CLINICO-PATHOLOGICAL STUDIES ON GASTROINTESTINAL DISORDERS ASSOCIATED WITH ADVANCED PREGNANCY IN BOVINES

Thesis

Submitted to the Guru Angad Dev Veterinary and Animal Sciences University in partial fulfillment of the requirements for the degree of

MASTER OF VETERINARY SCIENCE
in
VETERINARY PATHOLOGY
(Minor Subject: Veterinary Microbiology)

By

SWATI SHARMA
(L-2013-V-56-M)

Department of Veterinary Pathology
College of Veterinary Science

Ludhiana - 141004

2015

CERTIFICATE – I

This is to certify that the thesis entitled, "**Clinico-pathological studies on gastrointestinal disorders associated with advanced pregnancy in bovines**" submitted for the degree of **M. V. Sc.**, in the subject of **Veterinary Pathology** (Minor Subject: **Veterinary Microbiology**) of the Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, is a bonafide research work carried out by **Swati Sharma (L-2013-V-56-M)** under my supervision and that no part of this thesis has been submitted for any other degree.

The assistance and help received during the course of investigation have been fully acknowledged.

(Dr. A. P. S. Brar)
Major Advisor
Associate Professor
Department of Veterinary Pathology
College of Veterinary Sciences
Guru Angad Dev Veterinary and Animal Sciences University,
Ludhiana-141004, India.

CERTIFICATE – II

This is to certify that the thesis entitled, **"Clinico-pathological studies on gastrointestinal disorders associated with advanced pregnancy in bovines"** submitted by **Swati Sharma (L-2013-V-56-M)**, to Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, in partial fulfillment of the requirements for the degree of **M.V.Sc**., in the subject of **Veterinary Pathology** (Minor Subject: **Veterinary Microbiology**) has been approved by the Student's Advisory Committee after an oral examination on the same, in collaboration with an external examiner.

_____ _____

(Dr. A.P.S. Brar) **External Examiner**
Major Advisor

_____

(Dr. H.S. Banga)
Head of the Department

_____

(Dr. Simrat Sagar Singh)
Dean, Postgraduate Studies

ACKNOWLEDGEMENTS

*First and foremost, I thank the **Almighty** who granted me an opportunity and patience to make successful accomplishment of my research work and I express my indebtedness towards my respectable **parents** and **brother** for their utmost support, love and being together in all my good-bad times. It's beyond my vocabulary to express my veneration towards my family. Without their love, affection and encouragement, this work would not have been possible.*

*I extend my deep regards and immense indebtedness to my esteemed major advisor **Dr. A. P. S. Brar,** Associate Professor, Department of Veterinary Pathology, GADVASU, Ludhiana for his judicious technical guidance and suggestions, constant supervision, constructive enthusiasm, ever-willing and keen interest in inculcating in me the spirit of self-reliance so that I could work independently. I will always remain indebted to him for his untiring efforts in the successful completion of this investigation.*

*Words are inadequate to express my deep sense of gratitude and regards from the core of my heart to **Dr. N. K. Sood*** (Dean PGS Nominee), *Senior Veterinary Pathologist, Department of Teaching Veterinary Clinical Complex, for his patient hearing, parental attitude, sincere advice, supervision, constructive suggestions, whole-hearted encouragement and moral boosting which served as a constant source of inspiration throughout the course of my study and research work.*

*It is my privilege to express my gratitude to worthy members of my advisory committee namely, **Dr. Kuldip Gupta**, Professor, Department of Veterinary Pathology, College of Veterinary Science and **Dr. Deepti Narang**, Professor, Department of Veterinary Microbiology, for their encouraging attitude, meticulously scrutiny of this manuscript and for offering valuable suggestions.*

*I also express thanks to **Dr. H. S. Banga**, Professor and Head, Department of Veterinary Pathology, for their help.*

*Words are compendious to express my sincere and whole hearted thanks to faculty members of my department **Dr. N. D. Singh**, **Dr. C. K. Singh**, **Dr. B. S. Sandhu**, **Dr. R. S. Brar**, **Dr. Amarjit Singh, Dr. S. Deshmukh** and **Dr. Vishal Mahajan** for rendering help and moral support.*

*I am extremely thankful to the **Dean, Postgraduate Studies, Director of Research and Dean, COVS, GADVASU, Ludhiana** for providing financial support to complete the research work.*

*I am extremely thankful to **Dr. R. S. Singh**, Assistant Professor, Department of Teaching Veterinary Clinical Complex and **Dr. S. K. Uppal**, Professor, Department of Veterinary Medicine for helping me completing my research on time.*

*Special thanks to my friends **Salisha**, **Divya**, **Rashmi**, **Manjula**, **Abhinika**, **Ritika**, **Anjali** and **Gourav** for their constant support, encouragement and good times spent together.*

*I take this opportunity to express my heartfelt thanks to all seniors, especially, **Drs. Ejaz Ahmed Zargar, Omer Khalil Baba, Laltlankimi, Pankaj Goswami**, **Monika Thakur** for their unreserved help and constant acknowledgment.*

*I am also thankful to my colleagues, **Drs. Amrinder Kaur, Anamika Gupta, Payal Bhat, Sachin Dogra.***

*The help rendered by the laboratory staff (**Mr. Keval Singh, Mr. Dan Singh, Mr. Dilchain Singh** and **Mr. Harpreet Singh**) and other staff members (**Mrs. Inderjit Madam, Mr. Chintamani, Mr. Rameshwar, Mr. Ramesh, Mr. Nanua Ram, Mr. Budh Ram, Miss Chandni, Mr. Simran** and **Mr. Sanjeev Kumar**) is gratefully acknowledged.*

*I am also very thankful to the staff of Centralised Clinical Laboratory **Mr. Pritpal Singh, Mr. S. P. Singh, Mr. A. P. Bajaj, Mr. Raj Kumar, Mrs. Harpreet Kaur.***

*I am thankful to **Mr. Gurdeep Singh (ALPS)** for setting this manuscript neatly and meticulously.*

All may not have been mentioned but none is forgotten.

Place: Ludhiana

Date: _____ ***(Swati Sharma)***

Title of the Dissertation	:	Clinico-pathological studies on gastrointestinal disorders associated with advanced pregnancy in bovines
Name of the Student and Admission No.	:	Swati Sharma L-2013-V-56-M
Major Subject	:	Veterinary Pathology
Minor Subject		Veterinary Microbiology
Name and Designation of the Major Advisor	:	Dr. A. P. S. Brar Associate Professor
Degree to be awarded	:	M.V.Sc.
Year of the Award of Degree	:	2015
Total Pages of the Dissertation	:	101 + VITA
Name of the University	:	Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, India

ABSTRACT

The present study was conducted on 50 clinical cases presented in the large animal clinics GADVASU, Ludhiana, to investigate the pathology of various gastrointestinal (GIT) disorders affecting bovines in advanced pregnancy. Disorders were divided into different categories on the basis of clinical examination, radiography, ultrasonography, hematology, clinical chemistry and peritoneal fluid analysis. The various gastrointestinal disorders recorded were peritonitis (n=16), late pregnancy indigestion (n=7), traumatic pericarditis (n=7), diaphragmatic hernia (n=6), omasal impaction (n=3), paralytic ileus (n=3) and miscellaneous conditions (n=8). Higher occurrence of GIT disorders was seen in buffaloes (66.7%) than cows (33.3%). General clinical signs in GIT disorders included anorexia, reduced water intake, fever, tympany, depression and reduced faecal output or loss of defecation. The hematology revealed anemia, neutrophilic leukocytosis and thrombocytosis and the clinical chemistry, a significant increase in aspartate amino transferase, gamma-glutamyl transferase, total bilirubin, globulin and creatine kinase. Peritoneal fluid analysis showed presence of peritonitis in 18 cases characterised by an increase in total cell count, total protein, albumin and lactate levels as well as its cytology. The study also included 21 animals that died of GIT disorders in advanced pregnancy and the distribution of disorders confirmed at postmortem was similar to those recorded in the live animals. Histopathology revealed degenerative changes in rumen, reticulum and omasum, abomasal ulcers, ischaemic necrosis of intestine, hepatic cirrhosis, nephrosis, myocardial degeneration and rarefaction of lymphoid tissues. Immunohistochemistry done on the intestine of 21 cases affected with GIT disorders elucidated the presence of *Clostridium perferingens* in 11 cases showing necrotic enteritis. A significant finding of present study was marked liver damage in nearly all the cases of GIT disorders of live and dead animals possibly perpetuated by advanced pregnancy.

