	0-2 weeks		**2-4 weeks**		**Adults**	
	Vanaraja	Chabro	Vanaraja	Chabro	Vanaraja	Chabro
I.B.D	75.00	66.00	0.00	0.00	0.00	00.00
I.B.	50.00	29.00	100.00	99.00	100.00	80.52
Cyst adeno Carcinoma	0.00	00.00	0.00	0.00	100.00	100.00
Fowl Pox	0.00	00.00	100	0.00	0.00	0.00
Lymphoid Leucosis	0.00	00.00	50.00	50.00	60.00	50.00
C.I.A	55.00	50.00	33.00	23.00	0.00	0.00
R.D	2.30	2.70	58.00	5.00	12.30	15.80
Pasteurellosis	0.00	0.00	60.00	58.00	100.00	55.70
Oophoritis	0.00	0.00	0.00	0.00	52.00	63.40
Coryza	43.00	22.00	50.00	45.00	0.00	0.00
Enteritis	38.00	31.00	71.00	73.00	31,00	25.00
Collibacillosis	21.00	25.00	75.00	65.00	0.00	0.00
Yolk sac Infection	21.00	18.00	0.00	0.00	0.00	0.00
Pneumonia	12.00	17.00	2.00	1.50	0.00	0.00
Brooder Pneumonia	80.00	65.00	0.00	0.00	0.00	0.00
Ascaridiasis	27.00	25.00	19	28	33.3	33.3
Coccidiosis	20.00	22.00	27.00	24.00	0.00	0.00
C,O,D	100.00	100.00	61,00	58.00	0.00	0.00
Gout	80.00	84.00	0.00	0.00	100.00	100
Suspected for Mycotoxicosis	80.00	67.00	65.00	62.00	100.00	100.00
Nephritis	46.00	37.00	19.00	12.00	5.00	3. 00

Figure - 05: Bar graph showing Incidence of pancreatic pathology in different age group of poultry during different diseases.

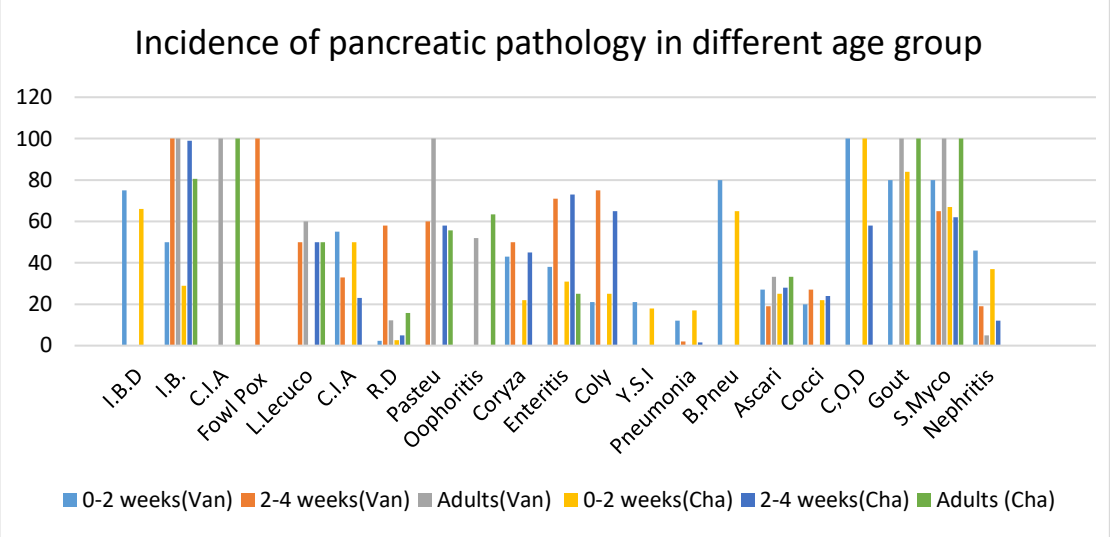


Table- 07: Chi square analysis for Incidence of pancreatic pathology in different age group of poultry during different diseases.

Comparison	**X^2 statistic**	**X^2 statistic**
	Vanaraja	Chabro
0-2 weeks vs 2-4 weeks	61.4321**	58.8602**
0-2 weeks vs Adults	0.2651NS	0.92421NS
2-4 weeks vs Adults	53.6303**	48.9870NS

NS: - Non-significant, $p>0.05$ **:- Highly Significant, $p<0.01$

4.5 Gross pancreatic pathology:

In the present study, Post-mortem examination of diseased birds revealed remarkable gross lesions in the birds in which involvement of pancreas was observed. The diseased pancreas exhibited congestion, bleached and mottled appearance with multifocal necrotic lesions, pancreatic deformity and atrophied and hyperplastic changes in pancreas. The overall gross pancreatic pathology and details of gross pancreatic pathology observed under different disease conditions are shown through graphical representation, given in table 05, 06 and fig. 06, 07 respectively. The important gross lesions seen during study period were following:

Bleached pancreas: This change was characterized by marked white discoloration of the pancreas as against pinkish to pale yellowish colour seen normally in pancreas. In majority of cases bleached appearance was seen independently in pancreas however in quite a few cases it was seen in conjunctions with pancreatic deformity, hyperplasic change, and atrophic changes with mild mottling (Fig. 39).

Congestion of pancreas: Congested pancreas were characterized by development of dark pinkish to reddish pink discoloration. Most of the time congestion was observed in the entire length of pancreas but in some cases there was intermittent dark pinkish and pale pinkish ting of congested pancreas. Congestion was not observed in atrophied pancreas. Concomitant development of pancreatic deformity and mild mottling due to multifocal necrosis was also seen some times (Fig 38)

Mottled Pancreas: This was an important gross pathological finding of diseased pancreas it was characterized by increased hardness in the consistency of pancreas with yellowish discoloration and formation of discrete multifocal white to creamy white, round necrotic lesion. These focal changes were either embedded in the parenchyma or showed discrete nodular swelling. Necrotic mottling was often associated with pancreatic deformity, bleached appearance, congestion and hyperplastic changes (Fig 46, 48).

Pancreatic deformity: Misshapen pancreas was important gross pathology in several birds. Pancreatic deformity was most commonly seen in grown-up birds as compared to chick and starter birds. A very wide range of pancreatic deformities were observed in the diseased birds. These deformity always occurred in conjunction with duodenal loop. Mostly, pancreatic deformity was found adjacent to duodenal loop giving distorted appearance to duodenal pancreatic loop. (Fig 45).

The nature of pancreatic deformity ranged from simple bending, curving folding. Single twisting, multiple twisting, coiling and wavy presentation of pancreodeodenal complex. These changes were seen mostly in the tip of the pancreas. However twisting. Lateral deviation and bending was also observed in the base or mid duodeno-pancreatic segment.

The primary deformity in such cases was seen in the pancreas which was mimicked by duodenum. This was proven by the fact that when pancreas was separated from duodenum it showed curving and twisting observed in the duodeno-pancreatic complex. The pancreatic deformity was often associated with bleached appearance, congestion, hyperplasia and multifocal necrotic mottling.

Pancreatic atrophy: Atrophy of pancreas was characterized by reduction in the width of pancreas most of the time and in the length of pancreas in some of the cases. In extreme pancreatic atrophy as seen in cystadenocarcinoma in which the entire intestinal mains formed a twisted bundle of intestinal loop with adhesion, island of

pancreatic tissue was found in the coiled and fused duodenal loop. Pancreas with atrophic changes always appeared pale or bleached with multifocal necrotic lesion very few cases (Fig 37).

Hyperplastic pancreas: These type of gross change was characterized by local widening of their pancreatic mass leading to deflection in the associated duodenum. In majority of cases such changes were observed at the base of duodenum or in the mid segment. Pancreatic hyperplasia was most commonly seen in young chicks. These pancreases were either pale or bleached in appearance (Fig 43).

Incidence of pancreatic pathology in decreasing order in terms of frequency is presented in table 08, which shows highest percentage of bleached pancreas and congested pancreas (both 23.07%Vanaraja and 33.33% Chabro) and lowest percentage of hypertrophied pancreas (15.38% Vanaraja and 9.50% Chabro) Significantly higher percentage of birds showed mottling of pancreas due to multifocal necrosis (15.23%Vanaraja and 9.52% Chabro).The main diseases in which bleached appearance of pancreas was prominent included coccidiosis (8300%Vanaraja and 32.00% Chabro), ascaridiasis and fowl pox (both 66.%Chabro and 66.00% Vanaraja) and IBD (55.51%Vanaraja and 46.00% Chabro). Congestion of pancreas was most marked in chicken infectious anaemia (71.42% Chabro and 61.20% Vanaraja), pneumonia (65.90%Vanaraja and 66.00% Chabro), chick odema disease (60.87%Vanaraja and 60.87% Chabro), coryza (45.00%Vanaraja and 53.13% Chabro) and IB (both Vanraja and Chabro 50.00%). Mottling was significantly high in cases of gout (70.00% Vanaraja And 66.12% Chabro), lymphoid leucosis (52.70%Vanaraja and 55.90% Chabro) and ascaridiasis (50% Vanaraja and 51.12% Chabro). In rest of the diseases mottling of pancreas was moderate to low. Pancreatic deformity was significantly higher in diseases like chick oedema disease (56.00%Vanaraja and 54.00%Chabro), colibacillosis (42.00%Chabro and 51.31%Vanaraja) and IBD (both breeds of poultry 50.00%) In rest of the diseases lower incidence of pancreatic deformity was seen. The statistical analysis to ascertain variation in incidence of gross pancreatic pathology of birds is shown in table 09.

Table- 08: Incidence of Gross pancreatic Lesion in Vanaraja and Chabro breeds of poultry.

Pancreatic Conditions	**Bleached**		**Congestion**		**Mottled With Necrotic Foci**		**Deformed Pancreas**		**Atrophic**		**Hyperplastic**	
Total (Vanraja 26 ,Chabro 21)	**Vanaraja**	**Chabro**	**Vanaraja**	**Chabro**	**Vanaraja**	**Chabro**	**Vanaraja**	**Chabro**	**Vanaraja**	**Chabro**	**Vanaraja**	**Chabro**
Number of specific gross lesion	6.00	7.00	5.00	4.00	4.00	2.0	3.00	3.00	4.00	3.00	4.00	2.00
Overall %	23.07	33.33	19.23	19.04	15.23	9.52	11.50	19.04	15.38	14.28	15.38	9.50

Figure - 06: Bar graph showing Incidence of Gross pancreatic Lesion in Vanaraja and Chabro breed of poultry in Vanaraja and Chabro breeds of poultry.

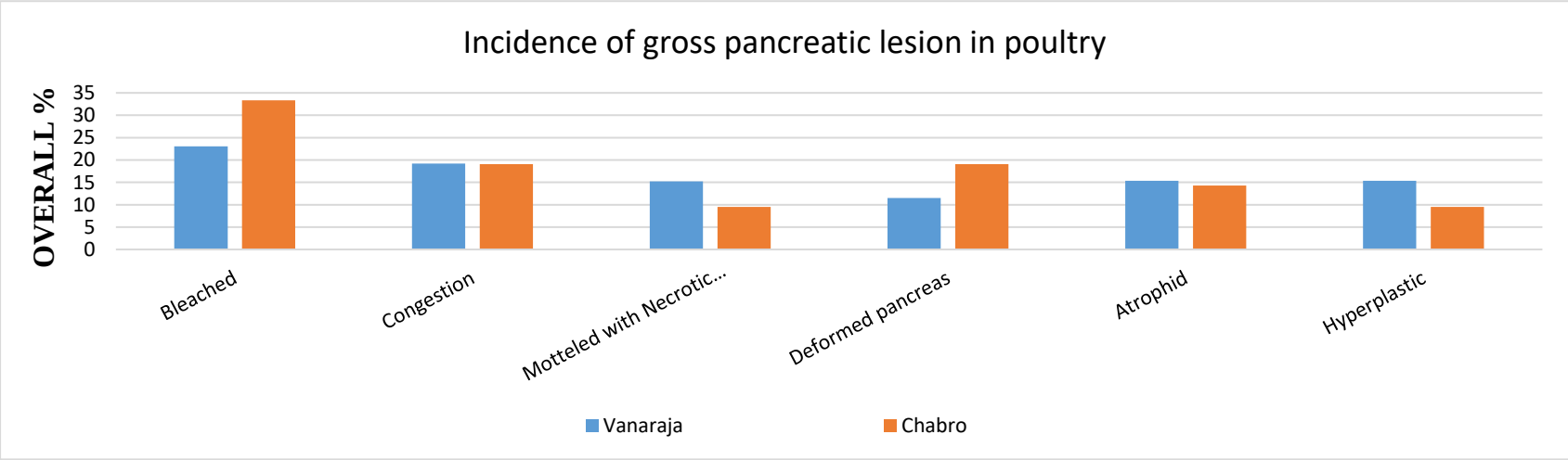


Table - 09: Incidence of gross pancreatic lesions in different disease conditions of Vanaraja and Chabro breed of poultry.

Diseases	**Bleached**		**Congestion**		**Mottled with Necrotic Foci**		**Deformed pancreases**		**Atrophic**		**Hyperplastic**	
	Vanaraja	**Chebro**	**Vanaraja**	**Chabro**	**Vanaraja**	**Chabro**	**Vanaraja**	**Chabro**	**vanaraja**	**Cabrao**	**Vanaraja**	**Chabro**
I.B.D	55.51	46.00	22.00	22.00	9.00	11,12	50.00	45.00	5.00	4.00	0.00	0.00
I.B	20.00	21.43	50.00	50.00	15.00	14.10	32.00	29.90	0.00	0.00	1.00	0.00
C.A.C	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	100.00	98.11	0.00	0.00
Fowl Pox	66.75	66.67	33.45	33.32	33.33	32.90	0.00	1.00	33.33	23.00	0,00	0,00
Lymphoid Leucosis	33.00	35.00	21.00	20.11	52.70	55.90	5.00	6.11	5.00	4.11	6.00	5.00
C.I.A	14.23	13.00	61.21	71.42	14.11	13.00	28.57	28.57	0.00	0.00	4.60	3.98
RD)	39.10	28.00	33.70	32.77	48.11	43.90	7.27	6.97	2.80	1.90	2.40	2.45
Pasteurellosis	21.80	23.00	43.80	47.37	37.30	34.00	0.00	0.00	0.00	0.00	0.00	2.42
Oophoritis	27.00	26.09	32.00	34.78	43.48	43.00	0.00	0.00	0.00	1.00	0.00	0.00
Coryza	14.00	16.67	45.00	53.13	37.12	37.50	25.00	24.00	4.17	3.12	2.30	2.30
Enteritis	32.00	15.00	56.00	42.65	7.90	7.79	16.41	15.43	2.60	4.10	4.00	3.90
Collibacillosis	33.33	33.80	46.76	44.87	46.67	43.91	51.31	42.00	0.00	2.10	0.00	0.00
Yolk sac Infection	32.00	36.00	48.12	48.00	9.20	9.20	5.75	5.75	1.15	0.00	0.00	1.00

Diseases	Bleached		Congestion		Mottled with Necrotic Foci		Deformed pancreases		Atrophic		Hyperplastic	
	vanaraja	Chebro	Vanaraja	Chabro	Vanaraja	Chabro	Vanaraja	Chabro	vanaraja	Cabrao	Vanaraja	Chabro
Pneumonia	28.54	30.00	65.90	66.00	16.39	16.38	11.48	11.48	1.64	1.11	2.14	0.00
Brooder Pneumonia	41.00	33.9	20.00	20.00	40.00	42.00	40.00	40.00	0.00	1.00	0.00	0.00
Ascaridiasis	66.00	66.00	0.00	0.00	50.00	51.12	0.00	0.00	0.00	0.00	1.00	0.00
Coccidiosis	83.00	32.00	6.90	6.90	10.00	10.32	10.00	10.33	10.32	0.00	1.16	1.00
Chick Oedema Disease	8.70	8.00	60.87	60.87	60.12	17.12	56.00	54.00	0.00	2.00	1.64	1.73
Gout	16.81	12.00	16.67	14.99	70.00	66.12	0.00	1.00	0.00	1.00	0.00	0.00
Suspected Mycotoxicosis	16.16	19.8	27.27	23.45	27.00	32.00	27.12	21.00	0.00	0.00	9.90	10.10
Nephritis	53.16	45.70	12.34	14.89	14.90	6.79	31.00	28.11	18.99	12.67	3.80	2.89

Figure - 07: Bar graph showing Incidence of gross pancreatic lesions in different disease conditions In Chabro and Vanaraja breed of poultry

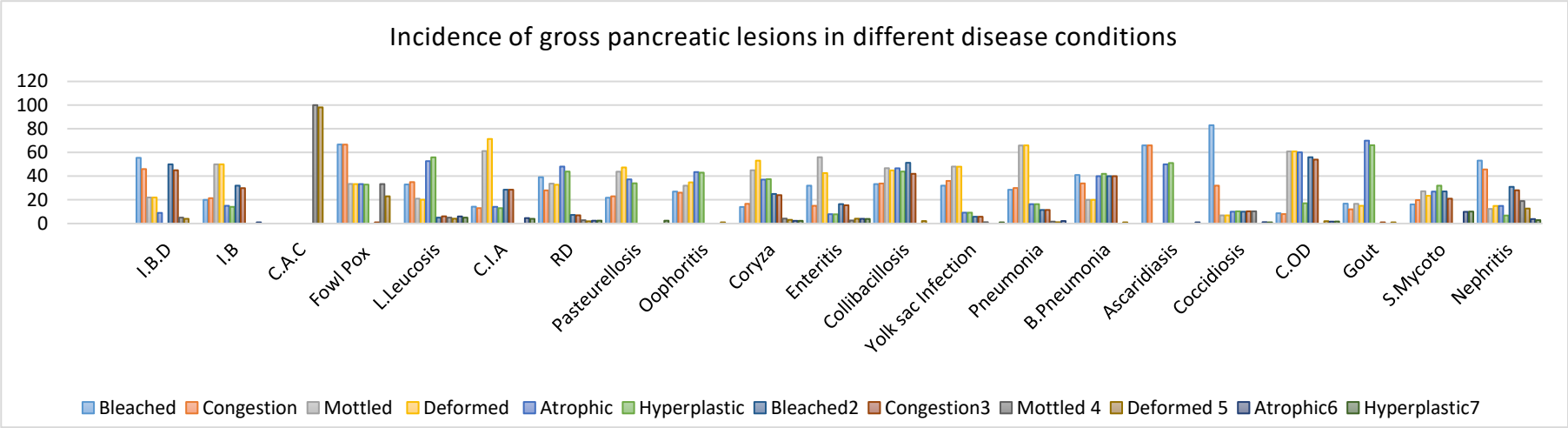


Table-10: Chi square analysis for comparison of gross pancreatic lesions in poultry Chabro and Vanaraja breed of poultry.