Keywords: Advanced pregnancy, Bovine, Clinical chemistry, Gastrointestinal disorders, Haematology, Immunohistochemistry, Immunosuppression, Live and dead animals, Peritoneal fluid analysis

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

CONTENTS

LIST OF TABLES

LIST OF FIGURES

Fig. No.	Title
18	Chronic active peritonitis characterized by large number of macrophages and active fibroblasts
19	Peritoneum showing lot of fibrin strands and adhesions and some exudate
20	Marked omental hemorrhage
21	Adhesive peritonitis characterized by adhesions between various abdominal organs and diaphragm along with hemorrhages
22	Heart showing epicardial hemorrhages and fibrinous exudation
23	Abomasal hemorrhages and erosions
24	Massive sloughing of ruminal mucosa (H&E, original magnification x 100 X)
25	Sloughing of reticular epithelium and marked dilatation of lymphatics (H&E, original magnification x 100 X)
26	Bile regurgitation in abomasum (H&E, original magnification x 400 X)
27	Type-1 ulcer in abomasum (H&E, original magnification x 100 X)
28	Section of intestine showing ischaemic necrosis (H&E, original magnification x 100 X)
29	Marked fibroplasia and mesothelial cell hyperplasia in peritoneum (H&E, original magnification x 400 X)
30	Centrilobular necrosis and early cirrhosis (H&E, original magnification x 40 X)
31	Fibrinopurulent perihepatitis (H&E, original magnification x 100 X)
32	Proliferative glomerulonephritis, tubular degeneration and nephrosis (H&E, original magnification x 400 X)
33	Lymph node showing congestion and lymphoid cell depletion (H&E, original magnification x 40 X)
34	Fibrinopurulent perisplentitis (H&E, original magnification x 100 X)
35	Suppurative pericarditis with inset showing recovered foreign body
36	Chronic gastritis with glandular hyperplasia (H&E, original magnification x 100 X)
37	Higher magnification of previous photo showing pronounced glandular hyperplasia (H&E, original magnification x 400 X)
38	TP case showing early cirrhosis and pseudolobulation besides chronic venous congestion (H&E, original magnification x 100 X)
39	A large abscess in a mesenteric lymph node in a case of TP (H&E, original magnification x 100 X)

Fig. No.	Title
40	TP case with massive epicarditis (superficially there is presence of bacterial colonies, massive necrosis, and fibrinopurulent exudation with granulation tissue at the base) (H&E, original magnification x 100 X)
41	Another case of TP showing moderate epicarditis (H&E, original magnification x 100 X)
42	Omasal impaction
43	Omasal impaction revealing early denudation of mucosa and hyperplasia of basal epithelial cells along with chronic inflammation in submucosa (H&E, original magnification x 100 X)
44	Higher magnification of previous photo showing hyperplasia and dysplasia of the basal epithelium (H&E, original magnification x 400 X)
45	Prolonged omasal impaction with marked epithelial sloughing, chronic inflammation and neovascularization associated with it (H&E, original magnification x 400 X)
46	DH showing herniation site
47	Marked aspiration of GIT contents in the lung
48	Chronic reticulitis with thromboembolism and fibroplasia besides extensive sloughing of epithelium (H&E, original magnification x 400 X)
49	Reticulum showing thromboembolism, sloughing of epithelium and chronic active inflammation with focal microabscesses (H&E, original magnification x 100 X)
50	Higher magnification of previous showing several coalescing microabscesses (H&E, original magnification x 400 X)
51	Histopathology of the case showing aspiration grossly, revealing microscopic evidence of aspiration in the bronchiole (H&E, original magnification x 100 X)
52	Marked chronic enteritis characterized by loss of villous epithelium and infiltration of mononuclear cells in case of paralytic ileus (H&E, original magnification x 100 X)
53	Section of intestine from volvulus case with massive hemorrhage and venous congestion (H&E, original magnification x 100 X)
54	Duodenal obstruction showing total loss of mucosa, presence of large amount of plant material in the necrotic mucosa and massive infiltration of chronic inflammatory cells (H&E, original magnification x 100 X)
55	Duodenal obstruction with total sloughing of mucosa and massive necrosis of underlying crypt epithelium along with chronic inflammation (H&E, original magnification x 40 X)

LIST OF ABBREVIATIONS

%	:	Per cent
A/G	:	Albumin to Globulin ratio
ALKP	:	Alkaline Phosphatase
AST	:	Aspartate Amino Transferase
AV	:	Abomasal volvulus
BUN	:	Blood Urea Nitrogen
C. perfringens	:	Clostridium perfringens
CBC	:	Complete Blood Count
CK	:	Creatinine Kinase
DAB	:	Diaminobenzidine
DH	:	Diaphragmatic Hernia
DLC	:	Differential Leukocyte Count
EDTA	:	Ethylene Diamine Tetra Acetic acid
Fig.	:	Figure
GGT	:	Gamma Glutamyl Transferase
GIT	:	Gastrointestinal Tract
H & E	:	Hematoxylin and Eosin
Hb	:	Hemoglobin
HIER	:	Heat Induced Antigen Retrieval
LDA	:	Left displaced abomasum
LDH	:	Lactate Dehydrogenase
LPI	:	Late Pregnancy Indigestion
MCH	:	Mean Corpuscular Hemoglobin
MCHC	:	Mean Corpuscular Hemoglobin Concentration
MCV	:	Mean Corpuscular Volume
N/L	:	Neutrophil to Lymphocyte ratio
PBS	:	Phosphate Buffer Saline
PCV	:	Packed Cell Volume
RBC	:	Red Blood Cell
SAAG	:	Serum-Ascites Albumin Gradient
TEC	:	Total Erythrocyte Count
TLC	:	Total Leukocyte Count
TP	:	Traumatic Pericarditis
TRP	:	Traumatic Reticuloperitonitis
Viz.	:	Namely
WBC	:	White Blood Count

LIST OF MEASUREMENTS

dl	:	Decilitre
º F	:	Degree Fahrenheit
fl	:	Femtoliter
g	:	Gram (s)
pg	:	Picogram
L	:	Litre (s)
mg	:	Milligram (s)
ml	:	Millilitre (s)
min	:	Minute (s)
µg	:	Microgroam
fl	:	Femtolitre
IU	:	International Unit
h	:	hour (s)
mmol	:	Millimole

CHAPTER – I

INTRODUCTION

Gastrointestinal disorders are one of the major causes of morbidity and mortality in bovines. Disorders of forestomach and intestine in adult cattle can result from a variety of causes, including dietary, inflammatory, and / or mechanical (Fubini and Ducharme 2004). Advanced pregnancy predisposes bovines to a number of gastrointestinal disorders, like traumatic reticuloperitonitis (TRP), diaphragmatic hernia (DH), traumatic pericarditis (TP), omasal and abomasal impaction, paralytic ileus, intestinal obstruction and late pregnancy indigestion (LPI) due to increased intra-abdominal pressure, hormonal and complex physiological changes during pregnancy. It has been documented that in laboratory animals gastrointestinal tract (GIT) motility is affected during pregnancy primarily by hormonal changes (Baron *et al* 1993).

Peritonitis is the inflammation of the peritoneal cavity and its serosal surface, the peritoneum. It can occur as a primary disease or secondarily as a part of a specific primary disease. As a primary disease it commonly results from injury of the gut serosal surface allowing the gut contents to enter the peritoneal cavity. In ruminants, it is mainly caused by penetrating metallic foreign body through the reticulum which initially causes acute local peritonitis or may spread to cause diffuse peritonitis or remain localized to cause subsequent damage including vagal indigestion and DH and is accompanied by abdominal pain, fever, toxaemia and a reduction in the amount of faeces (Radostitis *et al* 2007). Among various abdominal disorders, TRP has the major incidence among heifers and cows (Braun *et al* 989). TRP occurs more commonly in advanced pregnancy than in non-pregnant ones, because of increased intra-abdominal pressure of the gravid uterus (Radostits *et al* 2007). The leakage of gut contents may also occur through perforating abomaso-duodenal ulceration resulting into diffuse peritonitis (Palmer and Whitlock 1984).

Traumatic pericarditis in bovine is usually associated with perforation of potential metallic foreign bodies through the reticulum and diaphragm into pericardial sac. Accumulation of exudates due to inflammation and formation of adhesions causes cardiac temponade which leads to right side heart failure, systemic congestion and toxemia (Ramakrishna 1993 and Radostits *et al* 2007). The incidence of TP is

found more in pregnant or recently parturated animals, due to increased intraabdominal pressure (Athar *et al* 2012).

Diaphragmatic hernia is one of the important thoraco-abdominal disorders inducing mortality in buffaloes (Mohindroo *et al* 2007). It results in the passage of abdominal viscera into the thoracic cavity through a congenital or acquired opening in the diaphragm. DH is ranked next to the TRP amongst the various gastrointestinal disorders of bovine (Deshpande *et al* 1983) affecting the productivity of farm animals. Increase in intra-abdominal pressure during advanced stage of pregnancy is one of the major factors that cause DH (Dhablania *et al* 1971).

Abomasal impaction is common in pregnant cattle (Radostits *et al* 2007). It also occurs in feedlot cattle fed a variety of mixed rations containing chopped or ground roughage (straw, hay) and cereal grains and in late pregnant dairy cows on similar feeds.

Intestinal obstructions can be mechanical or functional in nature. A major cause of intestinal obstruction in cattle is functional distension of the bowel following loss of muscular contractility, without mechanical occlusion (Koller *et al* 2001). Paralytic ileus has been seen as a common disorder in late pregnancy (Pradhan *et al* 2008). Ileus results in a marked reduction in gastrointestinal motility and distension of the forestomach and abomasum due to the accumulation of excessive quantities of fluid, which results in dehydration.