Comparison	X^2 Statistic		Comparison	X^2 Statistic	
	Vanaraja	Chabro		Vanaraja	Chabro
Bleached v/s Congestion	0^{NS}	0.001^{NS}	Congestion v/c Atrophied	65.8383**	65.3378**
Bleached v/s Mottled with Necrotic Foci	14.5967**	10.487**	Mottled with Necrotic Foci v/s Deformed Pancreas	50.00034**	50.0092**
Bleached v/s Deformed Pancreas	101.0637**	101.1427**	Mottled with Necrotic Foci v/s Atrophid	111.5088**	111.0022**
Bleached v/s Hyperplastic change	313.9086**	289.0021**	Mottled with Necrotic Foci v/s Hyperplastic change	93.7818**	90.8819**
Bleached v/s Atrophied	278.1122**	10.5966**	Deformed Pancreas v/s Hyperplastic change	82.2321**	82.2123**
Congestion v/s Mottled with Necrotic Foci	10.0773**	9.0001**	Deformed Pancreas v/s Atrophied	58.7328**	57.2378**
Congestion v/s Deformed pancreas	98.0773**	99.0001**	Hyperplastic change v/s Atrophied	3.0236^{NS}	3.0851^{NS}
Congestion v/s Hyperplatic Change	111.000**	84.8750**			

NS: - Non-significant, p>0.05. **:- Highly Significant, p<0.01.

4.6 Pancreatic Histopathology:

Microscopic examination of diseased pancreas revealed several important histopathological alterations. The description of these microscopic abnormalities are following:

Interstitial and periductular fibrosis: These changes were most frequently seen in diseased pancreas. It was characterised by proliferation of fibrous connective tissue in the pancreatic Parenchyma. The degree and severity of fibrosis was variable in different disease conditions. It ranged from mild interstitial fibrosis in which fibrous connective tissue was observed in the intercellular or peri- acinar region. In more advanced cases significantly thick band of connective tissue was observed in the intercellular or peri-acinar region. This makes the Lobular pattern of pancreas prominent. In many cases significant periductular fibrosis was found adjacent exocrine pancreatic tissue. These changes often lead to considerably thick band of connective tissue forming prominent encroachment into the surrounding pancreatic parenchyma. In extreme cases of fibrosis which were very few in number, fibrous and collagenous tissue completely invaded the pancreas and created round island like lobular pattern. These trapped pancreatic cells due to cut off blood supply showed necrotic changes or were completely destroyed leaving behind tissue debris. Thickening of pancreatic capsule due to deposition of fibrous connective tissue was also observed in a few cases. Staining of the pancreas suspected for fibrosis with Mallory Trichrome stain, prominently displayed spectacular pattern of fibrosis in the interstitial, interlobular and periductular space (58, 68, and 69)

Congestion: Congestion of exocrine pancreas was characterized by vascular lumen being completely or partially filled with excess of erythrocytes. The capillaries present in the interstitial space also showed hyperaemic changes (Fig. 56 & 65).

Individualization of acinar cells: In several cases the acinar cells which normally should remain distributed in the form of acini with lumen in the central part showed dissociation of acinar cells, with disruption of acinar pattern of exocrine pancreas. In characteristic cases the cells appeared separated from each other. These changes were seen diffusely throughout the parenchyma. These developments were often associated with fibrosis in the interstitial or inter lobular space, necrosis with infiltration of inflammatory cells, apoptosis and vacuolar degeneration of the acinar cells (Fig. 55)

Diffuse pancreatic fat Necrosis: Necrosis in pancreatic parenchyma of birds due to different disease condition was an important microscopic finding. It was characterized by multifocal

loss of acinar cells and most of the time associated with infiltration of mononuclear cells of heterophils. The size of necrotic area was found to very between small to significantly large occupying almost half of the microscopic field. The necrotic zone were either round or irregular in shape. The acinar cells adjacent to zone of necrosis showed disruption of acinar pattern and individualization. Sometimes exocrine pancreas showed coagulation necrosis characterized by acidophilic discoloration of degenerating cells with pyknotic nuclei, however, such lesions often did not show infiltration of inflammatory cells. On few occasions, subcapsular necrosis has also been observed. In extreme cases the necrotic area of pancreas gave moth eaten appearance characterized by irregular empty areas in the necrotic zone. Proliferation of fibrous connective tissue in and around necrotic zone has also been recorded (Fig. 57).

Distainded zymogen granules: Zymogen granules were observed in both with hematoxylin and eosin (H & E) stain as well as periodic acid (PAS) stain. In these conditions the apical portion of acinar cells were filled with zymogen granules giving it bulging appearance. The nucleus of such cell was located at the base of the acinar cells. Contrary to hypertrophid acinar cells due to zymogen overload the atrophic acinar cell was considerably smaller in size with almost no zymogen granules in its cytoplasm. The nucleus in such cases took central position within the acinar cell. Atrophied acinar cells also showed vacuolar degeneration (Fig. 71).

Thickened capsule: The capsule of pancreas is normally a thin fibrous coating over its outer surface. Though, not a frequent finding capsule showed considerable thickening due to deposition of connective tissue. The thickened capsule often showed infiltration of inflammatory cells. Sub capsuler necrosis and individualization of acinar cells had also been observed (63).

Overall finding of microscopic abnormalities in the pancreas of birds suffering from different disease conditions and the variations observed in histopathological changes under different disease conditions are summarised in table 11, 12 and figure 8. 9. The major histopathological changes in pancreas in decreasing order of frequency were interstitial fibrosis (54.41% Vanaraja and 46.00% Chabro), congestion (46.37%Vanaraja and 30.00% Chabro), individualization of acinar cells (32.35%Vanaraja and 27.00% Chabro), periductular fibrosis (27.94% Vanaraja and 13.29% Chabro) Distainded zymogen granules (13.24% Vanaraja and Chabro), Interstitial fibrosis of exocrine pancreas was most marked in brooder's pneumonia, pasteurellosis, fowl pox, lymphoid leucosis, coryza, yolk sac

infection and chick oedema disease. Congestion of moderate to severe nature was observed in the pancreas in almost all disease conditions except in fowl pox and cystadenocarcinoma. Individualization of acinar cells was a significant feature of Pneumonia, coryza and lymphoid leucosis. It was not observed in conditions like cyst adenocarcinoma, fowl pox, brooder pneumonia and ascaridiasis. In the rest of the diseases, mild to moderate individualization was observed. Significantly high diffuse pancreatic fat necrosis was encountered in fowl pox, brooder's pneumonia and IB. On the other hand, Distainded zymogen granules was found to be significantly high in coryza, fowl pox, cyst adenocarcinoma, colibacillosis, ascaridiasis and coccidiosis. Periductular fibrosis and thickened capsule was most marked in brooder's pneumonia. Lastly, the thickening of the capsule was most frequently observed in Colibacillosis. The statistical analysis to ascertain variation in incidence of different microscopic changes of birds is shown in (Table 12).

Exocrine staining:

PAS staining of pancreas was done to see the excess or depletion of zymogen granules in the acinar cells. The zymogen granules were found to be PAS positive, however with routine H&E staining, zymogen granules were also distinctly visible as pink granular mass on the apical portion of acinar cells. Congo red staining was done on all the cases in which pancreas showed remarkable fibrosis, necrosis and atrophy. In none of the case, amyoid deposition was observed ruling out the possibility of amyloidosis as the cause of disease condition (Fig. 70, 71).

Endocrine staining:

In poultry as we observe in mammals the endocrine pancreas consists of islet of langerhans. However, the arrangement of cells in the islet of langerhans shows marked difference between avian and mammalian pancreas. Where as in mammalian pancreas the same islet of langerhans carries all the three cells of endocrine pancreas i.e. alpha, beta and delta cells which secretes glucagon, insulin and somatostatin respectively to regulate glucose metabolism in poultry on the other hand in avian pancreas, there are three types of islets viz light islet which is bigger with irregular outline and homes mainly glucagon secreting alpha cells, dark islets which are small round and oval with sharp outline and carries mainly insulin secretory beta cells and few delta cells and mixed islets in which all three cell types are present. Number of mixed islets is significantly low.

Histochemically staining was done to demonstrate and study the islet cells. Heidenhain's iron haematoxylin staining was done to differentiate between islet cells and

focal necrosis with inflammatory cells. Islets were characterized by light staining area without showing presence of any cellular population while areas of focal necrosis showed cellular population on the otherwise lighter stained zone of focal necrosis. Gomori's method of staining was done to observe the population of alpha cells and beta cells in the lighter or dark islets respectively and their cytoplasmic details (Fig. 64, 65). In the present study no remarkable pathology was observed in endocrine pancreas. Both light and dark islets were observed distributed sparsely in the midst of exocrine pancreas. Number of islets were found to be significantly high in the splenic lobe. However occasionally both light and dark islets showed marked reduction in cell population and infiltration of inflammatory cells. In the areas of massive necrosis and fibrosis there was complete destruction of islets of langerhans. At few plees hyperplasia of particularly light islet was also observed.

Table - 11: Incidence of histopathological lesions in pancreas of poultry Chabro and Vanaraja breed of poultry.

Diseases	**Histopathological Conditions**	**Interstitial fibrosis**		**Congestion**		**Individualization**		**Diffuse pancreatic fat necrosis**		**Periductular fibrosis**		**Thickened capsule**	
No of pancreas Examine	195,00	Vanaraja	Chabro	Vanaraja	Chabro	Vanaraja	Chabro	Vanaraja	Chabro	Vanaraja	Chabro	Vanaraja	Chabro
Number of Condition Specified		106.0	89.00	90.00	27.00	58.00	52.00	58.00	25.00	54.00	12.00	25.00	10.00
Overall %		54.41	46.00	46.37	30.00	32.35	27.00	30.88	13.00	27.94	13.29	13.24	5.39

Figure - 08: Bar graph showing Incidence of histopathological lesions in pancreas of poultry in Vanaraja and Chabro breed of poultry.

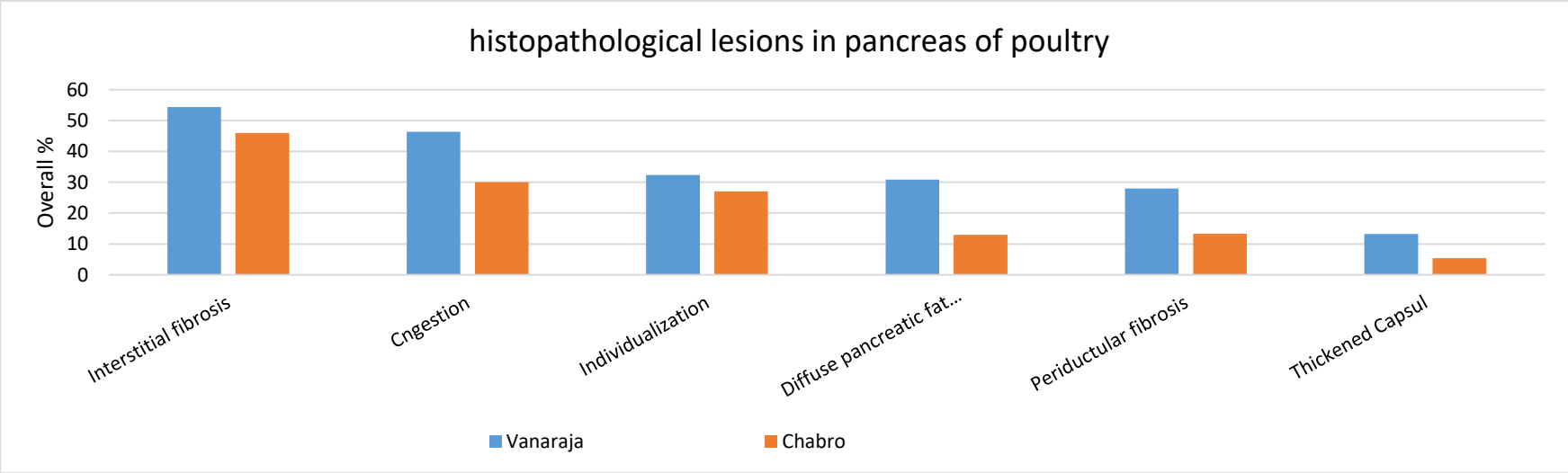


Table - 12: Incidence of histopathological lesions in different disease conditions of Vanaraja and Chabro breed of poultry.

Disease	**Histopathology Conditions**	**Interstitial fibrosis**		**Congestion**		**Individualization**		**Diffusepancreatic necrosis**		**Periductular fibrosis**		**Thickened capsule**	
		Vanaraja	Chabro	Vanaraja	Chabro	Vanraja	Chabro	Vanaraja	Chabro	Vanaraja	Chabro	Vanaraja	Chabro
I.B.D		41.86	38.00	36.85	41,00	25.00	28.57	23.00	22.00	27.00	25.57	0.00	0.00
I.B		13.24	14.29	41.86	40.00	40.86	41.00	70.45	70.00	27.37	25.57	0.00	0.00
Cyst adeno Carcinoma		13.70	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00
Fowl Pox		96.12	100.00	0.00	0.00	0.00	0.00	0.00	100.00	0.00	0.00	0.00	0.00
Lymphoid Leucosis		84.00	80,00	3.33	33.33	45.00	50.00	48.00	50.00	0.00	10.00	0.00	11.00
C.I.A.		55.00	61.00	50.00	49.00	32.00	30.00	55.00	63.00	9.00	5.88	0.00	0.00
RD)		51.00	45.00	33.33	33,33	46.10	45.10	21.50	21.57	5.66	0.00	24.69	23.400
Pasteurellosis		100.00	100.00	33.33	42.86	33.33	33.33	33.33	33.33	0.00	12.29	0.00	0.00
Oophoritis		26.57	27.00	42.67	67.66	23.97	26.00	13.39	11,29	14.25	0.00	13.29	13.00
Coryza		66.67	66,62	67.70	37.00	67.76	65.00	33.33	33.44	0.00	0.00	0.00	0.00
Enteritis		35.00	38.00	35.00	39.00	37.50	36.30	0.00	0,00	0.00	20.00	0.00	0.00
Collibacillosis		40.00	39.00	41.00	31.00	40.00	25.00	42.00	39.00	20.00	9.00	0.00	0.00
Yolk sac Infection		55.00	52.00	36.00	36.00	21.00	23.00	13.00	11.00	9.00	0.00	9.00	10.00
Pneumonia		32.00	33.33	67.88	66.67	65.68	65.00	68.62	62.00	0.00	100.00	0.00	0.00
Brooder Pneumonia		100.00	100.00	100.00	100.00	0.00	0.00	99.00	100.00	99.00	0.00	99.00	100.00
Ascariasis		50.00	51.00	45.00	46.00	0.00	0.00	0.00	0.00	0.00	30.00	0.00	0.00
Coccidiosis		41.00	41.00	60.00	69.00	11.00	10.00	41.00	35.00	30.00	23.00	0.00	19.00

Disease	HISTOPATHOLOGY	Interstitial fibrosis		Congestion		Individualization		Diffusepancreatic necrosis		Periductular fibrosis		Thickened capsule	
		Vanaraja	Chabro	Vanaraja	Chabro	Vanraja	Chabro	Vanaraja	Chabro	Vanaraja	Chabro	Vanaraja	Chabro
Chick Oedema Disease		50.00	51.00	85.00	78.23	22.00	26.00	11.00	11.90	25.00	0.00	9.00	11.50
Gout		100.00	100.00	55.00	45.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Suspected Mycotoxicosis		44.00	36,00	31.00	55.00	0.00	20.00	0.00	40.00	0.00	11.00	0.00	12.90
Nephritis		82.00	72.00	0.00	54.74	0.00	23.27	0.00	32.43	0.00	21.00	10.00	2.27

Figure- 09: Bar graph showing incidence of histopathological lesions in the pancreas of poultry in different disease condition

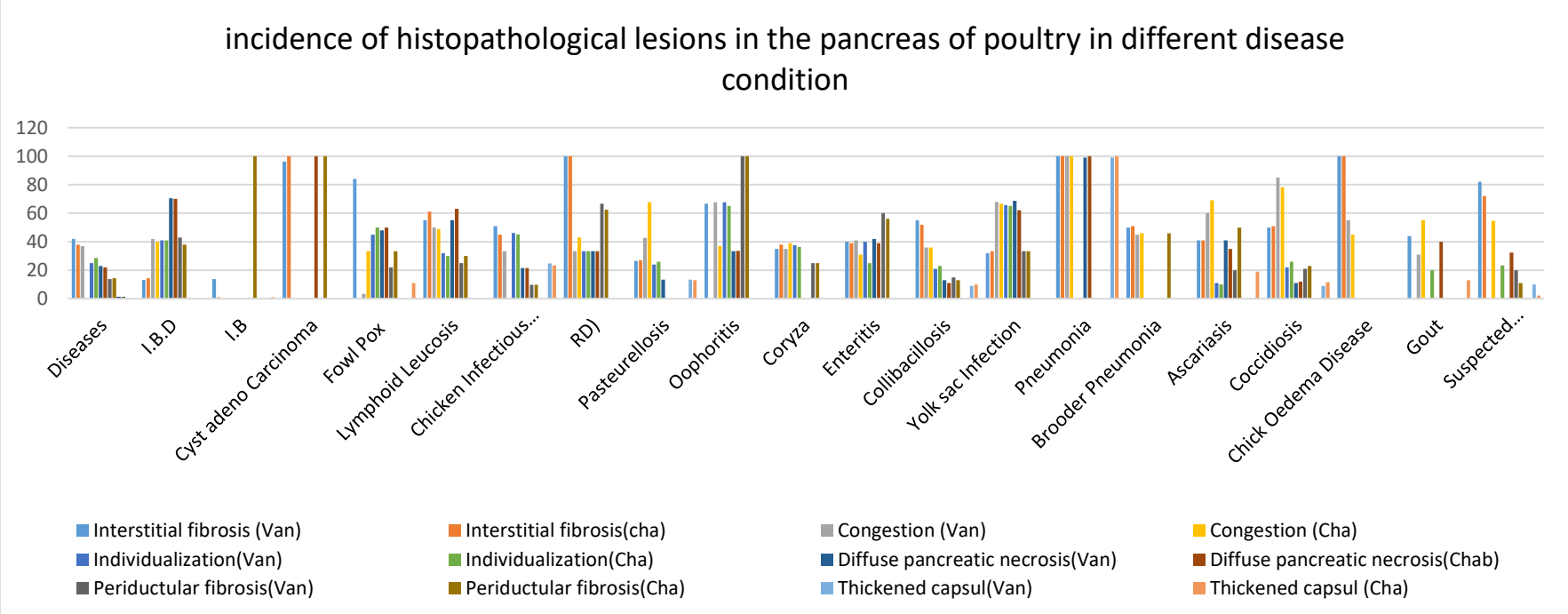


Table - 13: Chi square analysis for comparison of histopathological lesions in pancreas of Vanaraja and Chabro breeds of poultry.

Comparison	**X^2 statistic**		**Comparison**	**X^2 statistic**	
	Vanaraja	Chabro		Vanaraja	Chabro
Thickened capsule v/s Individualization	8.5341**	6.5301**	Individualization v/s Diffuse pancreatic fat necrosis	0.105^{NS}	2.0012^{NS}
Thickened capsule v/s Multifocal necrosis	42.0123**	38.161**	Individualization v/s Congestion.	19.4321**	17.9813**
Thickened capsule v/s Congestion	19.2311**	42.4311**	Individualization v/s Periductular Fibrosis	22.4321**	23.5431**
Thickened capsule v/s Periductular Fibrosis	62.5000**	86.3321**	Individualization v/s Interstitial fibrosis	2.81^{NS}	2.02^{NS}
Thickened capsule v/s interstitial fibrosis	2.31^{NS}	4.0214^{NS}	Diffuse pancreatic fat necrosis v/s Congestion	11.5435**	31.232**
Individualization v/s Diffuse pancreatic fat necrosis	33.2341**	22.4312**	Diffuse pancreatic fat necrosis v/s Periductular Fibrosis	17.4622**	21.0123**
Congestion v/s Interstitial fibrosis	43.2101**	40.3212**	Diffuse pancreatic fat necrosis v/s Interstitial fibrosis	23.0671**	31.0211**
Congestion v/s Vacuolar degeneration	54.3201**	61.3212**	Congestion v/s Periductular Fibrosis	54.0761**	72.01215**
Periductular Fibrosis v/s Interstitial fibrosis	89.3321**	41.3212**			

NS:- Non- Significant, P>0.05 ** :- Highly Significant P<0.01

4.7 Study of pancreatic pathology in chronic diseases conditions of poultry:

Chabro and vanaraja: birds suffering from chronic disease were Characterised by scanty pectoral and thigh muscles Showing complete wasting condition with prominent Kell (Fig 10) bone the affected Adults birch either chabro or and Vanaraja breeds Shown reduced weight gain. Poor performance, growth as well growth Necropsy
Revealed Atrophied visceral organ specifically. Heart however birds suffering from wasting diseases don't not had pathological changes. Suggestive of infectious diseases or even inflammatory conditions the present study revealed 2.9% poultry birds suffering from chronic poultry diseases. In Chabro and Vanaraja breeds most of the pancreas of poultry birds were Congested. However Bleached, Mottled, Atrophid and Hyperplastic changes were commonly found lesions.

Fig. 10: Photograph showing extreme wasting of sternal muscle in chronic poultry diseased of birds.

Comparative analysis of gross pancreatic pathology of and overall gross pancreatic lesions are presented in table 12 and figure 10. The statistical analysis to ascertain variation in the incidence of different gross pathological lesions in chronic diseases conditions**,** shown in table 13.

Table- 14: Comparison gross pancreatic pathology of chronic poultry diseases verses overall gross pancreatic pathology.

Total No Birds	Debilitating birds	Debilitating birds %	Pancreatic condition	Bleached	Mottled with Necrotic foci	Congestion	Deformed pancreas	Atrophid	Hyperplastic
240	7	2.9							
No of Conditions Specified				01	01	02	01	01	01
% of Gross pancreatic Pathology of Chronic diseases birds				14.285	14.285	28.57	14.285	14.285	14.285
Total No Birds with Pancreatic Pathology			47.00						
No of condition Specified				13	9.00	6.00	6.00	7.00	6.00
% of overall Gross Pancreatic Pathology				27.65	19.14	12.76	12.76	14.89	12.76
X^2 Statistic				0.4321^{NS}	0^{NS}	6.66612**	0.176^{NS}	0.671^{NS}	0.136^{NS}

NS : - Non significant, P>0.05 ** :-Highly Significant, P<0.01

Figure- 11: Bar graph showing comparison between gross pathology of chronic poultry diseases bird's verses overall gross pancreatic pathology.

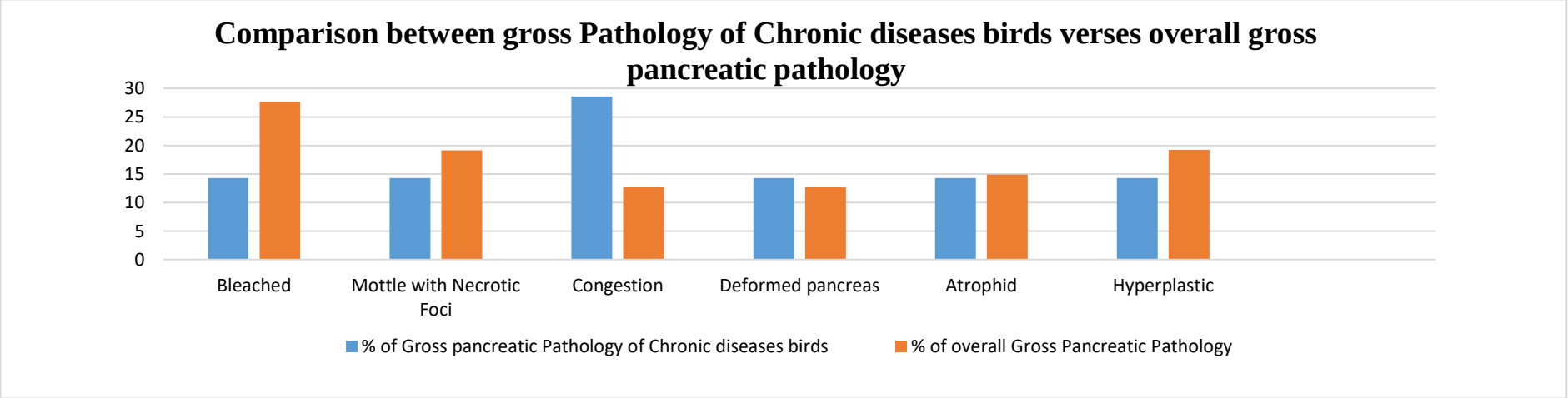


Table - 15: Chi square analysis for comparison of gross pancreatic lesions seen in chronic poultry diseases of birds.

Comparison	**X^2 Statistic**		**Comparison**	**X^2 Statistic**	
	Chabro	**Vanaraja**		**Chabro**	**Vanaraja**
Bleached v/s Congestion	9.0566**	8.9874**	Congestion v/s Atrophid	9.4325**	9.3251**
Bleached v/s Mottled with Necrotic foci	7.024**	7.547**	Mottled with Necrotic foci v/s Deformed pancreas	0.9134^{NS}	0.6543^{NS}
Bleached v/s Deformed pancreas	12.5793**	11.9873**	Mottled with Necrotic foci v/s Hyperplastic changes	17.8651**	19.3212**
Bleached v/s Hyperplastic changes	45.8753**	40.6231**	Mottled with Necrotic foci v/s Atrophid	11.5432**	12.6512**
Bleached v/s Atrophid	32.324**	31.9981**	Deformed pancreas v/s Hyperplastic changes	11.7612**	9.8979**
Congestion v/s Mottled with Necrotic foci	0.2323^{NS}	0.3241^{NS}	Deformed pancreas v/s Atrophid	5.7612**	5.0201**
Congestion v/s Deformed pancreas	0.2342^{NS}	0.012^{NS}	Hyperplastic changes v/s Atrophid	1.5435^{NS}	0.8768^{NS}
Congestion v/s Hyperplastic changes	17.1231**	17.0982**			

NS : - Non significant, P>0.05 ** :-Highly Significant, P<0.01

4.8. Histopathology of chronic poultry diseases:

The occurrence of major histopathological changes in chronic diseases birds in comparison to overall percentage of those changes in diseased birds is presented in table 24 and figure 21.

The major microscopic pancreatic abnormalities in chronic diseases birds birds in descending order of frequency were interstitial fibrosis (64.71%), Congestion (47.06 %), multifocal necrosis of exocrine pancreas (47.06%), individualization of acinar cells (35.29%), vacuolar degeneration of acinar cells (35.29%), periductular fibrosis (23.53%), increased zymogen granules (17.65%) and capsular thickening (17.65%). It is evident from table 22 that the incidence of all major histopathological abnormalities were higher in cachectic birds as compared to their overall percentage, with interstitial fibrosis being most frequent histopathological alteration (64.71%). However, variation in most of the histopathological lesions were non- significant in nature (table 25) except variation between periductular fibrosis and interstitial fibrosis.

Table-16: Comparison histopathological pancreatic pathology of chronic poultry diseases verses overall gross pancreatic Pathology.

Histopathological Conditions	**Interstitial fibrosis**	**Congestion**	**Individualization**	**Diffuse pancreatic fat necrosis**	**Periductular fibrosis**	**Thickened capsule**
No of chronic diseases poultry Examined	18.00					
No of Condition Specified	12.00	9.00	8.00	7.00	5.00	3.00
%of Pancreatic Histopathology of chronic diseases Poultry	66.66	50.00	44.44	38.88	27.77	16.66
Overall no of Birds Examined	195.00					
No of Condition Specified	106.0	89.00	90.00	27.00	58.00	52.00
% of Overall Pancreatic Histopathology	54.41	46.00	46.37	30.00	32.35	27.00
X2 Statistic	0.4501^{NS}	0.0458^{NS}	1.2141^{NS}	0.0004^{NS}	0.1337^{NS}	0.9871^{NS}

Figure - 12: Bar graph showing comparison between pancreatic histopathology of Chronic diseases birds overall pancreatic histopathology.

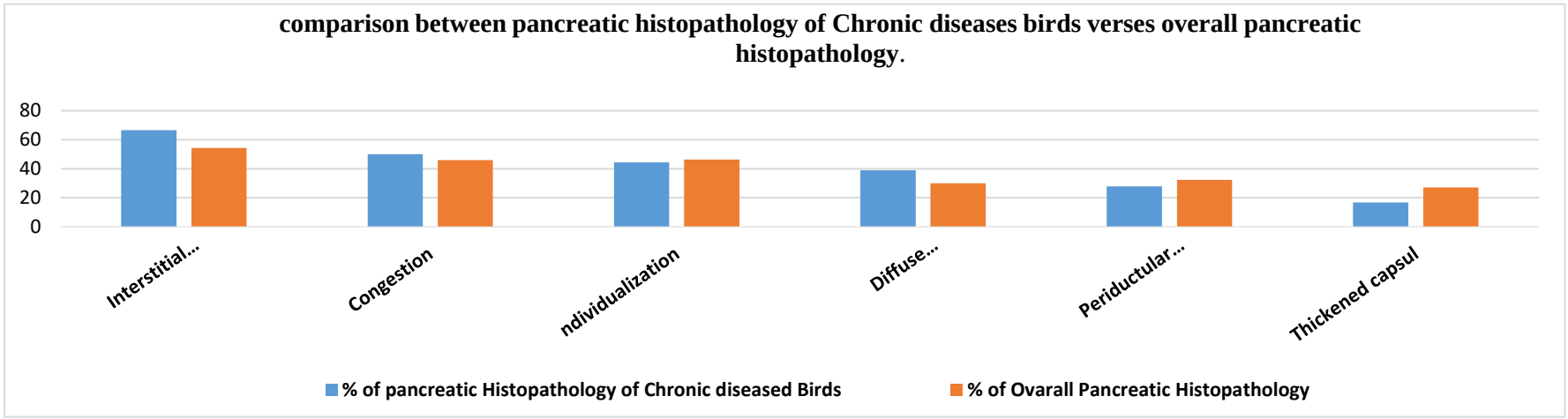


Table- 17: Chi square analysis for comparison of histopathological lesions seen in pancreas of chronic poultry diseases birds.

Comparison	**X^2 statistic**		**Comparison**	**X^2 statistic**	
	Vanaraja	**Chabro**		**Vanaraja**	**Chabro**
Zymogen granules v/s T. Capsule	0.2104^{NS}	4.2104^{NS}	Individualization v/s	0.211^{NS}	0.211^{NS}
Zymogen granules v/s Individualization	0.6162^{NS}	0.9166^{NS}	Individualization v/s Congestion.	0.141^{NS}	0.3011^{NS}
Zymogen granules v/s Multifocal necrosis	2.1401^{NS}	0.9501^{NS}	Individualization v/s Periductular Fibrosis	1.6621^{NS}	1.6242^{NS}
Zymogen granules v/s Congestion	2.1401^{NS}	2.5401^{NS}	Individualization v/s Interstitial fibrosis	0.1288^{NS}	0.4086^{NS}
Zymogen granules v/s Periductular Fibrosis	0^{NS}	0.65^{NS}	Multifocal necrosis v/s Congestion	0.2281^{NS}	0.4261^{NS}
Zymogen granules v/s Interstitial fibrosis	5.95*	3.85*	Diffuse pancreatic fat necrosis v/s Periductular Fibrosis	1.2541^{NS}	1.4001^{NS}
Zymogen granules v/s Vacuolar degeneration	0.5022^{NS}	0.5022^{NS}	Diffuse pancreatic fat necrosis v/s Interstitial fibrosis	0.424^{NS}	0.0014^{NS}

NS : - Non significant, P>0.05

** :-Highly Significant, P<0.01

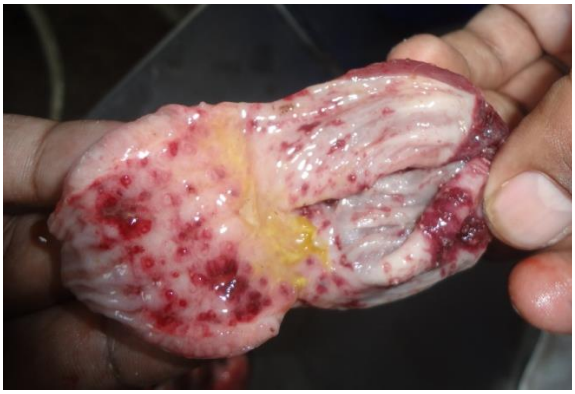


Fig:-13 : Photograph showing Pin point haemorrhage at the tip of proventricular glands in Chabro Bird (Ranikhet Diseases)

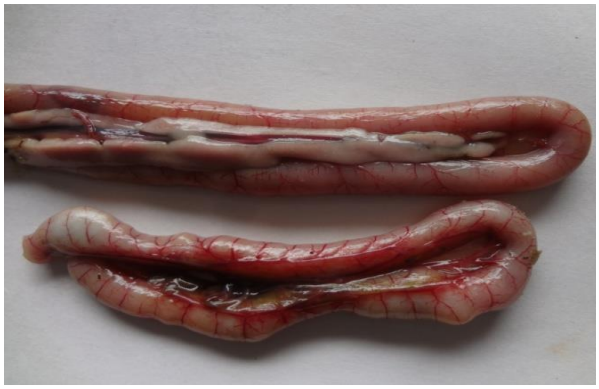


Fig: - 14: Photograph showing haemorrhagic enteritis in (Ranikhet Diseases) Vanraja Bird

Fig: -15 : Photograph showing Fowl Pox lesion Chabro Birds.

Fig: -16: Photograph showing Fowl Pox lesion in Vanraja Birds.

Fig: -17: Photograph showing enlarged liver with diffuse greyish nodules formed by abnormal growth of tissue (Marek's Disease) in Chabro birds

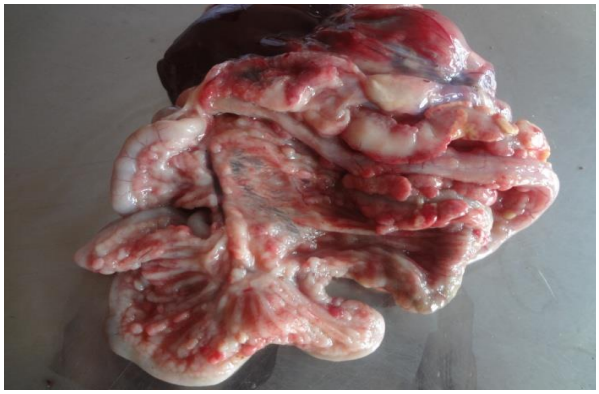


Fig:-18: Photograph showing : Multiple firm white tumorous nodules scattered throughout omentum/peritoneum (Marek's Disease) in Vanaraja birds

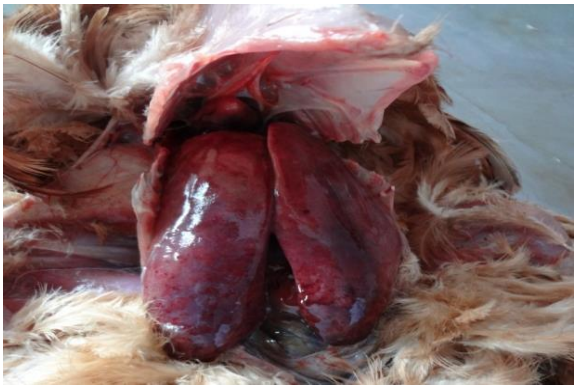


Figure- 19: Photograph showing *Avian Leucosis complex* (Big liver Disease) in Chabro.

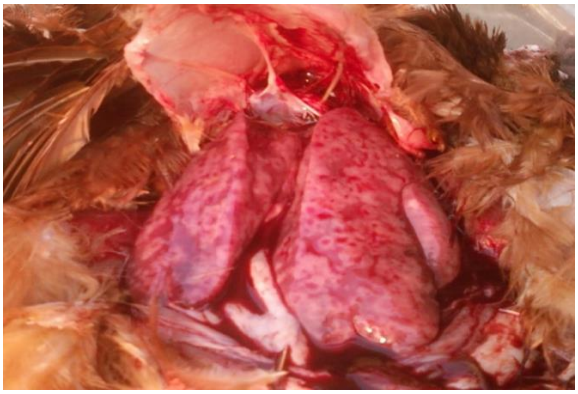


Figure- 20: Photograph showing haemorrhagic & necrotic lesions on liver in (Big Liver disease) in Vanaraja poultry bird.

Figure- 21: Photograph showing haemorrhages at thigh muscles (Gumboro Disease) in Chabro breed of poultry.

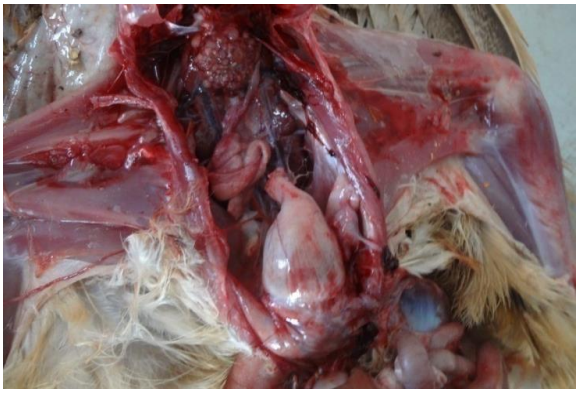


Figure- 22: Photograph showing haemorrhagic & necrotic lesion on bursa (Gumboro Disease) in Vanaraja breeds of poultry.

Figure- 23: Photograph showing shell less eggs (Infectious Bronchitis) Chabro birds.