Abomasal ulceration occurs in mature cattle and calves and may cause acute abomasal hemorrhage with indigestion, melena and sometimes perforation, resulting in a painful acute local or diffuse peritonitis followed by rapid death, or a chronic indigestion with only minimal abomasal hemorrhage. The stress of parturition, the onset of lactation and high-level grain feeding is associated with acute ulceration in dairy cows (Radostits *et al* 2007).

Gastrointestinal dysfunction due to advanced pregnancy is considered to be type IV vagal indigestion and is termed as late pregnancy indigestion. LPI is the reversible abomasal motility/outflow disorder that results from compression of the abomasum or proximal portions of the small intestine by the gravid uterus (Van Metre *et al* 1995). The diagnosis of LPI is usually made by excluding the other causes of

vagal indigestion and amelioration of clinical signs after parturition (Hussain *et al* 2014b).

General signs of indigestion including anorexia, ruminal tympany, scanty faeces, reduced milk production and atony of rumen are considered common signs between different gastrointestinal disturbances especially those of reticulorumen origin like TRP, perireticular abscess and DH (Braun *et al* 1998, Saini *et al* 2007 and Abdelaal *et al* 2009). Many abdominal disorders can be diagnosed based on the results of a thorough clinical examination (Braun 2006).

Digestive disorders in ruminants affect the rumen ecosystem, thereby affecting the other metabolic processes in the ruminants (Madan *et al* 2013). During course of impaction, fermentation and other ruminal activities get disturbed (Garry 2002). Excessive production of toxic substances in rumen and other parts of digestive tract cause over taxation of liver and lead to severe hepatic dysfunction (Kaneko *et al* 1997). Hematological analysis and blood chemistry profile are useful in making a definitive diagnosis of abdominal disorders. They are helpful in quantitating the degree of inflammation and disease diagnosis.

Peritoneal fluid analysis has considerable potential as an aid to the hematological and clinical examination in the diagnosis of bovine abdominal disorders (Hirsch and Townsend 1982). It can be helpful in establishing a diagnosis and determining a prognosis. Biochemical and cytological examination of the peritoneal fluid is a useful tool to evaluate the nature and magnitude of intra-abdominal inflammatory processes (Grosche *et al* 2012).

Thus, various gastrointestinal disorders may induce specific haematological, biochemical and peritoneal fluid alterations in dairy animals. These disorders are common during late pregnancy in bovines, yet no systematic study has been undertaken on this important aspect till date.

Therefore, the present study was envisaged with the following objectives:

1. To study the pathology of various gastrointestinal disorders affecting bovines in advanced pregnancy.
2. To correlate pathological alterations with specific disorders associated with advanced pregnancy in bovines.

CHAPTER – II

REVIEW OF LITERATURE

2.1 Occurrence of gastrointestinal disorders in advanced pregnancy in cattle and buffalo

2.1.1 Peritonitis

Divers and Peek (2008) observed that physical factors may contribute to perforation and subsequent clinical signs in TRP. The prime example of such a physical factor contributing to perforation is advanced gestation and a heavily gravid uterus. During the last trimester, the combined weight and size of the gravid uterus may allow the organ to act like a pendulum as a cow gets up and down, contributing to perforation by an existing sharp metallic object.

Abdelaal *et al* (2009) performed a study on 62 animals (29 Holstein Friesian cows and 33 buffaloes) to document the clinical and ultrasonographic differences between cattle and buffaloes with various sequelae of TRP. Fifteen cows and 22 buffaloes were pregnant over six months, 10 cows and 10 buffaloes had calved less than four weeks ago and four cows and one buffalo were non pregnant. Acute local peritonitis, chronic local peritonitis, acute diffuse peritonitis, reticular abscesses, thoracic abscesses and pericarditis were detected as sequelae of TRP in both cattle and buffaloes.

Athar *et al* (2010b) studied 21 clinical cases (4 cows and 17 buffaloes) of TRP. They divided animals into 2 groups based on clinical, haemato-biochemical, radiographic and ultrasonographic findings. Group 1 (n=12) included animals having localised peritonitis while group 2 (n=9) included animals suffering from diffuse peritonitis. Seven animals of group 1 had either recently calved or were in mid to late stage of pregnancy while in group 2, seven animals had recently calved or were in mid stage of pregnancy.

Shahin and Ghane (2010) conducted study to determine the risk factors and clinical findings of TRP. They examined 104 cattle with TRP. Based on their results, 48 (46.15%) cases were observed in last trimester of pregnancy.

Chanie and Tesfaye (2012) found that traumatic reticuloperitonitis is relatively common diseases in adult dairy cattle. Compression of the reticulum and pressure of the calf during late pregnancy, straining pregnancy or the efforts of parturition promote penetration of the wall by foreign objects.

Aref and Abdel-Hakiem (2013) performed clinical studies on sharp foreign body syndrome (SFBS) in buffaloes. In their study, 49 (25%) cases out of 196 cases were confirmed as SFBS by clinical and radiographical examination. Age of affected animals was ranged from 4-9 years old. Out of the 49 animals, 21 were pregnant (42.85%), 16 were recently calved (32.6%), 7 were non-pregnant (14.28%) while 5 cases were noted in males (10.2%).

Hussain and Uppal (2014) conducted study on 32 clinical cases of peritonitis in cattle. Majority of animals (17/ 32) were in 1st or 2nd lactation, 10 cows were in 3rd to 6th lactation and five cows were heifers. Twenty one cows were non-pregnant, four cows were 4-7 month pregnant (3 heifers) and 7 cows were more than 7 month pregnant.

2.1.2 Traumatic pericarditis

Tharwat (2011) conducted study on sixteen cows with traumatic pericarditis. Fifteen cows were pregnant (94%), of which two (13%) were in the first trimester of pregnancy, three (20%) in the second and 10 (67%) in the third trimester. Only one cow (6%) was non-pregnant.

Athar *et al* (2012) concluded that the incidence of traumatic pericarditis is found more in pregnant or recently parturated animals, due to increased intra-abdominal pressure pushes the foreign body towards the thorax.

Kumar *et al* (2012b) conducted study on adult female bovine (29 cows and 26 buffaloes) suffering from pericarditis. Among these 16 animals (7 cows and 9 buffaloes) were in advanced stage of pregnancy (>6 month of gestation) and 10 (3 cows and 7 buffaloes) had a history of recent calving of less than a month duration.

2.1.3 Diaphragmatic hernia

Prasad *et al* (1979) reported two rare cases of internal hernia in bovines *(Bubalus bubalis)*, in which reticulum and abomasum had herniated into the thoracic

cavity. They observed that involvement of abomasum did not exhibit any additional symptoms other than those commonly observed in cases where only reticulum is herniated. One animal had calved four weeks back and the other was seven months pregnant.

Deshpande *et al* (1982) observed significantly higher incidence of DH in females (96.4%) as compared to males (3.6%). In their study, in a series of 49 clinical cases, 33 animals had a definite history of either parturition or advanced pregnancy before the onset of symptoms. They revealed highly positive correlation between the frequency of calving and the number of cases registered during their study.

Netto *et al* (2008) reported a case of DH associated with traumatic reticuloperitonitis in a Jersey cow. The animal was presented at approximately 90 days of pregnancy with history of ruminal bloat, anorexia, emaciation and weakness.

Athar *et al* (2010a) conducted study on twenty seven animals (twenty six buffaloes and one cow) diagnosed with DH based on clinical signs, radiography, ultrasonography, and left flank laparorumenotomy. Fourteen (51.8%) animals had recently parturated, six (22.2%) were in advanced stage of pregnancy, while rest of the animals were in different stages of lactation.

Kumar *et al* (2012a) observed higher incidence of DH in adult recently calved and advanced pregnant cows and buffaloes. They conducted study on 24 animals (22 buffaloes and 2 crossbred cows) in which 13 were pregnant (12 buffaloes and 1 cow) and 11 were non-pregnant (10 buffaloes and 1 cow).

Abdelaal *et al* (2014) investigated each 10 of healthy non pregnant, pregnant and buffaloes with DH. The presence of foreign bodies and peri-reticular adhesion in 6 animals indicated high DH prevalence following traumatic reticuloperitonitis.

2.1.4 Omasal impaction

Simkins and Nagele (1997) reported severe omasal and abomasal impaction in a group of late gestation suckler cows which were housed in straw yards and had been fed solely on pea haulm for the previous three weeks. Four cows became ill, with a variety of clinical signs, two died, one was euthanized and one recovered spontaneously. Postmortem examination revealed severe omasal and abomasal impaction.

Radostits *et al* (2007) concluded that impaction of the omasum occurs in advanced pregnancy and is characterized by anorexia, scant feces, normal rumen movements, moderate dehydration and an enlarged omasum that may be palpable per rectum.

Turkar and Uppal (2007) studied blood biochemical and ruminal fluid parameters of 5 buffaloes showing omasal impaction. All buffaloes (n = 5) diagnosed for omasal impaction were female and were aged 3–12 years. Three animals were in mid pregnancy (3–6 months) and two animals were in advanced pregnancy (6–9 months). All animals were in second to fifth lactation.

Toor and Saini (2008) reported omasal impaction in 12 of 46 female buffaloes with abdominal disorders. Six of these animals had been pregnant for between 8-9½ months, and the other six had calved between 2½-6 months before they were examined.

Hussain *et al* (2013) conducted study on 8 buffaloes (5 Murrah and 3 Niliravi) and 3 cows (crosses of Holstein Friesian) diagnosed with primary omasal impaction with mean age of 6.70 ± 0.65 years and age ranging from 2.5 to 12 years. Two cows had recently parturated and 1 cow was a non-pregnant heifer, 4 buffaloes were 5–7 months pregnant, while the other 4 were in different stages of lactation.

2.1.5 Intestinal obstruction

Hanks *et al* (1993) evaluated effects of pregnancy on digesta kinetics and ruminal fermentation in beef cows. They included four pregnant and four non pregnant, ruminally cannulated beef cows in the study. They observed that during the third trimester of pregnancy, even with feed intake held constant, digesta passage rate increased and ruminal retention time decreased without affecting dry mater digestion. They further noticed that these changes in digesta kinetics with advancing pregnancy in beef cows seem to be related to changes in circulating estradiol and progesterone concentrations.

Desrochers and St-Jean (1995) reported a case of cecal impaction in 7 months pregnant Hereford cattle, which was showing signs of anorexia and progressive abdominal distension.

Koller *et al* (2001) reported a rare form of ileus in two Swiss Braunvieh cows in late pregnancy. It was found to be due to strangulation of the duodenum at its caudal flexure by the gravid uterus.

Dharmaceelan *et al* (2012) reported that out of 32 females with stomach obstruction, 31.25 % were pregnant and 68.75 % were non-pregnant. Among 10 pregnant animals, 9 animals were pregnant above 5 months and one animal was below 5 months. Out of 35 female animals with intestinal obstruction 48.57 % (17 animals) were pregnant and 51.43 % (18 animals) were non-pregnant.

Muggli *et al* (2014) reported herniation of gravid uterus through a tear in the mesoduodenum with subsequent displacement of the descending duodenum around the uterus combined with internal omental herniation in a three-year-old, five-month-pregnant Swiss Braunvieh cow.

2.1.6 Late pregnancy indigestion

Van Metre *et al* (1995) reported type IV vagal indigestion in 8 months pregnant Hereford cow, admitted to the hospital for evaluation of anorexia and abdominal distension. Diagnosis of type IV vagal indigestion was made on the basis of physical examination. CBC and peritoneal fluid analysis were not indicative of an underlying active inflammatory process.

Radostits *et al* (2007) considered indigestion of late pregnancy of cows, a type of vagus indigestion in which the rumen and abomasum are grossly distended, but the cause is uncertain. They concluded there is no evidence that the effects of an advanced pregnancy alone will cause a vagus-indigestion-like syndrome.

Hussain *et al* (2014b) conducted prospective study on 15 animals (eight buffaloes and seven cows), diagnosed with late pregnancy indigestion. Ten buffaloes and 10 cows served as the control group. The animals were in advanced pregnancy and had partial or complete anorexia, reduced water intake, loss of defecation or scanty faecal output, and mild to moderate dehydration.

2.2 Clinical findings

2.2.1 Peritonitis

Gokce *et al* (2007) observed that cattle with TRP showed signs of anorexia, reduction in milk yield, grunting, constipation, repeated chronic tympany and weight loss and these signs had usually been observed for more than 2 weeks before the animals were brought to the clinic by the owners. Clinical signs observed upon examination were dehydration, ruminal stasis and impaction, abdominal pain and tension.

Abdelaal *et al* (2009) observed that animals with TRP had reduced appetite, decreased milk production and ruminal atony, most of them had scanty faeces and ruminal bloat. Foreign body tests were positive in all cows and buffaloes with different sequelae of TRP while signs of pain and systemic reactions were observed more commonly in cows than in buffaloes.

Smith (2009) concluded that TRP in the most severe, acute form is characterized by fever, anorexia, decreased or absent ruminal contractions, and evidence of cranial abdominal pain. Some cattle grunt spontaneously when forced to move or when defecating or urinating. Lactating cows show a sudden decrease in milk production. Some cows regurgitate ruminal fluid, especially if the oropharynx is mechanically stimulated. Mild bloat, constipation or abducted elbows may also be seen.

Ghanem (2010) reported that cows with TRP had anorexia, arched back and a sharp decrease in milk production, were reluctant to rise or move and exhibited anxiousness. Abduction of the elbow joints was also observed, indicating cranial abdominal pain, as well as repeated chronic tympany.

Hussain and Uppal (2014) observed clinical signs like anorexia, hypodipsia, fever, one or other form of tympany, depression, congested or anaemic mucosa, various degrees of abdominal distension and dehydration, loss of defecation or reduced faecal output in cases of peritonitis in cattle.

Abdelaal and Floeck (2015) reported clinical findings in buffaloes with traumatic reticuloperitonitis. All buffaloes with perforating foreign bodies showed reduced appetite and positive pain tests. The majority of animals had recurrent tympany, elevated body temperature, reduced or absent reticular motility and scanty feces.

2.2.2 Traumatic pericarditis

Braun *et al* (2007) observed that the heart rate was higher than normal in 24 of them, and in 18 of these it ranged from 100 to 130 bpm. Both jugular veins were distended in 24 of the cattle, and 15 had oedema of the throat region, brisket and ventral abdomen. Twelve of them showed signs of pain, including bruxism, muscle fasciculations and grunting. Six of them stood with their elbows abducted. The rectal temperature was lower than normal in three animals and higher than normal in 16.

The ruminal motility was reduced or absent in 23 and intestinal motility was reduced or absent in 15. Signs of pain were elicited by upward pressure on the xiphoid in 17 of the animals, by percussion of the reticulum in 16 and by pinching the withers in 13.

Braun (2009) found that the primary clinical sign in cattle affected with TP is tachycardia with muffled heart sounds. There is a varying degree of distension of the jugular veins depending on the degree of cardiac tamponade and oedema of the submandibular region, brisket and ventral abdomen. The general demeanour, condition and appetite of cattle with traumatic pericarditis are always abnormal and sometimes cattle stand with their elbows abducted. Cattle often have signs of pain such as bruxism and grunting, and the majority has a fever of upto 40.2ºC.

Athar *et al* (2012) described clinical signs in pericarditis in bovines which include venous distension and pulsation, tachycardia, and bilaterally muffled heart sounds, edema of jaw dewlap and ventral abdominal region extending up to the udder, anorexia, drop in milk yield, reluctance to walk or walk with short steps and with stiff gait, pyrexia, increased pulse rate, abdominal respirations, arched back and abducted elbows. Mucous membrane may be congested and have a prolonged capillary refill time. On auscultation, the most consistent findings are tachycardia, muffling of heart sounds, and absence of lung sounds in the ventral thorax.

Kumar *et al* (2012b) conducted study on 6 clinically healthy bovine and 55 adult female bovine (29 cows and 26 buffaloes) suffering from pericarditis. They observed that 71% animals were anorectic for 1–4 weeks while remaining had a history of partial anorexia. Majority of the animals (69.1%) were passing scanty loose or hard faeces and 5.5% animals were not passing faeces while normal defecation was seen in 25.4% cases and tympany was recorded only in 11% cases and brisket edema was present in 81.8% (27 cows and 18 buffaloes) cases.

2.2.3 Diaphragmatic hernia

Saini *et al* (2007) reported DH in a 5-year old, Holstein Friesian cow which was admitted with primary complaint of partial anorexia, scanty feces, recurrent tympany for 12 days and drop in milk yield. The animal was in 2nd parity and had calved normally 1.5 months previously. Physical examination revealed general weakness, distended left flank, moderate dehydration, elevated rectal temperature

(40°C), normal heart rate (74 beats/min), normal respiratory rate (14 breaths/min) and abnormally high rumen motility (5 contractions/2 min).

Athar *et al* (2010a) concluded that the animals suffering from DH had mean age of 6.30 ± 0.37 years with duration of illness ranged from six days to four weeks with a mean of 21.04 ± 5.21 days. Fifteen (55.55%) animals were dull and depressed at the time of presentation and had a dry muzzle while the others appeared alert. Nineteen (70.4%) animals were passing hard, black faeces; seven (25.9%) animals had reduced faecal output while one animal was passing loose faeces. Recurrent tympany was observed in majority of the animals (63%) while three animals had persistent tympany and five did not show any tympany. Regurgitation was observed in two cases. Rumen was hyper motile (4.88 ± 0.21/2 minutes) but with reduced strength of ruminal contractions in seventeen (62.9%) cases while in three animals rumen was hypomotile (2.00±0.00/2 minutes). No rumen motility was appreciated in four animals while in three animals rumen motility was (3.00±0.00/2 minutes).

Kumar *et al* (2012a) conducted study on 24 animals (22 buffaloes and 2 crossbred cows) operated for DH. They observed that the affected animals had a mean age of 5.98±0.37 years. Majority of the animals had normal rectal temperature, respiration and heart rates. Major clinical signs observed were partial (75%) or total anorexia (25%), mild to moderate dehydration, recurrent tympany in all the animals, passing of scanty black constipated faeces (66.7%; n, 16) and mean loss of milk yield by 91%.