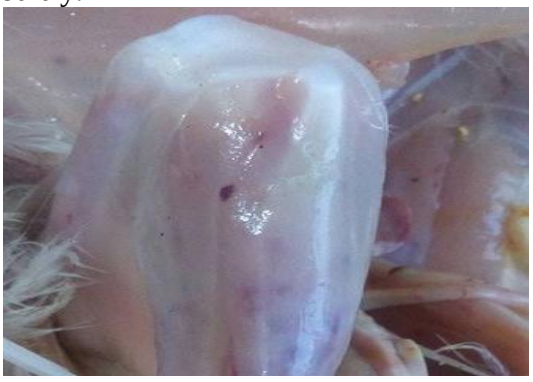


Figure -24: Photograph showing Chicken Infectious Anaemia (Vanaraja).

Figure-25: Photograph showing irregularly shaped and pedunculated ovary in Pulloram Disease in Vanaraja.

Figure -26: Photograph showing greenish brown or bronze swollen liver (Fowl Typhoid) in Chabro poultry bird.

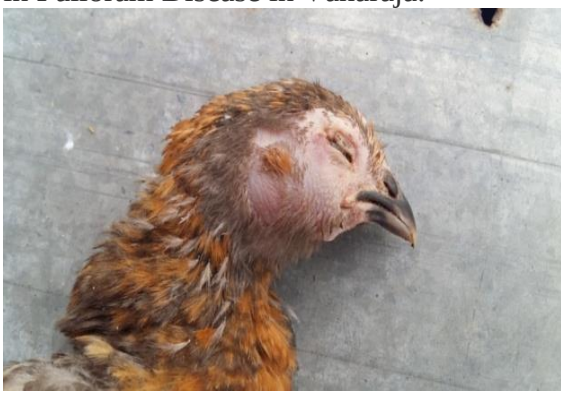


Figure -27: Photograph showing swollen face (Infectious Coryza) in Vanaraja bird.

Fig. - 28: Photograph showing haemorrhagic oophoritis and egg bound condition in Chabro breed of poultry.

Figure- 29: Photograph showing typical lesion of Caecal Coccidiosis (engorged with clotted blood) in Vanaraja.

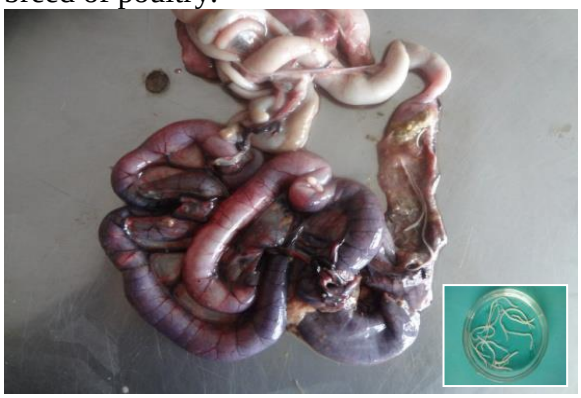


Figure -30: Photograph showing several immature round worm (*Ascaridia galli*) in intestinal lumen of Chabro bird.

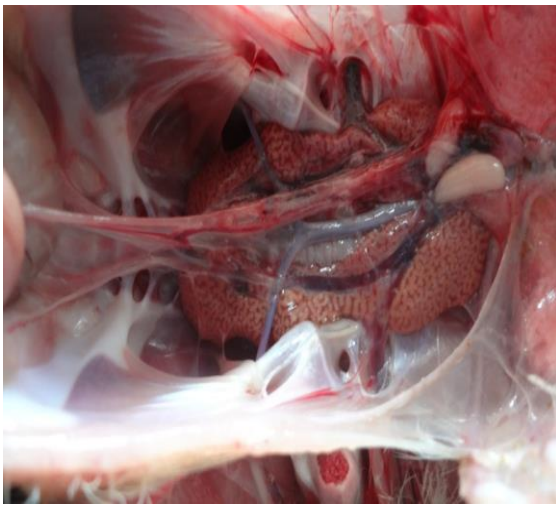


Figure -31: Photograph showing Aavian Nephrosis in Vanaraja.

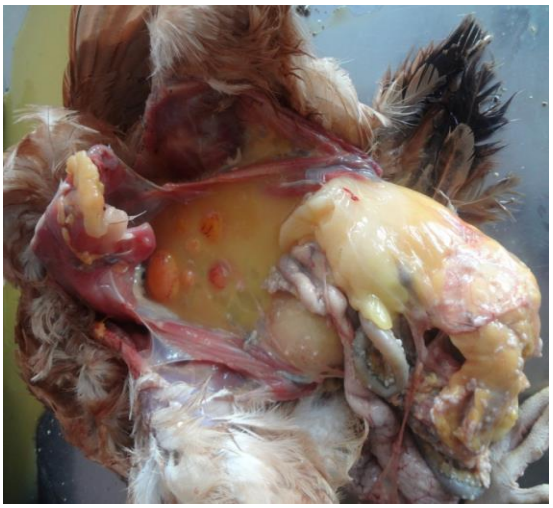


Figure -32: Photograph showing Ascitis in poultry in Chebro breed of poultry.

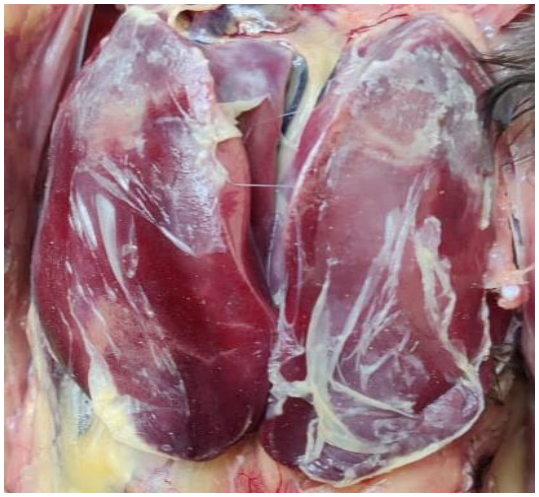


Figure-33: Photograph showing fibrinous hepatitis in Vanaraja breed of poultry.

Figure- 34: Photograph of kinked/deviated keel bone showing wasting of pectoral muscle in cachectic Chabro birds.

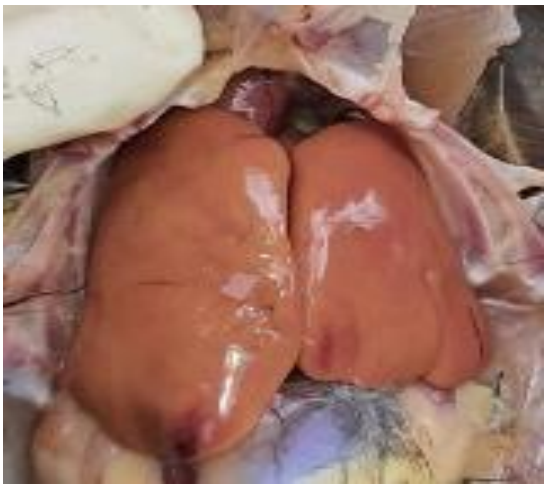


Figure -35: Photograph showing enlarged and pale liver in Vanaraja (Toxicosis).

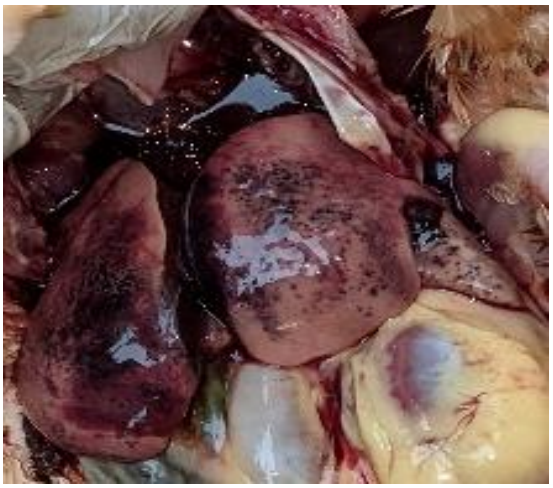


Figure -36: Photograph showing *Fatty liver* haemorrhagic syndrome in Chabro.

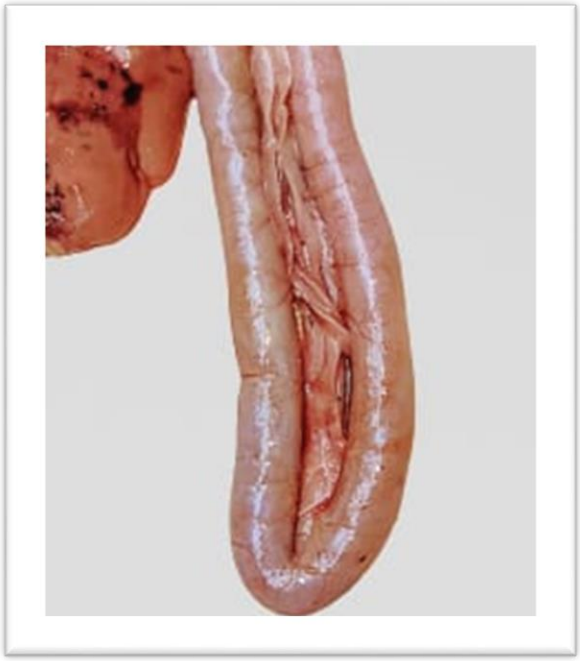


Figure-37: Photograph showing marked shortening of pancreas due to atrophy, in Vanraja poultry bird.

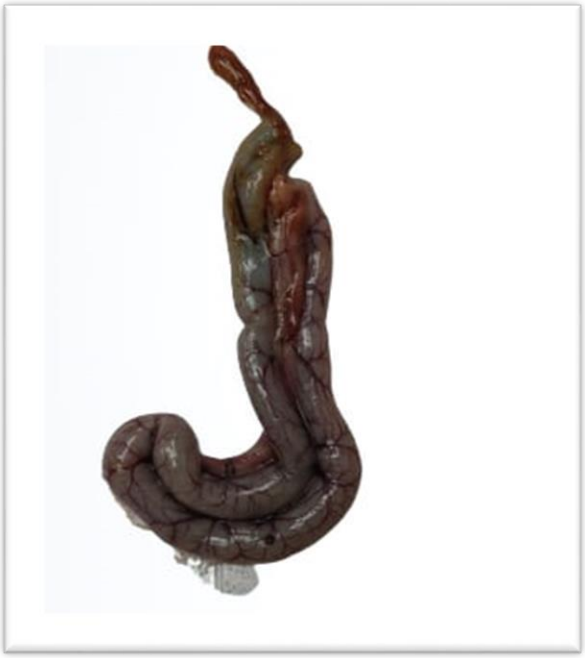


Figure-38: Photograph showing congested and acutely folded duodenum pancreatic loop in Chabro poultry bird.

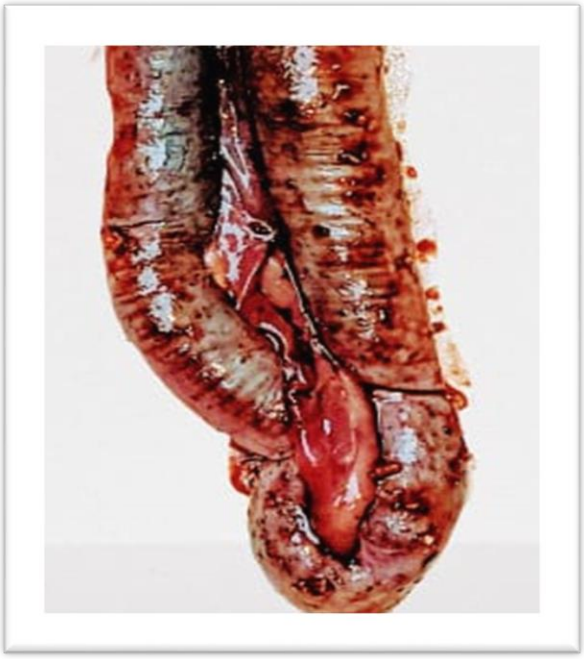


Figure - 39: Photograph showing bleached appearance of pancreas with mild end segmental deviation. (Vanaraja).

Figure-40: Photograph showing marked shortening of pancreas due to atrophy in Chabro poultry bird.

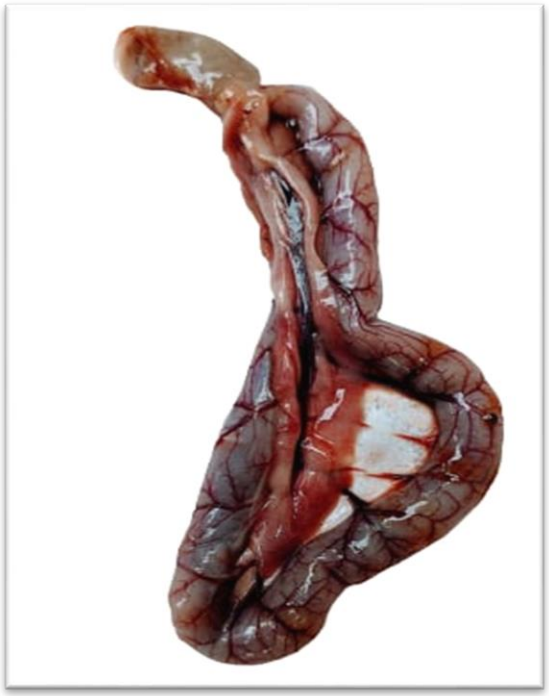


Figure-41: Photograph showing mid segmental curving of duodeno pancreatic complex with congested, in Chabro bird.

Fig. -42: Photograph showing severely congested pancreas of Vanaraja bird.

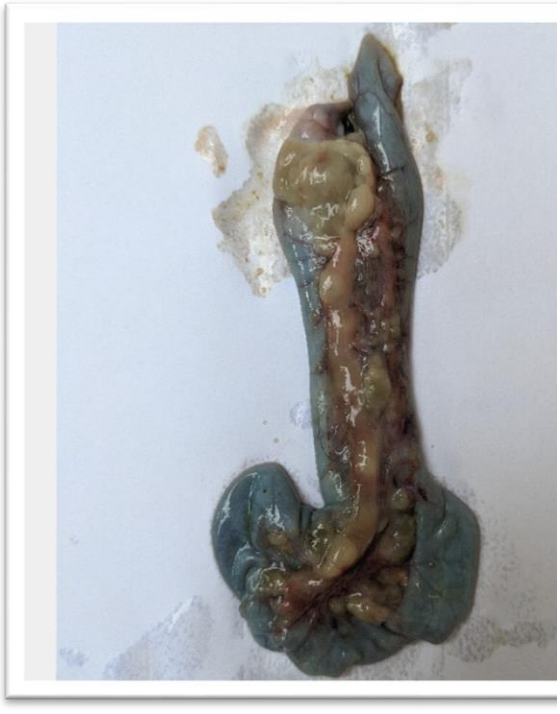


Figure - 43: Photograph showing hyperplasia of pancreas in Chabro poultry bird.

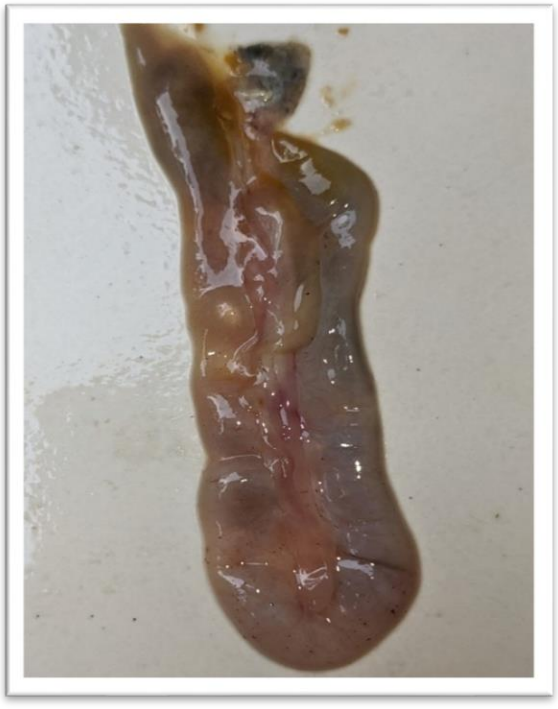


Figure -44: Photograph showing shrinked pancreas of Vanraja birds.

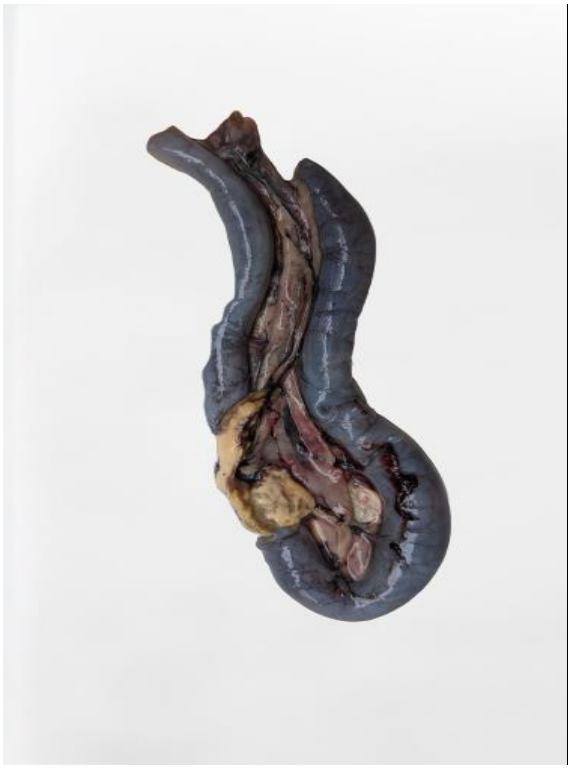


Figure-45: Photograph showing of duodenopancreatic deformity. (Chabro bird.)

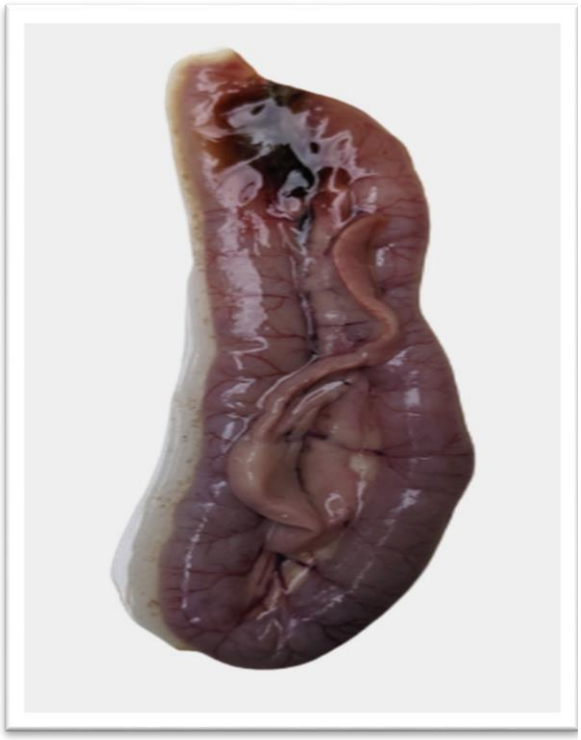


Figure- 46: Photograph showing folding and mottling of duodenopacreatic complex. (Vanaraja)

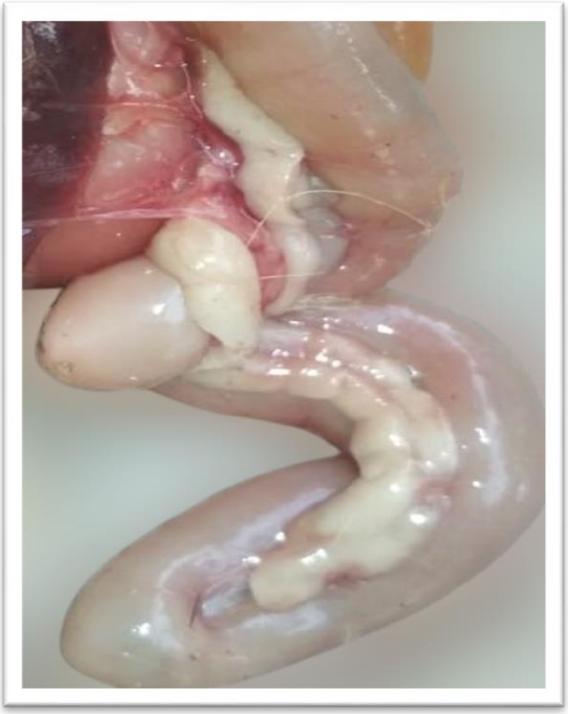


Figure - 47: Photograph showing curving of duodenum with bleached appearance of pancreas. (Chabro).

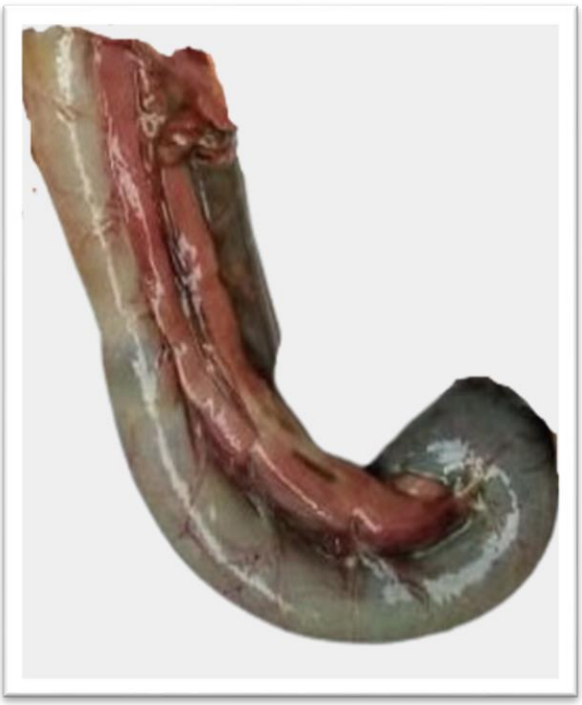


Figure- 48: Photograph showing mottling and coiling of pancreaticoduodenal loop (Vanaraja).

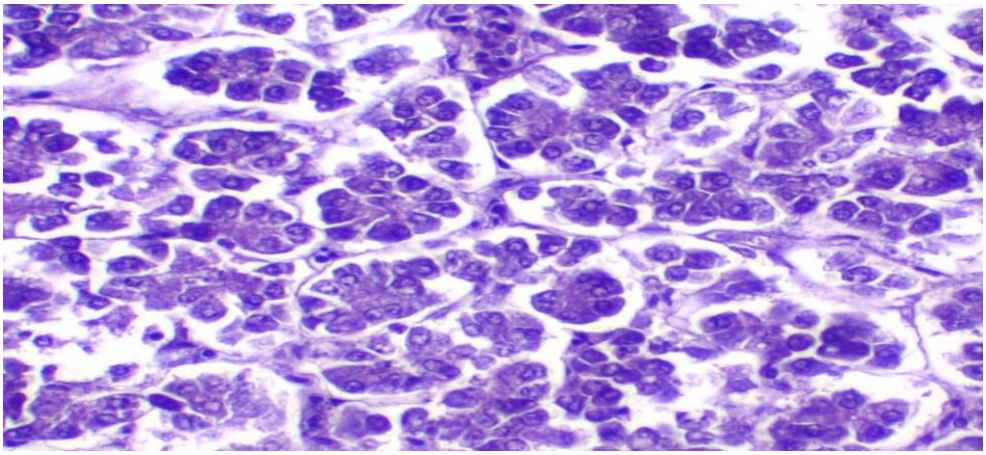


Fig. 49: Photomicrograph showing normal acinar pattern of exocrine pancreatic gland of Vanraja poultry bird (H&E Stain; 400X).

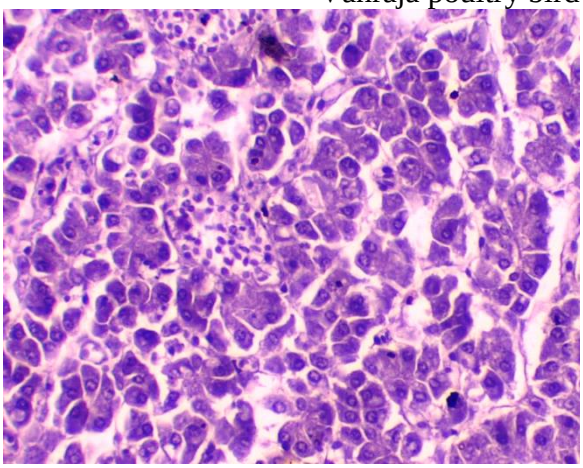


Fig. 50: Photomicrograph showing acinar cells over distended with zymogen granules with nucleus situated in the basal zone and Islets of langerhans (H&E Stain; 400X).

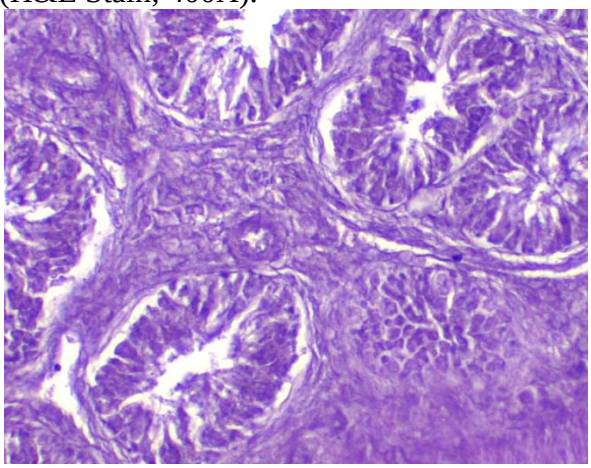


Fig. 51: Photomicrograph showing Inter-lobular fibrosis in exocrine glands of pancreas in Chabro Poultry Birds (H&E Stain; 100X).

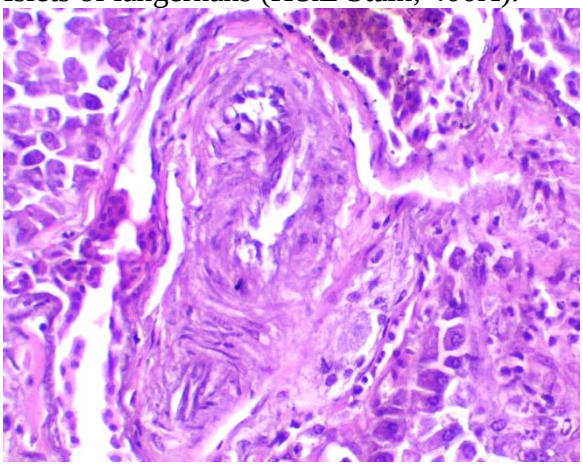


Fig. 52: Photomicrograph showing atrophy of exocrine pancreatic glands due to Inter-lobular fibrosis in Chabro Poultry Birds (H&E Stain; 400X).

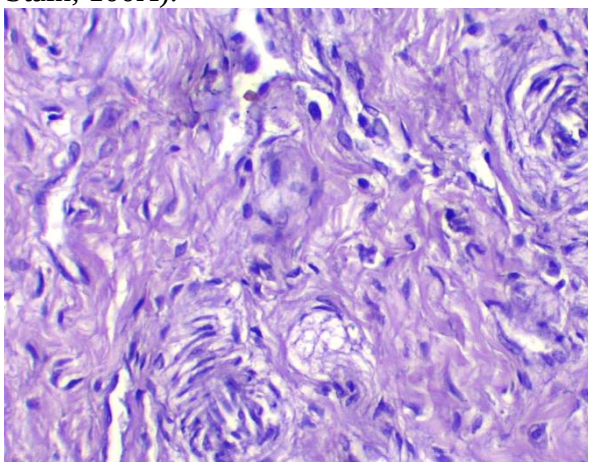


Fig. 53: Photomicrograph showing massive fibrosis and proliferation of collagen fibre tissue surrounding the degenerating acinar cells (H&E Stain; 400X).

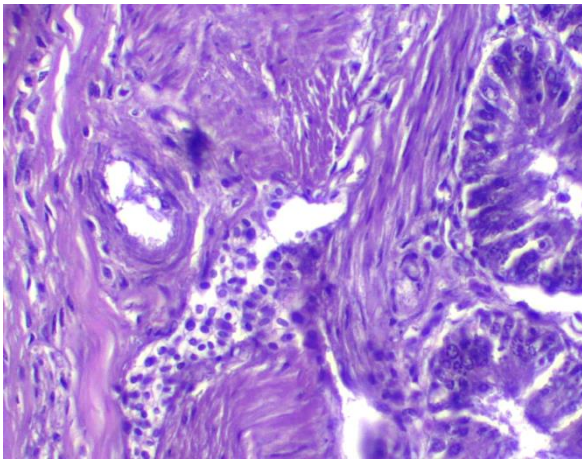


Fig. - 54: Photomicrograph showing massive sub capsular necrosis of acinar cells and infiltration of inflammatory cells surrounded by thick band of collagen fibre (H&E Stain; 100X).

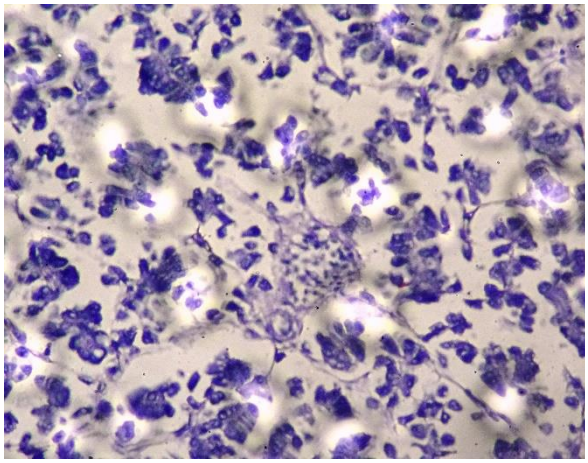


Fig. - 55: Photomicrograph showing individualization of acinar cells of pancreatic glands (H&E Stain; 400X).

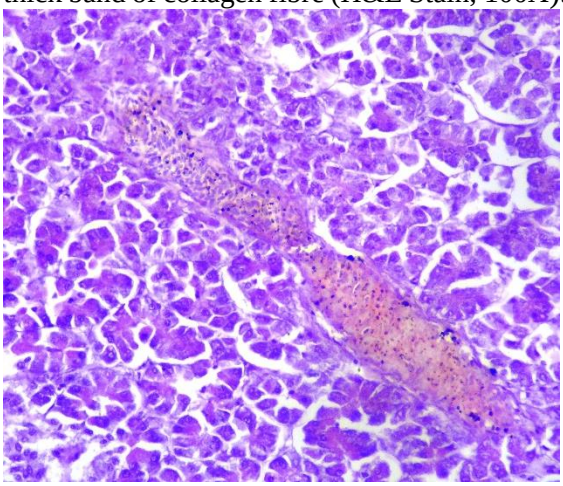


Fig. 56 Photomicrograph showing congested blood vessels and dissociated acinar cells in pancreatic glands of Vanraja birds (H&E Stain; 400X).

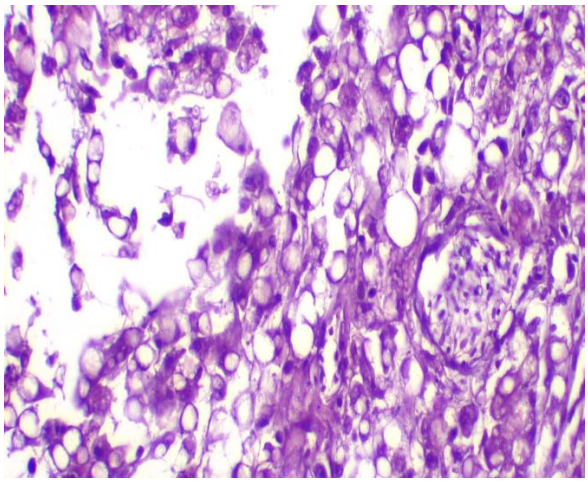


Fig. 57 Photomicrograph showing diffuse pancreatic fat necrosis and infiltration of inflammatory cells evident for acute pancreatitis in Chabro birds.

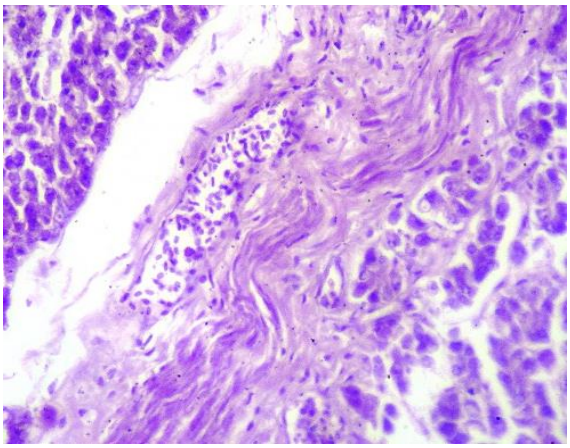


Fig.- 58: Photomicrograph showing marked interstitial fibrosis (H&E Stain; 400X).

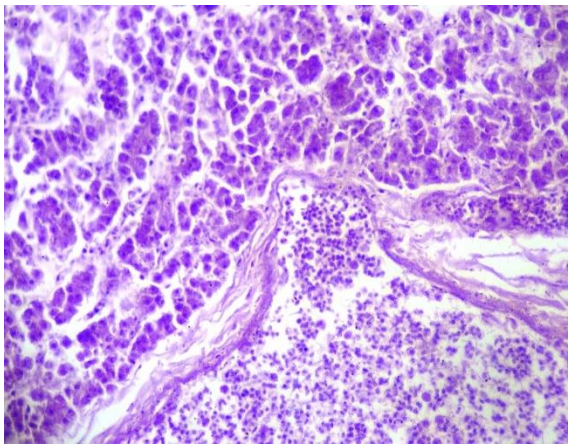


Fig.- 59: photomicrograph showing alpha and beta cells of Islets of langerhans(H&E Stain; 1000X).

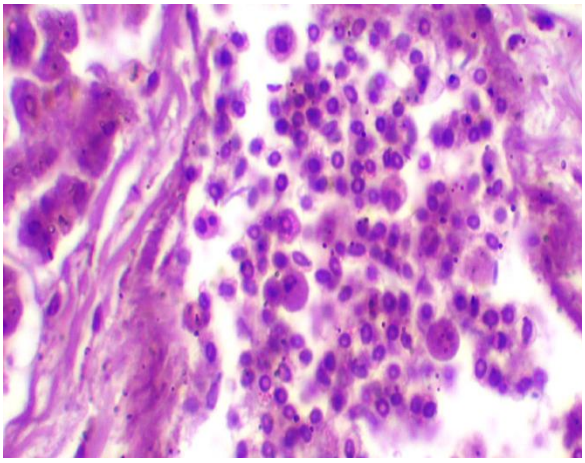


Fig. - 60: photomicrograph showing alpha and beta cells of Islets of Langerhans (H&E Stain; 1000X).

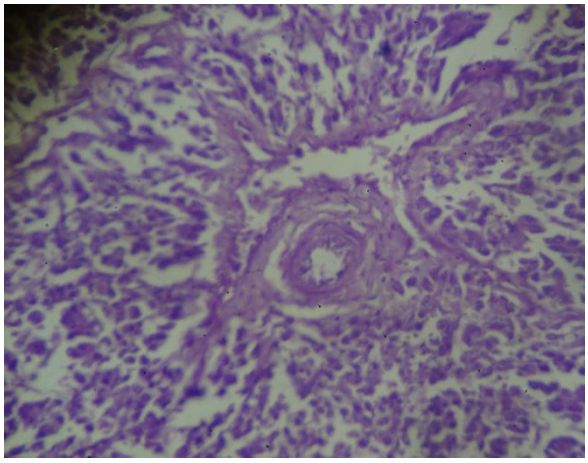


Fig. - 61: Photomicrograph showing marked periductular fibrosis in pancreatic gland of Vanraja chicks (H&E Stain; 100X).

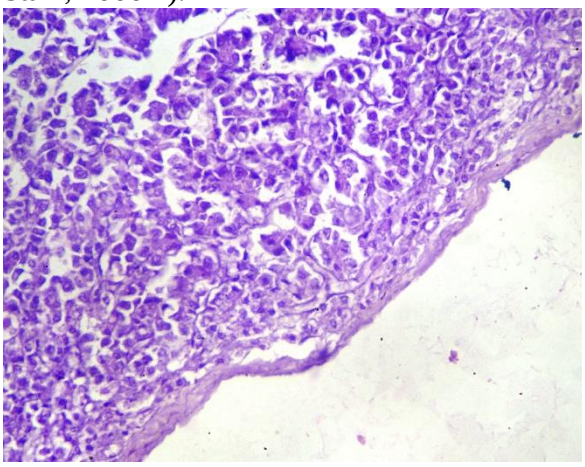


Fig. - 62: photomicrograph showing normal pancreatic capsule of pancreas in Chabro poultry bird. (H&E Stain; 100X).

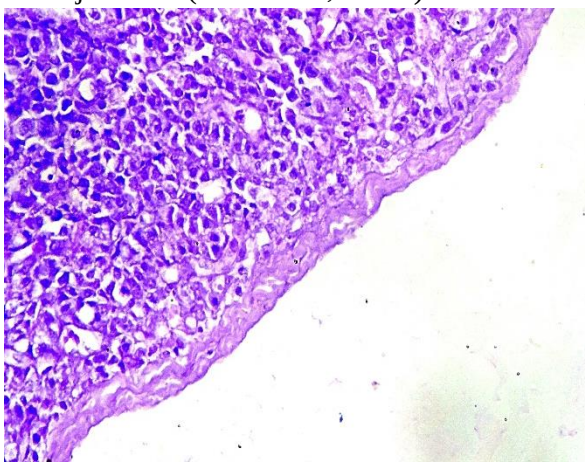


Fig. - 63: photomicrograph showing fibrous thickening of pancreatic capsule without infiltration of inflammatory cells in Chabro birds. (H&E Stain; 1000X).