2.2.4 Omasal impaction

Turkar and Uppal (2007) observed that all animals with omasal impaction were completely anorectic and three animals had history of gradual change in diet. Water intake was reduced in all cases. None had history of colic and cud dropping. Defecation was absent in two cases and three animals had history of passing constipated faeces. Clinical examination revealed that all animals were in depressed condition and rumination was suspended in all cases. The muzzle was dry in two cases while one animal had 4–6% dehydration and four animals were moderately (6–8%) dehydrated. Ruminal motility (0.40 ± 0.24 per 2 min) was reduced significantly ($p \leq 0.01$) compared to the control value. The mean rectal temperature (39.15 ± 0.24◦C) and respiration rate (22.0±3.52per min) did not differ significantly from the

control group, whereas heart rate (72.60±4.26per min) was significantly ($p \leq 0.05$) higher than in the control group.

Toor and Saini (2008) Omasal impaction in these 12 buffaloes was characterized by complete anorexia, an absence of defecation, loss of rumination, loss of rumen motility, dehydration, reduced levels of plasma electrolytes and increased levels of plasma proteins. These animals were introduced to finely chopped wheat straw as the only forage in the ration recently.

Hussain *et al* (2013) observed that the animals suffering from an omasal impaction had mean age of 6.70 ± 0.65 years and duration of illness was ranged from 5 to 20 days with a mean of 9.55 ± 4.74 days. The seven animals were dull and depressed and had a dry muzzle, while the others appeared alert. Six (54.5%) animals were passing hard feces and 5 animals had complete loss of defecation. The majority of the animals did not show any tympany, while 2 animals had persistent tympany and recurrent tympany was observed in 1 animal. Regurgitation was observed in 1 case. Rumination was absent in 10 animals, while 1 animal was ruminating irregularly. No rumen motility could be appreciated in 5 animals, while in 6 animals the rumen was hypomotile (1–2 contractions/2 min) with reduced strength of ruminal contractions.

2.2.5 Intestinal obstruction

Braun *et al* (1990) described clinical findings in 10 cows suffering from acute functional pyloric stenosis. The general condition of the cows was moderate to severely disturbed and abdomen of most of them was distended. Defecation was reduced or absent. The omasum, reticulum and rumen of most of the cows were dilated secondarily and filled with ingesta.

Braun *et al* (1993) described clinical signs, changes in blood and rumen fluid in 23 cows with obstruction or compression of the duodenum. The most important clinical findings included moderate to severe disturbance in the general behaviour and attitude, markedly reduced gastrointestinal activity, no or greatly reduced defaecation and abomasal reflux with metabolic alkalosis.

Cebra *et al* (1996) reported gravel obstruction in the abomasum or duodenum of two cows. They noticed clinical signs of depression, anorexia, and decreased milk

production. Bilateral abdominal distention and decreased fecal output were found on physical examination.

Nuss *et al* (2006) reported ileal impaction in 22 cows. The clinical signs included anorexia, decreased milk production, absence of faeces, restlessness and colic. The general condition of our patients was only mildly affected, the cows generally appeared calm and the mean heart and respiratory rates were within the normal range. On the other hand, the signs of colic caused by ileal impaction persisted and became more pronounced with time.

Hussain *et al* (2014a) conducted study on six cattle and two buffaloes with intestinal obstruction. Four cases had intestinal volvulus, three had intussusception and one case could have been volvulus or torsion. They observed that the animals were 2.5–8 years old females and had been ill for 2–10 days. Seven animals were completely anorectic while one cow was taking little fodder. All animals had reduced water intake and sudden reduction in milk yield in milking animals (6/8). Faeces were absent (6/8) or scant (2/8). Two animals had history of regurgitation. Physical examination revealed that the animals were depressed and dehydrated, and had congested mucous membranes, tachycardia (95.50 ± 6.25 beats/min), tachypnea (44.35 ± 3.98 breaths/min) and normal temperature (101.53 ± 0.61°F). Rumen was atonic in seven and hypomotile in one animal. Six animals showed severe and persistent abdominal pain characterized by kicking at belly, restlessness, frequently lying down and getting up and sometimes rolling. Persistent tympany was observed in four animals.

2.2.6 Late pregnancy indigestion

Van Metre *et al* (1995) reported late pregnancy indigestion in 5.5 year old Hereford cow that was eight months pregnant. Abnormalities detected on physical examination of the cow included lethargy, tachycardia (90 beats/min), and marked distension of the left dorsal and right ventral abdominal quadrants, increased rumen contractions (4 contractions/min) and feces were firm, mucus-covered, and contained abnormally long plant fibers. Examination per rectum revealed a viable later-term fetus and marked fluid distention of the dorsal and ventral ruminal sacs.

Hussain *et al* (2014b) conducted prospective study on 15 animals (eight buffaloes and seven cows), diagnosed with late pregnancy indigestion (Type IV Vagal

Indigestion). According to them, animals had partial or complete anorexia and reduced water intake. Complete loss of defecation was seen in 6 cases and the rest of the animals had scanty faecal output. Seven and three animals had history of fever and abdominal pain, respectively. Five cases had persistent tympany while two animals had single episode of tympany. Eight animals were dull and depressed at the time of presentation while seven animals appeared alert. Mean heart rate (84.13 ± 4.5/minute) and respiration rate (37.33 ± 2.9/minute) were increased and all animals were mildly to moderately dehydrated. Rumen was atonic in three, hypomotile in eight, and hypermotile in two cases, and rumen consistency was mushy or doughy. Five animals had moderate distension of left paralumbar fossa, two animals had papple shaped abdomen, and two animals had bilateral abdominal distension.

2.3 Diagnosis from blood of live animals

2.3.1 Haematology

Braun (2006) suggested analysis of blood, a helpful tool in the therapeutic decision in acute abdominal disorders of the cow. It often provides information about the localisation, nature and severity of the disorder.

Quiroz-Rocha *et al* (2009) determined reference limits for hematological analytes of dairy cows one week before parturition. No significant differences in leukocyte count were observed except decrease in eosinophils after calving. This change may result from the stress associated with parturition. Other typical changes of acute stress in cows like neutropenia or lymphopenia were not observed.

Roy *et al* (2010) performed hematological profile during gestation period in Sahiwal cows. They found that erythrocyte count, Hb, PCV and MCV were significantly decreased in the last period of pregnancy. The MCH increased during the last trimester of pregnancy. There was a significant decrease in the total leukocyte count during third gestation period along with significant increase in segmented neutrophils.

2.3.1.1 Traumatic reticuloperitonitis

Gokce *et al* (2007) observed that there was a significant reduction in the number of RBCs, and thrombocytes and an increase in total leukocytes and PCV values of the TRP group compared to the control group in cattle.

Radostits *et al* (2007) concluded that the total and differential leukocyte counts provide useful diagnostic and prognostic data in cases of peritonitis. In acute local peritonitis, neutrophilia and left shift are common. In acute diffuse peritonitis a leukopenia (total count below 4000/µL) with a greater absolute number of immature neutrophils than matur (degenerative left shift) occurs, which suggests an unfavorable prognosis if severe. The degree of lymphopenia (lymphocyte count below 2500-3000/µL) is an indication of a stress reaction to inflammation.

Athar *et al* (2010b) observed neutrophilic leucocytosis with left shift in clinical cases of TRP. The mean Hb and PCV levels found elevated in all animals. There was relative neutrophilia and lymphopenia indicative of stress.

Chanie and Tesfaye (2012) observed neutrophilic leukocytosis with left shift in cattle with TRP. Significant increase in total leukocyte, in the percentage of neutrophils and decrease in the percentage of lymphocytes are common hematological findings.

Aref and Abdel-Hakiem (2013) observed significant increase in total leukocytic count (10.28±1.36) in complicated cases of sharp foreign body syndrome as compared to uncomplicated (6.45±1.8) and control (5.17±0.80) ones. Highly significant increase in granulocyte count was observed in complicated cases as compared to control group.

2.3.1.2 Traumatic pericarditis

Braun *et al* (2007) observed clinical findings in 28 cattle with TP. The most common abnormal laboratory findings that indicated inflammation were a reduced clotting time in the glutaraldehyde test in 26 of the animals, leucocytosis in 22 and a high concentration of fibrinogen in 19.

Ghanem (2010) repored that there was significant erythrocytopenia (reduced RBCs) and lower Hb concentrations in the cows with TRP and TP, as compared to the control group. On the other hand, PCV was significantly higher in the cows with TRP and TP than in the control. There was significant leukocytosis, neutrophilia, and lymphopenia in the cows with TRP, as compared to the controls. The cows with TP had significant leukocytosis, neutrophilia, and monocytosis, as compared to the control and TRP groups.

Athar *et al* (2012) concluded that hematological examination in pericarditis in bovines reveal a pronounced leukocytosis, with a TLC of 16,000-30,000/µL with shift to left accompanied with neutrophilia and eosinopenia is usual.

Mohamed (2010) did clinicopathological studies in 40 water buffaloes (*Bubalus bubalis*) with TP. Findings included neutrophilic leucocytosis, elevated total protein concentration, hypoalbuminaemia, hypergammaglobulinaemia and increased concentration of free fatty acids.

2.3.1.3 Diaphragmatic hernia

Turkar and Uppal (2006) observed that the mean Hb level in buffaloes with DH (7.6 ± 0.39 gm%) was significantly decreased whereas mean PCV and TLC did not differ significantly from their respective control values. The mean relative neutrophil count and absolute neutrophil count were significantly higher whereas the mean relative and absolute lymphocytic count were significantly lower than their respective control values.

Saini *et al* (2007) evaluated a five year old Holstein Frisian cow suffering from DH. The hematological analysis revealed Hb, PCV, TLC, neutrophils, lymphocytes, monocytes and eosinophils as 10.4g/dL, 36 percent, 7.5×10^3/µL, 1.8×10^3/µL, 5.1×10^3/µL, 15×10^3/µL and 0.45×10^3/µL, respectively.

2.3.1.4 Omasal impaction

Turkar and Uppal (2007) observed no significant difference in mean Hb and PCV level whereas the mean TLC value ($8.65 \pm 0.38\times10^3$/µl) was significantly different from the control value. The mean relative neutrophil count and absolute neutrophil count were significantly higher than the control level.

Imran *et al* (2011) did clinicopathological studies in cows with omasal impaction. The results of a CBC included polycythemia, leukocytosis with mature neutrophilia, and lymphopenia, consistent with dehydration and stress.

Hussain *et al* (2013) observed significant increase in the number of WBC and neutrophils and a decrease in the number of lymphocytes of the primary omasal impaction group compared to the control group. Leukocytes count ranged from 9500–21,650/µL. It was found to be than 12,000 in 7 animals, and 8000–12,000 in 4 animals. They also observed toxic changes in neutrophils in 5 animals which were

mild to moderate in 4 and severe in 1. Left shift was found mild to moderate in 3 and marked in 3 animals. Leukocytes count more than 15,000/µL with marked left shift and toxic changes in neutrophils was a consistent finding in non survivors.

2.3.1.5 Intestinal obstruction

Makhdoomi *et al* (2002) observed increase in mean values of PCV and Hb in simple intestinal obstruction in buffalo calves.

Mohan *et al* (2006) observed that Hb, PCV levels and TLC count showed no variation from normal in simple intestinal obstruction in buffaloes. DLC showed mild neutropenia and mild lymphopenia, whereas monocytic and eosinophilic counts were within normal limits.

Nuss *et al* (2006) reported ileal impaction in 22 cows. The haematocrit was >38% and ranged from 39% to 47% in five cows, and 10 had a mild leukocytosis.

Hussain *et al* (2012) concluded that the mean values of Hb, PCV and TLC did not differ significantly from their respective control values in buffaloes suffering from caecal dilatation. The mean relative neutrophil count was significantly higher than control value whereas the mean relative lymphocyte count was significantly lower than control value. Neutrophil to lymphocyte ratio was significantly higher than respective control value. They further observed relative neutrophilia in eight animals and absolute in one animal. Moderate to marked left shift was also observed in four animals while toxic changes in neutrophils were observed in two animals only.

Hussain *et al* (2014a) found out that mean PCV and neutrophil count were significantly higher whereas lymphocyte count was significantly lower in bovine intestinal obstruction cases. The mean WBC was higher than the normal reference range but did not differ significantly from control value. Seven out of eight animals showed moderate to marked left shift along with moderate to severe toxic changes in the neutrophils.

2.3.1.6 Late pregnancy indigestion

Hussain *et al* (2014b) did hematological studies in bovines with late pregnancy indigestion (Type IV Vagal Indigestion). There were no significant differences from control values with respect to hemoglobin, packed cell volume (PCV), and total white blood cell (WBC) count. Neutrophil and lymphocyte counts

were significantly higher and lower than the control value, respectively. Hematological analysis revealed neutrophilia in 11 and neutrophilic leukocytosis in three animals. They observed mild to moderate toxic changes in neutrophils in 6 cases and severe in one case. Left shift was found mild to moderate in five cases and marked in two cases.

2.3.2 Clinical chemistry

Radostits *et al* (2007) listed normal biochemical parameters such as total bilirubin (0-0.8 mg/dL), AST (43-127 U/L), ALKP (27-107 U/L), GGT (15-39 U/L), BUN (7.8-25 mg/dL), creatinine (1-2 mg/dL), glucose (42-75 mg/dL), Na (132-152 mmol/L), K (3.9-5.8 mmol/L), Cl (97-111 mmol/L) Ca (9.7-12.4 mg/dL), P (5.6-6.5 mg/dL), Mg (1.7-3 mg/dL) in cattle.

Roy *et al* (2010) did biochemical profile during gestation period in Sahiwal cows. Glucose concentration remained unchanged during pregnancy. ALT decreased in the third period of gestation. AST activity remains decreased throughout the gestation period. There was a non-significant decrease in plasma total protein content in third period of gestation. Their results demonstrated no alterations in the calcium and inorganic phosphorus values.

Madan *et al* (2013) conducted study on metabolic alterations in buffaloes suffering from digestive disorders. They observed a significant increase in BUN in buffaloes suffering from digestive disorders as compared to healthy animals. A non-significant difference in mean values of calcium was observed in buffaloes suffering from TRP. The AST enzyme activity increased significantly in anorectic animals as compared to normal buffaloes. A decrease in plasma protein concentration was observed in anorectic buffaloes, though the difference in comparison to controls was not significant. A non-significant difference was observed in plasma calcium and phosphorus concentrations between the diseased and healthy buffaloes.

2.3.2.1 Peritonitis

Gokce *et al* (2007) observed significant increase in the concentrations of total protein, total globulin and total bilirubin, and in the serum activities of AST and ALKP in the TRP group compared to the control group. The albumin to globulin (A/G) ratio was significantly lower in the TRP group than in the control group. The

calcium concentration was significantly lower, while the phosphorus concentration was significantly higher in the TRP group than in the control group.

Tabrizi *et al* (2007) did a survey on the biochemical parameters in serum of the Azarbaijan buffaloes with TRP. The mean levels of biochemical parameters in buffaloes with TRP were: sodium (112 mEq/Lit), potassium (3.11 mEq/Lit), Glucose (42.29 mg/Lit), phosphorus (3.84 mg/dl), albumin (544 mg/dl) and calcium (8.6 mg/dl). Chemistry abnormalities associated with TRP were hypochloremia, hypokalemia, and metabolic alkalosis. The mean levels of sodium, potassium and phosphorus in buffaloes with TRP was less than the normal buffaloes and the mean levels of calcium and albumin was higher than the normal buffaloes.

Athar *et al* (2010b) observed increase in levels of total protein, albumin, and fibrinogen and decrease in plasma concentration of sodium, potassium and chloride in majority of TRP cases.

Hussain *et al* (2011) reported a case of frank exudative peritonitis due to perforated abomasal ulceration in a crossbred cow. Blood biochemical analysis showed altered liver and kidney functions, increased glucose and lactate, and decreased levels of albumin, potassium, chloride, calcium and phosphorus.

Tharwat *et al* (2012) observed hypoalbuminemia and hyperglobulinemia in cases of chronic peritonitis in buffaloes and cattle.

Hussain and Uppal (2014) did biochemical analysis in clinical cases of bovine peritonitis. There were increased serum total bilirubin, AST, glucose and lactate levels whereas albumin, chloride and calcium concentrations were decreased.

2.3.2.2 Traumatic pericarditis

Braun *et al* (2007) observed that the activities of GGT and AST and the concentration of bilirubin were higher than normal in 20, 15 and 11 of the animals, respectively, in cattle with TP.

Braun (2009) reviewed that the activities of GGT and AST and the serum concentration of bilirubin are increased in cases of TP, indicating hepatic congestion.

Ghanem (2010) found that sodium, potassium, and chloride levels were significantly lower in the cows with TRP and TP than in the controls, however, fibrinogen, AST, ALT, CK, LDH, BUN, and creatinine were significantly higher in

the TRP and TP groups than in the control group. Enzymatic activity of AST and ALT was significantly higher in the TP group than in the TRP group, the level of CPK enzyme was significantly higher in the TP group than in the TRP group, and the glucose level was significantly lower in the TP group than in the control group.

Athar *et al* (2012) concluded that the activities of GGT and AST and the serum concentration of bilirubin are increased in cases of TP. Hyperfibrinogenaemia, is also a common finding. Electrolyte concentrations are usually normal, but serum calcium and potassium concentrations may be low because of anorexia.

2.3.2.3 Diaphragmatic hernia

Turkar and Uppal (2006) did haemato-biochemical studies in buffaloes diagnosed with DH. The mean plasma levels of AST (174.4 ± 17.68 IU/L), bilirubin (1.69 ± 0.36 mg%), BUN (66.60 ± 1.97 mg%) and creatinine (2.1 ± 0.48 mg%) were significantly higher than the control values. The mean plasma total protein (6.21 ± 0.15 gm%), albumin (2.68 ± 0.24 gm%) and globulin (3.53 ± 0.25 gm%) did not differ significantly from control values. The mean plasma calcium (8.71 ± 0.29 mg%), sodium (116.7 ± 2.51 mmol/L) and potassium level (3.38 ± 0.14 mmol/L) levels were significantly lower than those of control values.