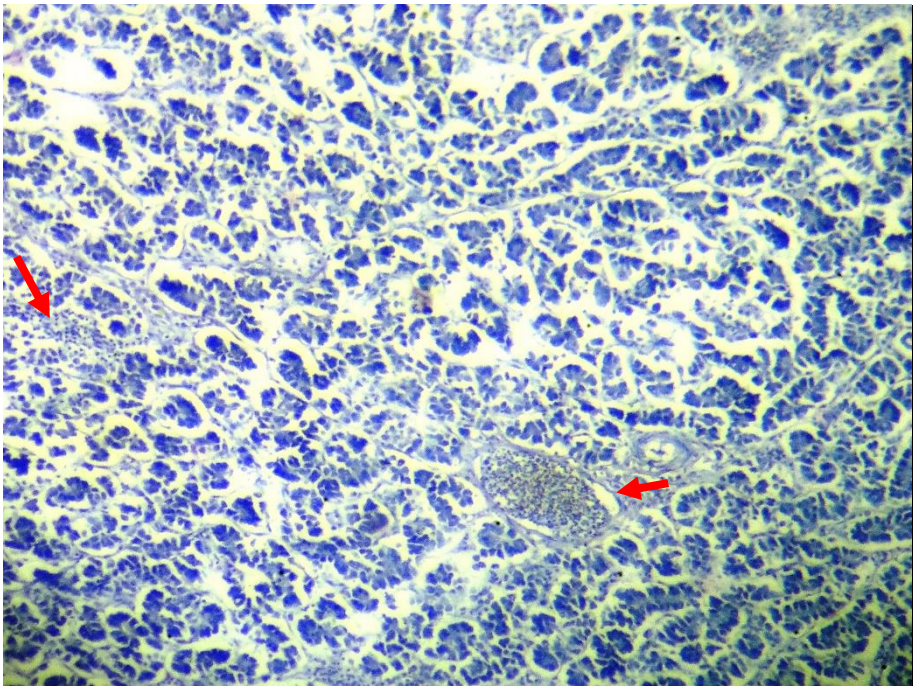


Fig.- 64: Photomicrograph showing Islets Langerhans (Red arrow) – Gomori's stain -100X

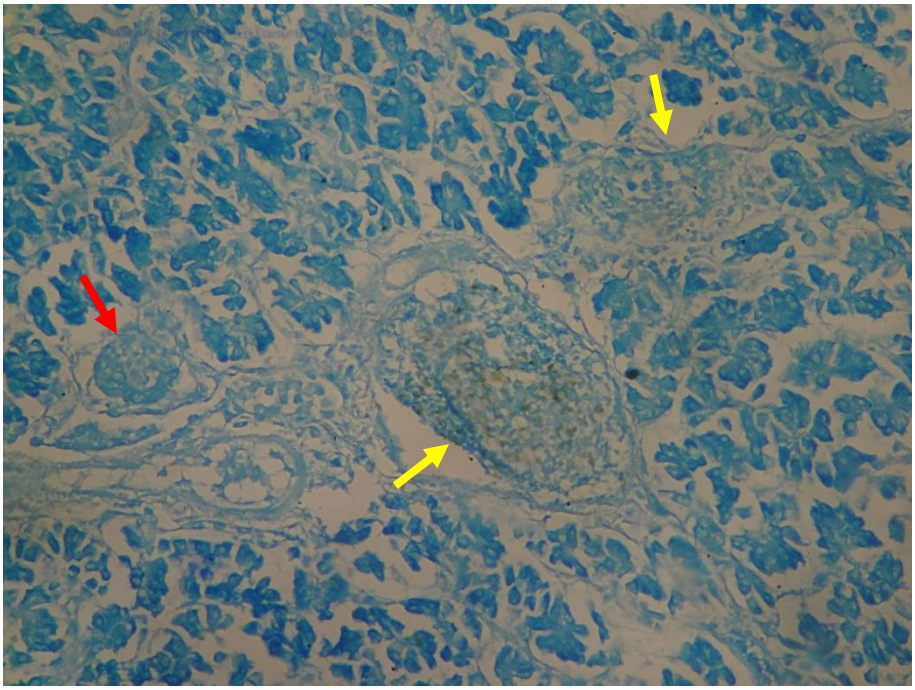


Fig.- 65: Photomicrograph showing Islets Langerhans (Yellow arrow) surrounded by a connective tissue capsule and marked congested blood vessels(Red arrow) - Gomori's stain -400X

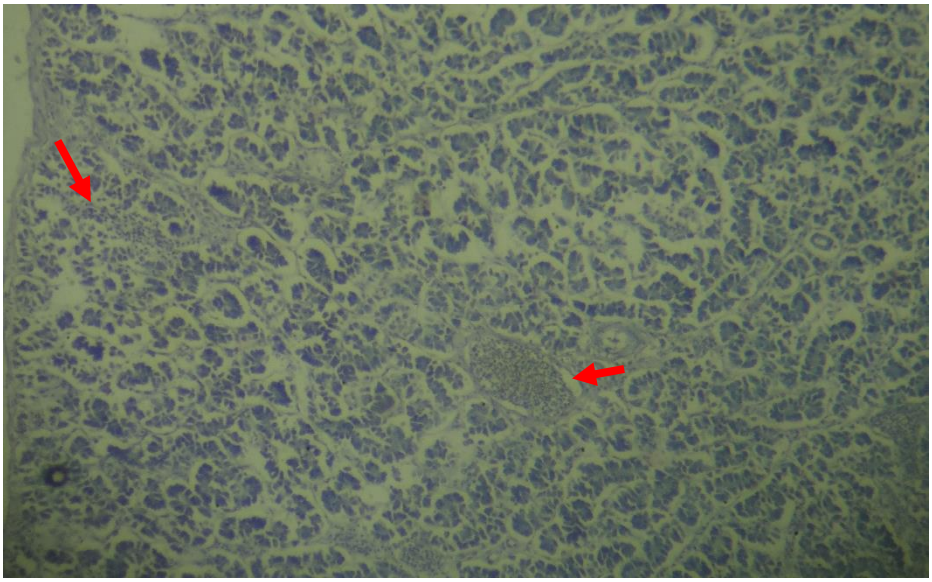


Fig.- 66: Photomicrograph showing Islets of Langerhans in pancraes (Heidenhain`s Iron Haematoxylin stain -100X)

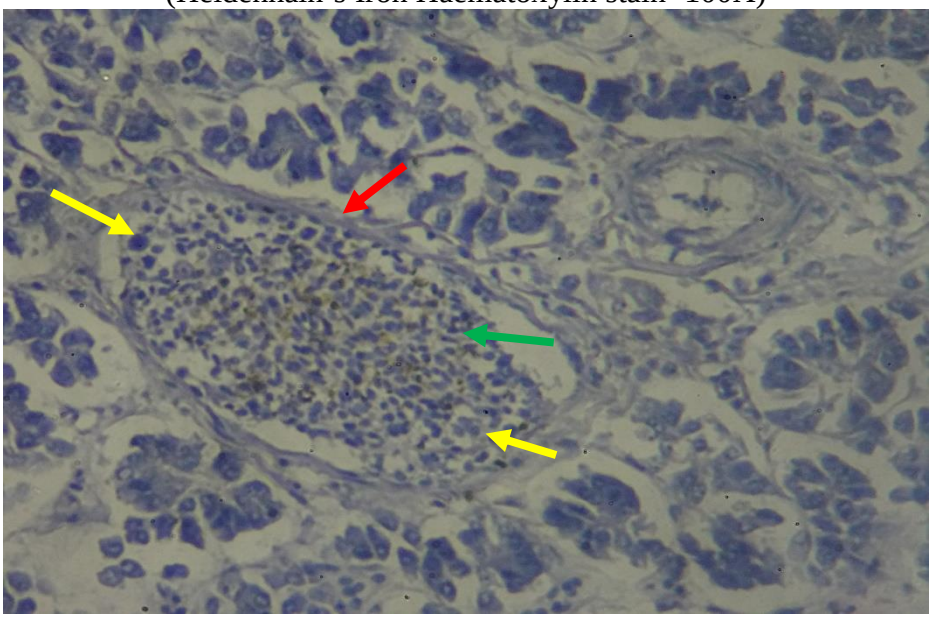


Fig.- 67: Photomicrograph of Heidenhain`s Iron Haematoxylin stained tissue section showing pancreatic Islets(Red arrow) distinguished from the surrounding exocrine tissue by a continuous connective tissue capsule. Glucagone secreting alpha cells (Yellow arrow)and insuline secreting beta cells(Blue arrow) -400X

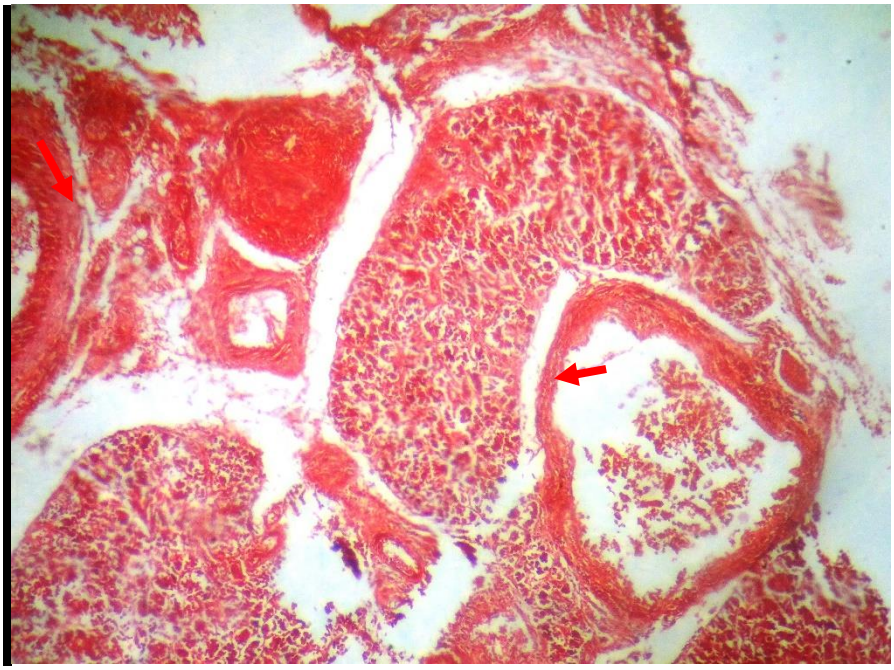


Fig.- 68: Photomicrograph showing large duct with pancreatic secretions periductular fibrosis (Mallory trichome stain -40X)

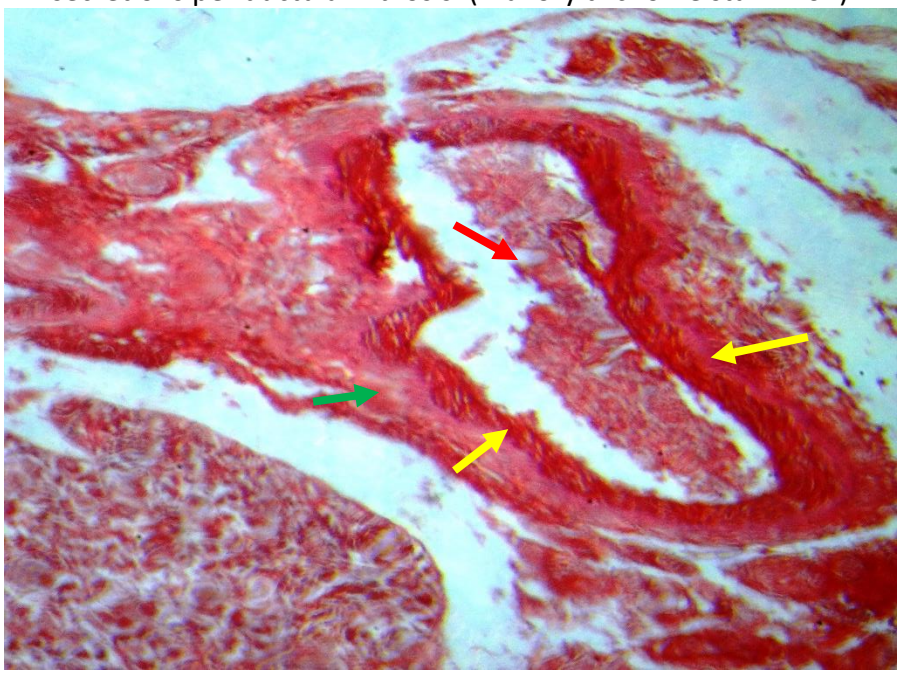


Fig.- 69: Photomicrograph showing large duct(Yellow arrow) with pancreatic secretions(Red arrow) and marked periductular fibrosis (Green arrow) - Mallory trichome stain 100X

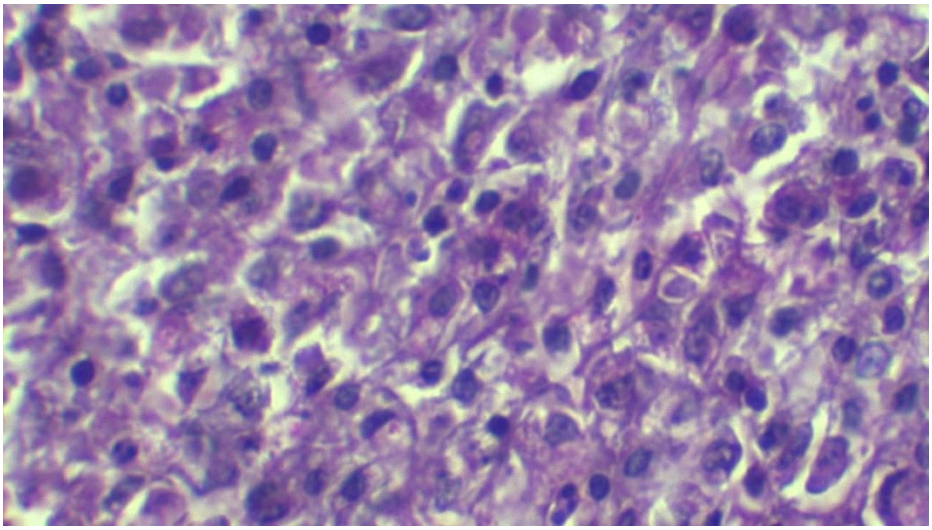


Fig.- 70: Photomicrograph showing Acinar cells of exocrine glands of pancraes. (PAS stain -400X)

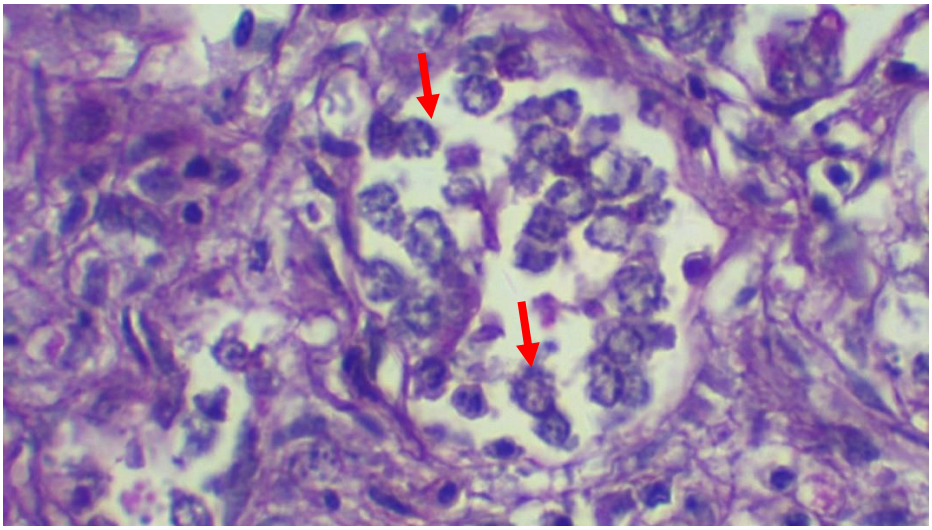


Fig.- 71: Photomicrograph showing Acinar cells distainded with Zymogene granules. (PAS stain -400X)

5 DISCUSSION

Pancreas is unique in its functions, both as an exocrine and endocrine organ as well. While the exocrine pancreas secretes digestive enzymes into the duodenum, the endocrine pancreas is composed of the islets of Langerhans, which contain specialized hormone-secreting cells. These cells include α cells, β-cells, δ-cells, and γ-cells, which secrete glucagon, insulin, somatostatin, and pancreatic polypeptide, respectively. The endocrine pancreas plays a critical role in regulating glucose homeostasis by secreting insulin and glucagon in response to high and low blood glucose concentrations, respectively. Birds displays the highest fasting blood glucose concentration among all vertebrates, with concentrations that are more than twice those in mammals (Braun and Sweazea, 2008; Scanes and Braun, 2012).

Since the viscera of poultry is not compartmentalized it is easy for the infection to spread in the entire organ system of birds in conditions like mushy chick disease, chick edema disease, egg peritonitis, visceral gout, air sacculitis etc. to name a few. Curving of duodenal loop (J-like appearance) and pancreatic atrophy, has been reported during Postmortem examinations of broiler chicken infected with parvovirus (Nunez *et al.*, 2016) and zinc toxicosis in ostrich as well. It was also found that major histological lesions were acute pancreatic necrosis and their atrophy due to different strain of influenza viruses in duck (Brojer *et al.*, 2009), swan (*Cygnus cygnus*) (Teifke *et al.*, 2007) and chicks (Shinya *et al.*, 1995). Avirulent Newcastle Disease Virus was also shown to replicate in the pancreas of chickens causing acute pancreatitis. Nakamura *et al.*, *(*2002) reported pancreatic multifocal necrosis in pancreatic acinar cells in adenovirus-associated hydro pericardium syndrome (Leechi Heart Diseases) of broiler chicken. Pancreatic Adenocarcinoma has been evidenced in guinea fowl and chicken by (Okoye *et al.*, 1993) and Abdul-Aziz (1995) respectively

In general, acute pancreatitis is an inflammatory disease of the pancreas. The etiology and pathogenesis of acute pancreatitis have been intensively investigated for centuries worldwide. Many causes of acute pancreatitis have been discovered, but the pathogenetic theories are controversial. On the other hand, chronic pancreatitis is a chronic condition characterized by pancreatic inflammation that causes fibrosis and the destruction of exocrine and endocrine tissues. Chronic pancreatitis is a progressive disease, and no physiological treatment is available to reverse its course.

Despite of the vital role being played by pancreas in maintaining health of the birds, very little attention is being given to pancreatic pathology at the time of post mortem examination, unless the changes in diseased pancreas are remarkable and noteworthy to catch the attention of attending poultry pathologists. This is further substantiated by the fact that primarily diseases reported on pancreatic pathology deals with secondary involvement of pancreas in all kinds of infectious and non-infectious conditions.