Bellavance *et al* (2010) observed elevated levels of BUN (7.71 mmol/L), creatinine (134 mmol/L), AST (136 U/L), and CK (959 U/L) in a case of DH in a newborn calf. There was also a decreased blood level of GGT (63 U/L) and Chloride 94.6 mmol/L.

2.3.2.4 Omasal impaction

Turkar and Uppal (2007) studied blood biochemical profile in buffaloes showing omasal impaction. The mean value of AST, ALP, total bilirubin, BUN, creatinine, total protein, globulin, albumin in plasma were 227.0 ± 36.78 IU/L, 184.2 ± 28.07 IU/L, 3.08 ± 0.68 mg%, 83.16 ± 22.23 mg%, 2.24 ± 0.48 mg%, 8.3 ± 0.14 gm%, 4.46 ± 0.3 gm% and 3.84 ± 0.22 gm%, respectively. The mean value of calcium and inorganic phosphorus were 7.73 ± 0.87 mg% and 5.80 ± 0.22 mg%. The mean value of sodium, potassium and chloride in plasma were 121.80 ± 3.35 mmol/L, 2.99 ± 0.10mmol/L and 81.98±3.35mmol/L, respectively.

Hussain *et al* (2013) found that there was a significant increase in the concentration of total bilirubin, BUN, glucose, lactate, and fibrinogen as well as serum activities of AST, ALP, and GGT in the primary omasal impaction group compared to the control group. The concentrations of albumin, fibrinogen ratio, calcium, and chloride were significantly lower in the primary omasal impaction group compared to the control group. The concentrations of potassium and phosphorus were lower than the reference values but did not differ significantly from the respective control values.

2.3.2.5 Intestinal obstruction

Doll (1991) observed clinico-chemical findings in calves and young cattle with ileus. There were markedly raised ruminal fluid chloride levels, combined with hypochloraemia and a metabolic acidosis, indicative of an "abomasoruminal reflux syndrome" resulting from an obstruction in the region of the abomasum or cranial small intestine.

Singh *et al* (2000) did acid-base, blood gases and biochemical analysis in crossbred cow calves (*Bos indicus*) with strangulated ileal obstruction. BUN values increased significantly at last stages. Sodium and potassium values in the blood showed insignificant declining trend, whereas plasma chloride values dropped significantly at terminal stage. Blood glucose and lactate values were elevated at the end stage. Significant increase in arterial pH, $HCO3^-$ and base excess (ECF) indicated severe metabolic alkalosis.

Mohan *et al* (2006) studied changes in different body fluids during simple intestinal obstruction in buffaloes. They observed that plasma total protein and plasma albumin levels were decreased, whereas plasma globulin did not vary. Plasma calcium concentration was decreased whereas, plasma inorganic phosphorus concentration was increased. Plasma sodium and potassium levels were within the normal limits, whereas, plasma chloride was low.

Vogel *et al* (2012) reported duodenal obstruction caused by duodenal sigmoid flexure volvulus in 29 dairy cattle. They observed severe hypokalemia (2.9±0.5 mol/L), hypochloremia (69.7± 11.1 mmol/L) metabolic alkalosis (44.5 ± 7.4 mmol/L) and hyperbilirubinemia (32.4 ± 29.0 μmol/L).

Kahnamoii *et al* (2013) evaluated hepatic serumal enzyme activity in dairy cattle with cecum dilatation and twisting. They observed that the average rate of ALKP in diseased cattle was 497.6 ± 41.2 U/L and in healthy group was 384.7 ± 48.5 U/L. The average levels of ALT and AST in diseased cattle were 51.09 ± 9.2 U/L and 159.5 ± 20.3 U/L, respectively.

Hussain *et al* (2012) did blood biochemical analysis in buffaloes suffering from caecal dilatation. They observed significant increase in concentrations of total bilirubin, AST, globulin, BUN and lactate and significant decrease in mean calcium, potassium and chloride levels as compare to their respective controls.

Hussain *et al* (2014a) did serum C-reactive protein, biochemical and blood gas analysis in bovine intestinal obstruction cases. Serum biochemical analysis showed significant increase of AST, fibrinogen, lactate and C-reactive protein levels and significant reduction in albumin, fibrinogen ratio, potassium, chloride, calcium and phosphorus levels. Blood gas analysis revealed hypochloremic hypokalemic metabolic alkalosis with compensatory respiratory acidosis.

2.3.2.6 Late pregnancy indigestion

Hussain *et al* (2014b) performed serum biochemistry in animals diagnosed with late pregnancy indigestion. Total bilirubin, AST, total protein, globulin, BUN, and calcium were significantly lower than the control values. ALP and GGT were higher than the reference values, but they did not differ significantly from control values. Potassium and phosphorus were lower than reference values but did not differ significantly from control values.

2.4 Peritoneal fluid analysis

Drisken (1979) found that in cattle with forestomach diseases, peritoneal fluid was reddish in colour, slightly cloudy, odourless to foul smelling, having a medium to high cell count with predominance of leucocytes. In normal animals peritoneal fluid was colourless to light yellow, odourless and clear with cell count of 2000 to 5000/cumm.

Hirsch and Townsend (1982) conducted a retrospective study of peritoneal fluids in cattle with abdominal disorders. Out of 66 cattle studied, 31 had a non-septic peritonitis, 11 acute bacterial peritonitis, eight ascites and 16 with miscellaneous

disorders such as abomasal impaction, enteritis and lymphosarcoma. In 80% of cases with peritoneal inflammation, nucleated cell count was higher than 6000 cells/µL and total protein content was higher than 3 g/dL.

Dubensky and White (1983) analyzed the value of total plasma protein in diagnosis of TRP in dairy cattle using the concept of sensitivity, specificity and predictive value of test. They concluded that sensitivity of the total plasma protein in diagnosis of TRP decreased from 97 to 6.3% as the cut off value for a positive test rose from 65g/L to 110g/L, while specificity increased from 11.3% to 99.1% over the same range of cutoff values.

Wilson *et al* (1985) started a new technique for collection of peritoneal fluid in cows using a sterile Nelson trocar and cannula. It was observed that the greater than 10% eosinophils were typical of normal peritoneal fluid and peritoneal fluid with a relative neutrophil count greater than 40% and a relative eosinophil count of less than 10% was frequently associated with the diagnosis of peritonitis. Parturient cattle had large volumes of peritoneal fluid with low total protein and white cell counts. Growth of Gram-negative or anaerobic organisms was found to be associated with mortality.

Ziemer (1989) found that a nucleated cell count of 5000/cumm or less was normal for large animals. Total protein concentration less than 3.5 g/dL was also considered normal.

Ward and Ducharme (1994) confirmed that a relatively accurate diagnosis of peritonitis was made with nucleated cell count >6000 cell/µL and total protein concentration >8 g/dL. It was also confirmed that differential leukocyte count with neutrophils >40% was more diagnostic.

Anderson *et al* (1995) compared the peritoneal fluid constituents of clinically normal young calves, to those of adult cattle. Calves had significantly higher peritoneal fluid nucleated cell and mononuclear cell counts, but lower peritoneal fluid eosinophil cell count than the adult cows.

Singh (2004) observed increased total protein, total nucleated cell count and neutrophil count in peritoneal fluid cytology of buffaloes suffering from reticular abscess while in DH a significantly lower total nucleated cell count was seen.

Zadnik (2010) did retrospective study of peritoneal fluids in cows with abdominal disorders. A total of 779 cows were studied, in which 444 had hardware disease, 212 had left abomasal displacement, 98 cows had right abomasal displacement with or without abomasal torsion, and 25 cows had problems with the uterus. He examined three parameters of the abdominal fluid viz, physical appearance, differentiation to transudate or exudate and cytological characteristics.

Wittek *et al* (2012) compared peritoneal inflammatory response to surgical correction of left displaced abomasum using different techniques. Evaluation of peritoneal fluid revealed aseptic inflammatory response within the abdominal cavity indicated by increased peritoneal fluid leukocytes, D-dimer concentrations and enzyme activities. The increased blood and peritoneal L-lactate concentrations were decreased immediately after surgery. Laparotomy resulted in an increase of blood and peritoneal fluid CK on day 1 after surgery, whereas, laparoscopy caused an increased peritoneal fluid CK only.

Hussain and Uppal (2014) did peritoneal fluid cytology in clinical cases of bovine peritonitis. The mean values of specific gravity, total protein and total cell count of peritoneal fluid in peritonitis were 1.03± 0.001, 4.54± 0.21 g/dL and 4740.31± 558.50/ µL, respectively. The neutrophil, lymphocyte and esinophil counts were 83.44± 1.65%, 16.16± 1.65% and 0.25± 0.11%, respectively. Peritoneal fluid cytology revealed degenerated neutrophils with engulfed bacteria and leakage of gut contents in to abdominal cavity. Presence of bacteria indicated septic peritonitis.