Another important fact which acts against the detection of pancreatic pathology in poultry as compared to mammals is complete absence of performing pancreatic function test in live conditions by histochemical estimation of pancreatic enzymes such as amylase and lipase to assess the functional status of pancreas in diseased birds. Moreover, poultry are not subjected to any kind of physical examination nor is it possible to keep track of the nature of excreta being passed by birds, making diagnosis of pancreatic dysfunction in live birds almost impossible. This brings to the lobe important need for analysing if there is any involvement of pancreas in a plethora of diseases which birds suffer from while kept in farm conditions or when the poultry farm faces outbreak of a particular disease irrespective of aetiology. It also necessitated the need to analyse what are the main gross and histopathological alterations exhibited by diseased pancreas and whether there is any correlation between particular disease and pancreatic pathology being exhibited.

With these facts it is envisaged to study the correlation of pancreatic pathology with different spontaneous disease conditions of variable etiology, age of locally available beeds of Vanaraja bad Chabro poultry birds. Effort was also being made to study the extent of pancreatic pathology in chronic poultry diseases.

5.1 Overall pancreatic pathology

The present study was conducted on Vanraja and Chabro poultry birds (120 each) irrespective of their age, sex and breed revealed distinct pancreatic pathology in 21.00% Vanaraja and 17.00% Chabro of total birds examined which shows that ona an average more than one bird out of five diseased birds suffered from definite pancreatic pathology. Scanty literature is available on the role and significance of pancreatic pathology in diseases of poultry birds. However, Qamar *et al.,* (2013) has described

histopathological alterations in 16.65% birds suffering from runting stunting syndrome in which birds exhibited poor and stunted body growth.

In the present study, twenty one different types of disease caused by various infectious agents were recognized on the basis of gross and microscopic examination which had been grouped under broad heading of viral, bacterial, fungal, parasitic, protozoal, metabolic and toxicological / mycotoxicosis group. The pancreatic pathology was observed in most of the disease conditions though there was distinct variation in the incidence of pancreatic lesions under different disease conditions. However, Nunez *et al.,* 2016, Carreira *et al.,* 2011 and Nakamura *et al., (*2002) reported incidences of panctreatic pathology in various bacterial and viral diseases only.

It was observed that the highest incidence of pancreatic pathology was found in fungal (66.70%) and metabolic (66.66%) diseases which mainly included brooder pneumonia, gout and chick oedema disease. However, in various disease conditions under different groups clearly revealed that it was the viral diseases like IBD, IB and bacterial disease like pasteurellosis in which the incidence of pancreatic involvement was also consistent i.c. 75.00%, 50.00% and 66.70% respectively. Such high incidence of pancreatic pathology in many of the disease conditions highlights the importance of pancreatic abnormality as one of the major contributory factors in disease development and outcome as well as in the poor production performance of diseased birds.

A number of reports are there which has observed significant pancreatic involvement and pathological alteration in different viral diseases such as avian adenovirus,paramyxovirus type 1, herpes virus, Newcastle disease virus, avian influenza virus, avian parvo virus, avian encephalomyelitis virus, paramyxo-3 virus, polyoma virus, turkey viral hepatitis, west nile virus, psittacid herpes virus (Capua 1994, Chaslton and Bickford 1995, Goodwin *et al*., 1996, Barton *et al*., 1992, Simpson, 1993, Hooper *et al.*, 1995, Nunez et al., 2006, Mundhenk *et al*., 2009,Teifke et al., 2007, Phalen *et al.,* 2007, Legler *et al*., 2008, Nakamura *et al*., 2002, Ritchey *et al*., 1997, Meulemans *et al*., 1998, Shinya *et al*., 2007, Okoye and Ilochi 1993, Nakamura 2008, Caricchioli 2015,)

Reports of pancreatic pathology in viral diseases in the present study which includes Infectious bursal diseases, Infectious Bronchitis, cystoadenocarcinoma, lymphoid leucosis, Fowl pox, and chicken infectious anaemia and Ranikhet diseases. In the present study, it was found that RD showed least (2.7%) involvement of pancreatic

pathology which is contrary to the observation of (Nakamura 2008 and Caraccioli 2015) who have reported distinct susceptibility of pancreatic parenchyma to ND virus. Meulemans *et al.,* 1998 found that non-virulent Newcastle disease virus (strain APMV-1 96/89 VB) replicates in the pancreas of chickens and the consistent histological lesions were observed in terms of acute pancreatitis.

In both the varieties of birds, namely Vanraja and chabro, it was observed that incidence of pancreatic pathology was higher (21.00%) in vanraja birds as compared with chabro (17.00%).Chabro poultry birds were found to be moderately reluctant to pancreatic alteration as compared to Vanraja. The studies had been done by the earlier workers…….renu *et al.,* 2020, Jain 2009 and Vaish (2005) on afore mentioned varieties of birds but there is paucity in the literature on their incidence rate.

5.2 Effect of age on pancreatic pathology:

The study revealed that the younger birds (0-2weeks) have poorly developed pancreas, on one hand, the adult or older birds on the other hand have significant replacement of exocrine and endocrine pancreas by fibrous connective tissue as a senile changes. Meulemans *et al.,* 1998 and Mujumdar *et al.,* 1997 reported that the pancreatic function in younger and older age are suboptimal.)

In the present investigation, the highest incidence of pancreatic pathology was observed in 2-4 weeks of age in both the verieties of birds namely Vanraja and Chabro in the range of 27% to 30%. This shows higher susceptibility to diseases and pancreatic pathology in 2-4 weeks of age groups of birds. However it is important to keep in consideration the fact that the nature of disease varies amongst the three age groups due to age susceptibility of certain diseases and other important contributory factors such as management, biosecurity, vaccination practices etc. Meagre information on age susceptibility to various diseases and related pancreatic pathology was mentioned previous reports though disease incidences and pancreatic pathology in chicks and grower has been reported by (Meulemans *et al.,* 1998, Nakamura *et al*., 2008).

5.3 Gross pancreatic pathology:

The pancreas in poultry are collection of three elongated lobes viz dorsal, ventral and splenic, each of which empties the content of digestive enzymes through separate

duct in the terminal part of ascending duodenal loop alongside bile duct and hepatic duct. Any deviation in the colour, consistency, texture or size of pancreas is indicative of adaptive or pathological process. In the present study, the major gross pathological changes comprises bleached & mottled appearance, pancreatic congestion, deformities, atrophy and hyperplasia. Somewhat similar description of gross pancreatic pathology has also been reported by Rantzer *et al.,* 1997; Majumdar *et al*., 1997 and Klar *et al.,* 1990.

In the present study, the highest incidence among the gross pancreatic lesions, were recorded for bleached (33.33% in Chabro and 23.07% in Vanraja) & congested appearance (almost 19 % in both the breeds of poultry). Similar findings of bleached appearance of diseased pancreas as a major gross pathological lesion has also been reported by Ruff, 1982 and Qamar *et al*, 2013.

Mottling of pancreas due to small pinpoint nodular or non- nodular appearance of multifocal necrosis was found to be a consistent feature in diseased pancreas. Mottling of pancreases was easily detectable at the time of necropsy in Vanraja (15.23%) and Chabro (09.52%) breeds of poultry. It was also reported by Wallner-Pendleton *et al.* (1993) in a spontaneous case of diabetes mellitus in red-tailed hawk (Buteo jamaicensis) and it was due to markedly vacuolated islet cells which were histochemically proven to be beta cells of endocrine glands of pancreas.

Another feature of pancreatic pathology which is its deformity, was frequently found in different age groups of Vanraja (11.50%) and Chabro (19.04%) breeds of poultry. Pancreatic deformities ranged from simple bending of terminal pancreas along with duodenal loop to complex twisting of duodenal pancreatic complex often assuming the features of torsion. Further, these pancreatic deformity in the majority of cases affects the terminal part of duodenal loop. The deformity were found to be more frequent in Chabro breeds as compared with Vanraja breeds of birds. Pancreatic deformities may be attributed to stronger connective tissue stroma and more proliferation of fibrous connective tissue with aging of the birds. Nunez *et al.,* 2016 also reported curving of duodenal loop (J-like appearance), pancreatic atrophy, and mesenteritis during Postmortem examinations of broiler chicken infected with parvovirus.

It is a well-established fact that avian exocrine pancreas has poor intra-acinar and intralobular connective tissue framework (Aziz and Fletcher, 2016). Thus, any persistent injury to pancreas may bring about tissue necrosis and fibrous connective

tissue proliferation which is often quite extensive as evidenced in histopathological study and histochemical demonstration of massive interstitial fibrosis with mallory's trichrome stain. The resisting nature of interstitial and interlobular pathological fibrosis and proliferating nature of developing and regenerating exocrine pancreatic tissue may lead to distortion in the shape of pancreas which bring about concomitant spontaneous twisting of duodenal loop matching each and every curve being developed in diseased pancreas. This was proven by the fact that when duodenum was detected from pancreas at the time of post mortem, the pancreatic tissue showed deformity in conformity with the twisted appearance of duodenal pancreatic complex.

Pancreatic deformity has the potential to bring obstructive changes in pancreatic duct and blood supply. Any obstruction in the ductular passage may interfere with secretory function of exocrine pancreas. Degenerative changes and proliferative changes have been observed in ductular epithelium of birds examined along with periductal fibrosis on histopathological examination. Congestion has also been a consistent change in pancreatic parenchyma in the present study. All these points are clearly indicative of abnormal pancreatic function, mainly exocrine segment in the affected birds thereby directly or indirectly responsible for poor body weight gain due to improper digestive and absorptive processes.

It has also been observed that pancreas on several occasion exhibited a combination of gross pancreatic pathology such as simultaneous development of bleached appearance, pancreatic deformity, congestion, mottling with multifocal necrotic lesions in various combinations. The magnitude of pancreatic deformity exhibited by birds suffering from different disease conditions had been the high point of present study. Except for report of parvovirus initiated terminal bending of pancreas and duodenum published by Nunez *et al*., 2006.

In the present study the highest incidence of pancreatic deformities were observed in chick edema disease (56% in Vanraja & 54% in chabro birds) and colisepticemia (51.3% in Vanraja & 42% in chabro birds). Both of these conditions mainly affect growing birds and are associated with increased intra-abdominal pressure due to accumulation of fluid or exudate. Such pathological conditions are slow developing and provide ample opportunity for growth of fibrous connective tissue and passive hyperaemia. Fibrosis of pancreatic parenchyma may interfere with secretory functions of pancreas. Corroborating all these facts, along with similar findings of Renu *et al* (

2020) Teifke *et al.*, 2007 Mundhenk *et al*., 2009 and Nunez *et al*., (2016), it is obvious that pancreas suffer major pathological change and plays an important role in the deranged physiology of the digestive system.

Bleached appearance of pancreas was most prominently seen in coccidiosis (83% in Vanaraja & 32% in chabro birds), ascaridiasis(66% in both breeds of birds), fowl pox (almost 66% in both breeds of birds), IBD (55.50% in Vanaraja & 46.00% in Chabro birds), and nephrosis(53% in Vanaraja & 45% in Chabro birds),. Anaemia is the consistent findings among the aforesaid diseases which may constitute an important underlying factor in giving bleached appearance to pancreas. Ruff (1982) had also reported bleached appearance of pancreas as major finding (47%) in stunted broilers. Such lesions may be attributed to patchy disposition with autolysed area giving bleached looks which tend to resist eosin and gives slate grey colour under the influence of hematoxylin while histopathological evaluation (Charles *et al*., 2007 and Qamar *et al.*, 2013

Congestion of pancreas in Vanaraja and Chabro breeds of poultry was a consistent pathological features of various viral, bacterial, parasitological, metabolic and toxicological poultry diseases in the present study. Acute pancreatitis which is an inflammatory disease of the pancreas could be a probable reason behind the congestion of pancreas (Wang *et al* 2009). The aetiology and pathogenesis of acute pancreatitis have been intensively investigated. The most common cause of acute pancreatitis is obstruction of the distal common bile-pancreatic duct. Acute pancreatitis occurs when intracellular protective mechanisms to prevent trypsinogen activation or reduce trypsin activity are overwhelmed. However, little is known about the avian acute pancreatitis. We hypothesize that acute biliary pancreatitis and other causes of acute pancreatitis possess a common pathogenesis. Pancreatic hyperstimulation and pancreatic duct obstruction increase pancreatic duct pressure, active trypsin reflux, and subsequent unregulated activation of trypsin within pancreatic acinar cells. It is related to passive hyperaemia associated with pulmonary disorder such as pneumonia or septicaemic conditions such as infectious bronchitis, pasteurellosis, colisepticemia, volk sac infection and oophoritis. Congestive changes have the potential to bring about significant histopathological alteration due to hypoxic injury such as cellular swelling and necrosis of parenchymal cells. Similar to our findings congestive changes in pancreatitis has also been reported by Chuston and Bickford, 1995

Atrophic changes in the pancreatic parenchyma were not a consistent finding and considered as a minor pathological change characterized by shrunken white pancreas giving lesser gross visibility of the organ during necropsy. It was most evident in cases of adult Vanaraja (33.33%) and Chabro(23.00%) breeds of poultry suffering from fowl pox A condition in which the ovary showed replacement by multiple cystic neoplastic lesions of variable size. It is often developed into conjunction with lymphoid leukosis (big liver disease), a pathological expression of type C RNA retrovirus. In such cases the whole intestinal mass underwent adhesive changes due to massive proliferation of fibrous connective tissue which invaded the pancreatic mass and destroyed both exocrine and endocrine pancreas. In such cases the pancreas appeared as a small island of white broken atrophied structures buried in the intestinal knot formation and only partially visible from the surface. Nunez *et al* (2016**)** also found the pancreatic atrophy and curving of the duodenal loop (J-like appearance) in the chickens screened for avian nephritis virus (ANV), chicken astrovirus (CAstV), avian rotavirus (ArtV), avian reovirus (AReoV), infectious bronchitis virus (IBV), fowl adenovirus group I (FAdV-1), and chicken parvovirus (ChPV).

Hyperplasia of pancreas was seen in 15.38% in Vanraja & 09.50% in Chabro breeds of birds. It was mostly seen in birds suffering from Lymphoid Leucosis (5-6%) Chicken Infectious Anaemia (3-4%) and mycotoxicosis (9-10%). A segment of pancreas either at the base or middle part or tip of the pancreas showed increase in volume which often was associated with curving of the duodenal loop at the site of hyperplasia. Exact cause of such hyperplastic changes could not be ascertained. It may be considered a developmental anomaly or increased local availability of growth factors.

Pang et al (1986) also observed pancreatic interstitial edema, hyperplasia of ductular epithelium and other pancreatic lesions induced by sublethal doses of T-2 toxin in swine. Rantzer *et al*., (1997) also found similar lesions in the growing pigs and explained that the acinar cell hypertrophy and hyperplasia leading to organ enlargement occur in response to diet rich in protein and energy, when these substrates are withdrawn, reversal of organ and cells to its original size may be observed, in which autophagy and apoptotic changes play important role. On the other hand, pancreatic atrophy and atrophy of acinar cell is characteristically seen when the bird is subjected to dietary deficiency of protein and energy.

Thus, it is evident from the finding of gross pathological changes in pancreas under different disease conditions that the changes recorded are non-specific in nature, rather these are manifestations of secondary pancreatic involvement. None of the gross lesions could be taken as a change of diagnostic significance; however, their presence is a clear indication of pancreatic dysfunction.

5.4 Pancreatic Histopathology:

Histopathological alterations exhibited by diseased pancreas was critically observed during the study and correlation between particular disease and pancreatic pathology had been done extensively. The occurrence of the various microscopic lesions of pancreases, interstitial fibrosis (54.41%), individualisation (46.37%), congestion (46.00%), periductular fibrosis (32.35%), pancreatic fat necrosis (30.00%) and capsular thickening (27.00%) were in descending order. The principal histopathological finding reported in the present study was interstitial fibrosis in the cases of pancreatic deformities.

Marked interlobular and periductal fibrosis was also observed. The fibrous connective tissue consists of collagen tissue being synthesized by fibroblasts. Mallory's trichrome stain was especially used in the present study to demonstrate the presence of various amount of collagen fibre in interstitial and periductular region. Mahmood *et al* (2020) also found it as a perfect staining reaction to visualize collagen fibers in pathological samples in sheep diagnosed with chronic fasciolosis which is similar to that obtained by traditional Masson's trichrome stain. Reshma V *et al* (2016) also used Mallory's stain for distinguishing subepithelial hyalinization in the oral submucosal fibrosis. Sheep diagnosed with chronic fasciolosis

During acute inflammatory process, fibrin and serum proteins form a loose gel-like framework for migration of fibroblast. Various growth factors like FGF-I, FGF-2, EGF and TGF-B 1, 2, and 3 released during inflammation, results in fibroblast

proliferation and migration. Factors such as FGF, PDGF, IL-13 and TGF-ß induce fibroblasts to produce collagen (Ackermann, 2007).

Pancreatic stellate cells have also been recognized as an important participant in pancreatic fibrogenesis (Bechem *et al*. 1998. Aple *et al*. 1999. Harber er af 1999 and Ellenrieder *et a*l., 2004). These pancreatic stellate cells have similarity with hepatic cells in morphology and physiology. In case of pancreatic injury the stellate cells undergo myofibroblastic transformation with capability to synthesize fibrillar collagen. The myofibroblastic transformation is stimulated by PDFG and TGF-B (Shek *et al*, 2002). This increased collagen synthesis decreased collagen forms the ground work for overt interstitial fibrosis. However this mechanism in avian pancreatic fibrosis needs further exploration and confirmation.

Thus, interstitial fibrosis may play important role in development of pancreatic deformity since collagenous tissue being firm and strong may resist expansive growth of pancreatic parenchyma particularly in growing birds grower with resultant curving, bending or twisting of duodeno-pancreatic complex, grossly being seen as pancreatic deformity. The pancreatic deformity was found to be most prominent and remarkable in growers.

Congestion was another consistent change in most of the disease conditions in vanraja and Chabro poultry birds, though there was variation in the degree of hyperaemia. Persistent congestion can bring about hypoxic damage to the parenchymal cells being drained by the veins of the affected pancreatic parenchyma. Hypoxia often leads to cellular swelling, vacuolar degeneration and necrosis. These changes were in corroboration with the findings of Jain (2009) and Renu *et al* (2020). Agonal congestion could be the other cause for pancreatic passive hyperaemia since pancreas examined in the present study were almost all from dead birds due to various disease conditions. Increased intraabdominal pressure in conditions like chick edema disease, oophoritis, egg peritonitis, enlisepticemia or mushy chick disease brings about passive hyperaemia due to compression of inferior vena cava. Different lesions in lungs, liver or kidney in conditions like pneumonia hepatitis and nephrosis /nephritis respectively can also bring about obstruction in blood supply and subsequently passive hyperaemia.

Significant number of diseased pancreas exhibited individualization of acinar cells. This condition is characterized by disruption of tubule-acinar pattern and the acinar cells appears separated from each other. Individualization of acinar cells has not

commonly being described in avian pancreatic pathology. However, Smith and Embling (1993) found individualization or loss of cell polarity has been described in severe chronic nutritional atrophy of acinar cells in exocrine pancreas of pig suffering from clinical copper deficiency.

Atrophy of the pancreas has been registered in animals subjected to starvation, prolonged anorexia, protein caloric deficiency, and malabsorption and mal-digestive syndrome. Atrophy of acinar cells may be due to protein energy mal nutrition. Deficiency of essential amino acids, vitamin-A, trace elements such as zinc, copper and selenium may also cause atrophic changes in pancreas (Gabrielson, *et al*., 1996; Graham, et al., 1988; Kazacos and Van vleet, 1989).

The cytoplasm of acinar cells and cytoplasmic granules exhibited mild to moderate and intense reaction for PAS (Periodic Acid Schiff), respectively in pancreatic parenchyma of Vanraja and Chabro poultry birds. Jain (2009) also reported that PAS activity was moderate in chicks of CARI Shyama, mild to moderate in chicks of Vanaraja, moderate in growers of CARI Shyama, intense in growers of Vanaraja and mild to moderate in pullets of both breeds which was in corroboration with our findings. Hamodi *et al.*, (2013) and Renu *et a*l (2020) stated that the pancreatic acinar cells were moderately positive for PAS in Common gull and Guinea fowl. Renu et al (2020) also stated that the pancreatic acinar cells were moderately positive for PAS in chabero chicken. However, according to Vaish (2005) in Kadaknath fowl negative reaction for PAS was observed in all the structures of pancreas.

PAS stain has been usually used to detect glycogen, which is normally present in Pancreas and other muscles. In the present study, PAS stain had been used to demonstrate zymogen granules which is acidophilic in nature and P.A.S. positive. Increased concentration of zymogen granules in the acinar cell was observed in small percentage of diseased Vanraja and Chabro birds, however this change was quite high in birds suffering from brooder pneumonia. Any deficiency or toxicity, especially zinc toxicity damage the basement membrane of acini and adjacent interstitium leading to acinar cell dissociation. It is preceded by progressive depletion of zymogen granules, cell shrinkage, cytoplasmic basophilia, and cytoplasmic vacuolations. Fell, *et al*., (1985) reported development of residual bodies within the acinar cells or are extruded in the interstitial space. The cells finally loses their polarity and get dissociated /

individualized with loss of glandular arrangement as a consequence with loss of glandular arrangement as a consequence.

These Individualization or loss of polarity of acinar cells represent the ultimate expression of acinar pathology which had undergone basement membrane damage, acinar cell atrophy, apoptosis and vacuolar degeneration prior to development of loss of polarity. It also suggest that birds would have been suffered from nutritional deficiencies, It is further substantiated by the fact that birds in present study were mostly in poor health. It is quite possible that nutritionally deficient birds might have suffered from various disease conditions thus aggravating the loss of polarity or individualization of acinar cells.In present study, the multifocal exocrine pancreatic fat necrosis has been registered in all age group of Vanaraja and Chabro birds, however their incidence was significantly higher in viral diseases such as IB, fowl pox, leucosis, Chicken infectious RD and IBD which suggests pancreas is habitually invaded by pathogenic viruses as reported by Charles (2007). Consistent to our finding multifocal necrosis of exocrine pancreas due to viral diseases such as canine distemper virus, unine parvovirus, felid herpes virus, FMD virus, classical swine fever virus, paramyxo virus type I avian influenza virus etc have also been previously reported by Iovanna, (1996)., Van Pelt and Crauded, (1987)., Capua, (1994)., Barton, *et al.*, (1992)., Hooper et al., (1995).The clinical out-come will be depending upon the extent of necrotic and degerative changes suffered by pancreas of the ailing birds, Diffuse pancreatic necrosis is definitely going to reduce the functional status of pancreas, resulting into production loss of the flock.

Thickened pancreatic capsule in the present study has been observed as a associated lesions with active inflammatory conditions in pancreas. Thickening of the capsule and associated fibrous changes may predisposes the organ to adhesive changes with the organ of the digestive system. This had been distinctly observed in cases of lymphoid leukosis or cystadeno carcinoma, where the pancreas becomes completely embedded in thickened capsule. Encapsulation brings about degenerative and necrotic changes in pancreatic acini, especially those which were completely surrounded by fibrous connective tissue. These changes normally are suggestive of obstruction in the network of pancreatic ducture, resulting in reduced excretion of pancreatic enzyme despite being continually produced by acinar cells. These end up in increased zymogen granules concentration in acinar cells (Pound *et al*., 1981).

Thus, it is obvious that the histopathological changes observed in current study were mostly non-specific in nature and doesn't carry any significant diagnostic value for a particular disease condition and there is no direct correlation between gross pathological change and histopathological changes. Often there is overlapping of both gross and histopathological lesions.

The exocrine enzyme-secreting cells of the pancreas supply digestive juice to facilitate digestion in the small intestine (Pilny, 2008; Schmidt and Reavill, 2014). The vacuolar degeneration and loss of zymogen granules shown in cases of LD/MAS are directly related to the diminished production of digestive enzymes, as previously reported (Nili *et al*., 2007). In the present study the pancreas of the affected birds showed the same characteristics and loss of zymogen granules, relating these findings to poor digestion and a reduction in the absorption of nutrient.

Gomori's Method of special staining was utilized for Pancreatic Islet Cells of endocrine pancreas. It was distinctly observed in pancreatic parenchyma of Vanraja and Chabro poultry birds. Increased number of islets were observed in the splenic lobe of the pancreas which is a finding consistent with the description of islets in the splenic lobe by Rideau, (1988). Also Modified Gomori's aldehyde-fuchsin staining of the pancreatic parenchyma was utilized by Pirmoradi *et al*. (2016) for evaluation of beta-cell numbers and islet volumes of normal and diabetic rats.

Heidenhain"s Iron Haematoxylin was another histochemical stain used to evaluate the pathology of Islets of Langerhans of pancreas under different poultry disease conditions of various age groups of Vanraja and Chabro birds. Zharkov, and Boĭko (1978) had studied modification of the Heidenhain's method of staining with iron hematoxylin for detection of the A-cells of the islands of Langerhans and found similar findings. In contrary Ferreira (2003) has demonstrated staining of intestinal protozoa with Heidenhain's iron hematoxylin. It has been found that only in occasional cases there was reduced cell population in both light and dark islets which is suggestive of compromised endocrine pancreatic function. However significant loss of islet was observed in pathological conditions associated with massive fibrosis of pancreatic parenchyma especially in cases of cystadenocarcinoma where both exocrine and endocrine pancreatic tissue showed necrotic changes, which must have significantly contributed towards poor performance and death of the affected birds.

Excessive vacuolar degeneration of islet cells which is a hallmark of birds suffering from diabetes mellitus was observed in none of the birds, nor were any neoplastic changes recorded affecting the islet cells. However, these pathological changes clearly suggest that pancreatic abnormality is one of the major underlying cause for propagation and complication of disease progress and production performance.

Reshma V *et al., (*2016) conducted aggrandizing oral submucous fibrosis grading using an adjunct special stain: A pilot study and found that on using a special stain, this distinction can be clearly visualized. Van-Gieson, Masson's and Mallory's stain could all distinguish subepithelial hyalinization and edema. The distinction was better delineated by Mallory's stain.

5.5 Pancreatic pathology in chronic poultry diseases of Vanaraja and Chabro poultry birds:

Poultry birds from either Vanaraja or Chabro breeds suffering from chronic diseases in the present study constituted 2.90% of 240 birds examined. These birds were characterized by almost complete wasting of sternal muscle and significant loss of thigh muscle. The visceral organs, particularly heart of such birds were markedly atrophied. The difference in gross pathological alterations observed in the pancreas of chronic poultry diseases as compared to other diseased birds did not show remarkable variation though incidence of incidence of bleached pancreas and pancreatic deformity was higher in chronic poultry diseases, while congestive changes in pancreas were found to be significantly higher in acute poultry diseases diseased birds.

However, histopathological studies revealed higher incidence of all the microscopic lesions viz. interstitial and periductal fibrosis, congestion, multifocal necrosis, individualization of acinar cells, vacuolar degeneration of acinar cells, enhanced zymogen granules in acinar cells and thickening of pancreatic capsule in chronic poultry diseases birds as compared to acute poultry diseases birds. These findings are in conjunction with the description given by Schmidt & Reavill (2014)

These changes are strongly suggestive of reduced functional parenchymal acinar cells in chronic poultry diseases birds. Cachexia was mostly observed in adult birds in which no significant disease process was observed. The organs were found to be atrophied, especially the heart. Thus. In the absence of any significant disease process, higher incidence of pancreatic abnormalities in chronic poultry diseases birds as

observed in this study becomes crucial. Histopathological alteration of high incidence of interstitial fibrosis, multifocal necrosis, individualization of acinar cells and vacuolar degeneration of acinar cells are strongly suggestive of chronic inflammatory process in Such changes are also suggestive of compromised functional status of pancreas contributing directly or indirectly in the development of chronic poultry diseases It also appear that a viscious cycle might have been created by the way of initial pancreatic damage being done by nutritional deficiencies or excesses or toxicities in the absence of any obvious infectious disease conditions followed by pancreatic dysfunction and as a fall out of necrotic and degenerative changes ensure further damage to the support system of pancreas like blood supply and nerve supply. Mal digestion and malabsorption in the face of deficient pancreatic enzymes would further hamper nutritional supply to already beleaguered pancreas.

Thus, a definite correlation has been observed in the present study between chronic poultry diseases and pancreatic involvement. However, it needs further scientific exploration of structure and function of pancreas at ultrastructural and molecular level to clearly define the role of pancreas in chronic poultry diseased conditions of birds. Bahrawyet al 2015 Kwon CI, *et al (2019)* Diagnosis and management of chronic pancreatitis

6 SUMMARY AND CONCLUSION

Pancreas is one of the most vital organs of the body in general and digestive system in particular. It is a dual function organ comprising of both exocrine and endocrine elements. In poultry, the pancreas appears as a compact elongated structure nested between the duodenal loops. Present study was taken up to evaluate the pancreatic pathology of birds suffering from different disease conditions and also to correlate the pancreatic pathology with their chronic disease body conditions. The study spanned three months, starting from March 2021 and finishing in August 2021. Pancreatic pathology was assessed by studying the various parameters after performing post-mortem examination of all the birds submitted to the department of veterinary pathology, BVC Patna. Parameters include gross pathology of the dead birds including detailed study of the pancreatic pathology. Tissue samples from affected pancreas along with from other affected organs were collected and preserved in 10% neutral buffered formalin. After processing the tissues histopathological studies were carried out by employing routine Hematoxylin and Eosin staining and confirming the lesions by histochemical examination using different stain viz. Heidenhain's Iron Haematoxylin stain, P.A.S. stain, Gomori's staining technique for pancreatic islet cells.

In our study 240 birds (120 Chabro and 120 Vanaraja) birds examined showed definite pancreatic pathology. Maximum incidence of pancreatic pathology was observed in fungal (66.66%Vanaraja) and metabolic (66.66% Vanaraja, 66.6 Chabro) diseases whereas least pancreatic pathology was registered in viral diseases (14.28% vanaraja 11.42 Chabro). The overall incidence of pancreatic pathology viral disease other than RD has been observed. Disease wise incidence of pancreatic involvement is significantly higher in cases of IBD (75% Vanaraja and 66.70 % Chabro) followed by IB (50.00%) and pasteurellosis (66.70%). Contrary to the rest of the viral diseases the pancreatic pathology was extremely low (2.70%) due to RD though mortality of birds registered as highest, followed by pneumonia of bacterial origin (14.28%).

Age group wise maximum pathology was registered in 2- 4 weeks birds (30.00%) followed by 0-2 weeks (20.00%) and adult birds (10.00%). A significant variation was also observed in susceptibility to increased pancreatic pathology under different disease conditions between all the three age groups.

In the present study, gross pancreatic pathology exhibited bleached appearance, congestion, mottled appearance with multifocal necrotic lesions, pancreatic deformity and atrophied or hyperplastic changes in pancreas. Pancreatic pathology shows the highest percentage of bleached pancreas (23.07 % Vanaraja and 33.33% Chabro) congested (19.04%Chabro and 19.24% Vanaraja) and lowest percentage of hypertrophicd pancreas (9.50% Chabrp and 15.38% Vanaraja).

The main diseases in which bleached appearance of pancreas was prominent included. Coccidiosis (83.00% Vanaraja and 32.00% Chabro ascaridiasis and fowl pox (both 66.675%) and IBD (55.51% Vanaraja and 46.00% Chabro). Congestion of pancreas was most marked in chicken infectious anemia (14.23%Vanaraja and 13.00% Chabro), pneumonia (28.54% Vanaraja and 30.00% Chabro), chick edema discuss (8.70% Vanaraja and 8.00% Chabro), Coryza (14.00%Vanaraja and 16.67% Chabro) and IB (20.00%Vanaraja and 21.43% Chabro). Mottling was significantly high in cases of gout (70.00% Vanaraja and 66.12% Chabro), lymphoid leukosis (52.70%Vanaraja and 55.90% Chabro) and ascaridiasis (50.00%Vanaraja and 51.12% Chabro). In the rest of the diseases the mottling of pancreas was moderate to low. Pancreatic deformity was significantly higher in diseases like chick oedema disease (both breed 60.87%), colibacillosis (46% &43%) and IBD (9%to 11.12%). In the rest of the diseases lower incidence of pancreatic deformity was seen.

The major histopathological changes in pancreas in decreasing order of frequency were interstitial fibrosis (54.41% Vanaraja and 46.00% Chabro), congestion (46.00%Vanaraja and 30.00 %Chabro), individualization of acinar cells (32.35%Vanaraja and 27.00% Chabro), Diffuse pancreatic fat necrosis in exocrine pancreas (30.85Vanaraja and 13%Chabro), periductular fibrosis (13.20% Chabro and 27.944% Vanaraja) and capsuler thickening (5.39% Chabro and 13.24% Vanaraja). No direct correlation was found between gross pathology and histopathology in our study.

Chronic diseases conditions birds were characterized by marked loss of muscle mass particularly in the sternum and thigh. Sternal muscle showed complete wasting with prominent exposure of sternal cartilage and keel bone. In the present study, 2.9% birds (07/240) showed cachexia. The incidence of gross change such as mottled necrotic appearance and congestion was found to be higher in birds other than that showing clinical cachexia, while changes such as bleached appearance and pancreatic deformity was found to be significantly higher in chronic diseases conditions birds.

The nature of microscopic pancreatic pathology in cachectic chronic diseases conditions birds was found to be similar as seen in overall pancreatic pathology. The incidence of all major histopathological abnormalities was higher in chronic diseases conditions birds as compared to their overall percentage with interstitial fibrosis being most frequent histopathological alteration (66.67%).

Thus we conclude that the incidence of primary pancreatic pathology is rare. Its involvement as a secondary complication is quite high and may constitute an important contributory factor in variable growth rate shown by birds in cases of disease outbreak and consequently the economic loss faced by the poultry industry.

Significant pancreatic pathology with subsequent pancreatic dysfunction is also an important contributory factor for development of cachectic conditions of birds.

For proper functioning of pancreas it is essential that all preventive measures and managemental care should be taken along with feeding of mycotoxin free feed to minimize the disease conditions and maximize economic profitability. Considering the consistent pathology in pancreas in different disease conditions which is suggestive of suboptimal functioning of pancreas, it would be beneficial to add preparations of pancreatic enzyme in the poultry ration in all cases of disease outbreak, which so far is not included as a feed supplement in poultry industry as well as to add vitamin A and vitamin E to support the regenerative process in damaged pancreas.

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APPENDIX

COMPOSITION OF THE GENERAL REAGENTS USED:

Ehrlich's hematoxylin:

Hematoxylin crystals ……	4.0 gm
Alcohol, 95% …….....	200,0 ml
Ammonium or potassium alu ……	6.0 gm
Distilled water ……...	200.0 ml
Glycerin ……	200.0 ml
Glacial acetic acid……..	20.0 ml

Hematoxylin was dissolved in the alcohol and the alum distilled water and After these are in complete solution glycerin and acetic acid were added.

Acid alcohol:

Alcohol.70% …….	1000.0 ml
Hydrochloric acid, concentrated……	10.0 ml

Ammonia water:

Tap water…….	1000.0 ml
Ammonium hydroxide, 28%.....	2-3 ml

Eosin solutions:

Eosin.....	1.0gm
Distilled water …….	20.0m1
Dissolve and add …….	
Alcohol, 95% ……..	80.0 ml

Bouin's solution

Picric acid, saturated solution…….	750.0mi
37-40% formalin……..	250,0 ml
Glacial acetic acid.....	50.0 ml

Potassium permanganate solution

Potassium permanganate…….	0.3 gm
Distilled water.....	100.0 ml
Sulfuric acid concentrated……	0.3 ml

5% sodium bisulfite solution

Sodium bisulfite 5.0 gm……..	0.3 gm
Distilled water.....	100.0 ml

Chromium hematoxylin solution

Hematoxylin, 1% aqueous solution....	50.0 ml
Chromium potassium sulfate, 3% aqueous solution ,,,,,,,,,,,,,,,,,,,,,,	50.0 ml

To 100 ml of chromium hematoxylin solution 0.1 gm of potassium iodate was added and boiled until a deep blue colour appeared. The mixture was ripened immediately and used as long as a film with a metallic luster forms on its surface in a coplin jar. Filtered before use.

1**% acid alcohol solution**

Alcohol, 70%.....	1000.0 ml
Hydrochloric acid, concentrated…..	10.0 ml

0.5% phloxine B solution

Phloxine B.....	0.5 gm
Distilled water …….	100.0 ml

5% phosphotungstic acid solution

Phosphotungstic acid…….	0.5 gm
Distilled water ……..	100.0 ml

1% congo red solution:

Congo red ……..	1.0 gm
Distilled water.....	100.0 ml

1% sodium hydroxide solution

Sodium hydroxide	1.0 gm
Distilled water	100.0 ml

Alkaline alcohol solution

Sodium hydroxide 1%	1.0ml
Alcohol 50%	100.0 ml

Mayer's hematoxylin solution

Hematoxylin crystals.	1.0gm
Distilled water...	1000.0ml
Sodium iodate:	..0.2gm
Ammonium or potassium alum.....	30.0gm
Citric acid.....	1.0gm.
Chloral hydrate.....	.50.0gm

Dissolved the alum in water, without heat; the haematoxylin was added and dissolved in this solution. Then the sodium iodate, citric acid, and the chloral hydrate was added, shaked until all components were in complete solution. The final color of the stain is reddish violet. Stain keep well for months.

Aniline blue-orange G mixture

Aniline blue.	0.5 g
Orange G.....	2.0 g
Phosphotungstic acid	1.0 g
Distilled water.....	to 100 ml

VITAE

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EDUCATIONAL QUALIFICATION

S. No	Name of Courses	Name of University	Year of Passing	Percentage Marks/OCPA
1.	B .V. Sc. & A.H.	CGKV Anjora, Durg.	2017	6.82
2.	M.V.Sc.	BASU, Patna, Bihar.	2022	7.6