Wittek *et al* (2010b) checked diagnostic accuracy of d-dimer and other peritoneal fluid analysis measurements in dairy cows with peritonitis. They observed that peritoneal fluid LDH activity was significantly increased in cows with septic peritonitis compared with nonseptic peritonitis whereas, activities of ALP and CK and concentrations of L-Lactate did not differ between cows with and without peritonitis. Peritoneal fluid D-Dimer was found most accurate in diagnosing peritonitis in cows. Low peritoneal fluid glucose concentration was found to be highly indicative of septic peritonitis. According to them, SAAG did not seem to be superior in detecting peritonitis in cows.

Grosche *et al* (2012) evaluated peritoneal fluid in 40 cows with left displaced abomasum (LDA) and 15 cows with abomasal volvulus (AV). Total protein, albumin,

glucose and cholesterol were normal in peritoneal fluid of cows with LDA and AV. Although L-lactate increased in both groups, cows with AV had significantly higher values. The number of leucocytes was normal, however, significantly more peritoneal neutrophils appeared necrotic or apoptotic after AV. Peritoneal fluid of cows with abomasal displacement showed distinctive features of ischaemia and inflammation.

Hussain *et al* (2014a) did peritoneal fluid analysis in cases of bovine intestinal obstruction. Total cell count was more than 10,000/µl with a mean of 14,200 ± 1977/µl in all the cases and there was massive neutrophilia (>90%) along with presence of markedly degenerated neutrophils. The specific gravity (1.035 ± 0.005) and total protein (6.2 ± 0.76 g/dl) concentration were increased. Bacteria (cocci) were present in two and pyoperitonium, characterized by severely degenerated neutrophils with engulfed bacteria, was diagnosed in one case. Leakage of gut contents was also observed in two cases.

2.5 Diagnosis of gastrointestinal disorders in dead animals

2.5.1 Gross lesions

As per Whitlock (1980), abomasal ulcers can be divided into four types. Ulcers with clinical signs are usually type 2 (bleeding ulcer), but occasionally may be type 3 (perforated ulcer with local peritonitis), or type 4 (perforated ulcer with generalized peritonitis). Type 1 ulcers (erosions and non-perforating lesions of the abomasal mucosa) are usually diagnosed at post-mortem, as affected animals frequently have only mild or no clinical signs.

Deshpande *et al* (1981) investigated 10 buffalo carcasses to know patho-anatomy of DH in bovines. They found that hernias had occurred at the musculotendinous junction of the diaphragm, ventral to the foramen venae cavae and slightly lateral to the median plane with diameter of the hernial ring varied from 7 cm to 20 cm. Herniation was more common in the right thoracic cavity with the reticulum firmly adherent to the hernia ring. Adhesions between the herniated portion of the reticulum and pleura, lung, pericardium or thoracic wall were present, while in a few cases thick fibrous tracts concealing metallic bodies were found.

Sojka *et al* (1990) observed an unusual case of TP in a cow. They observed a single chronic subcutaneous abscess along with osteomyelitis of the fifth sternebra

without evidence of reticulitis or peritonitis. There was penetration of the wire through the skin, with subsequent migration into the sternebrae and pericardial sac.

Roth and King (1991) described that TP is characterized by fibrinopurulent changes in the pericardium and epicardium, sometimes associated with abscessation.

Akkoç *et al* (2007) described an unusual case of reticulopericarditis traumatica complicated with pythorax in a Saanen goat. Grossly, he observed ascitic fluid accumulation, pyothorax, disseminated abscesses in the liver, spleen and lung, and severe fibrinopurulent exudation in the pericardial sac.

Braun *et al* (2007) diagnosed TP at postmortem in 28 animals. Ten of them had fibrinous pericarditis and epicarditis, suppurative pericarditis was diagnosed in 10 other animals, constrictive pericarditis in five and suppurative pericarditis with abscessation in three. Metal wire was identified as the cause in 14 of the animals, and a nail in nine, in three of these animals, the foreign body responsible had been removed from the reticulum during a previous rumenotomy.

Ghanem (2010) did comparative study on TRP and TP in Egyptian cattle. Postmortem examination in the TRP group revealed extensive fibrinous adhesions between the cranioventral aspects of the reticulum, the ventral abdominal wall, and the diaphragm. Adhesions and multiple abscesses were observed on either side of the reticulum. Postmortem examination in the TP group revealed thickening of the pericardial sac. Cross sections of the pericardium and heart muscle showed thickening of the pericardium, with accumulation of pus between the pericardium and cardiac muscle.

Tharwat (2011) conducted postmortem examination in 16 cows diagnosed with TP. He observed fibrinous adhesions between reticulum and diaphragm, reticulum and spleen or between rumen and left abdominal wall. Pus and fibrin were detected in the pericardium. Other postmortem findings included splenic abscessation and pleural effusions where the foreign body was seen.

Athar *et al* (2012) found that gross postmortem examination in acute cases of pericarditis show distention of pericardial sac with foul-smelling grayish fluid containing flakes of fibrin. There may be organization of the fibrinous exudate and epicardium may be covered with heavy deposits of fibrin giving appearance of "scrambled eggs". In chronic cases, the pericardial sac is grossly thickened and fused

with the pericardium by strong fibrinous adhesions surrounding loculi of varying size which contain pus or straw colored fluid.

Chanie and Tesfaye (2012) reviewed that localized TRP is characterized by varying degrees of locally extensive fibrinous adhesions between the cranioventral and the ventral aspects of the reticulum. Adhesions and multiple abscesses may extend to either sides of the reticulum involving the spleen, omasum, liver, abomasums and ventral aspects of rumen. Large quantities of turbid foul-smelling fluid containing clots of fibrin are usually present. Loops of intestine and omentum are commonly stuck together by thick layer of fibrin.

Hussain *et al* (2014b) did necropsy examination in two animals died of late pregnancy indigestion In both cases, the rumen and reticulum were distended with frothy contents compressing the diaphragm, abomasum was compressed cranially, and intestines were collapsed. The death was ascribed to respiratory failure due to compression of diaphragm caused by abdominal distension.

2.5.2 Histopathological studies

Johnson and Jamison (1984) diagnosed wide spread amyloid deposition in various organs like kidneys, liver, adrenal glands and spleen in dairy cows due to foci of inflammation related to traumatic reticuloperitonitis, traumatic pericarditis, salpingitis, mastitis and metritis.

Jensen *et al* (1994) demonstrated gastrointestinal mycosis in 73 lesions of 32 cattle without a history of engorgement. They observed that omasum was the target organ for infection, followed by the rumen and reticulum. Acute necrohemorrhagic lesions dominated, with infiltration of neutrophils and thrombosis.

Akkoç *et al* (2007) observed that liver, spleen, and lung sections in case of TP revealed variably sized abscesses surrounded by a wide zone of fibroblast proliferations and connective tissue capsule. Severe fibrous tissue reaction, accumulation of degenerated neutrophils, macrophages, and diffuse fibrin strands were seen in the pericardial sac and epicardium.

Chvojka *et al* (2008) did study on histopathological responses to peritonitis-induced septic shock in pigs. All septic pigs developed hyperdynamic shock with acute renal injury as evidenced by a 30% increase in plasma creatinine levels. Renal

histology revealed only subtle changes. Only minor histological changes encompassing mild brush-border loss and vacuolisation of tubular cells were present at 22 hours of the experiment in kidney. No signs of acute tubular necrosis or tubular cast formation were found.

Ghanem (2010) did histopathology in cases of TP which revealed accumulation of fibrinous inflammatory exudate between the pericardium and myocardium. The myocardium had severe inflammatory cell infiltration replacing the cardiac muscle that had atrophied and in severely affected cases myocardium exhibited hyalinosis.

Chanie and Tesfaye (2012) observed that histopathological examination in TP reveal variably sized abscess in liver, spleen and lung sections surrounded by a white zone of fibroblast proliferation and connective tissue capsule, severe fibrous tissue reaction, accumulation of degenerated neutrophils, macrophages and diffused fibrin strands in the pericardial sac and epicardium. Local fibrous tissue proliferation with numerous macrophages dominated the inflammation in the reticulum.

Kumar *et al* (2012) did histopathology of ultrasound guided biopsy (USGB) and ultrasound guided fine needle aspiration biopsy (USG-FNAB) of liver, which confirmed sinusoidal dilatation in all the animals along with chronic cholangiohepatitis (n=3), amyloidosis (n=2) or chronic sepsis, purulent hepatitis (n=2), chronic active hepatitis (n=2) and fatty liver (n=1).

2.3. Immunohistochemistry studies

Bacciarini *et al* (2003) conducted a retrospective study to assess the presence of the *C. perfringens* β2-toxin in tissues of the equine gastrointestinal tract. They produced monospecific polyclonal antibodies against recombinant β2-toxin in rabbits and used to demonstrate the β2 -toxin in sections of the gastrointestinal tract by immunohistochemical methods. Sections from 69 horses were stained and β2-toxin was observed immunohistochemically in 40 animals. Sections from the stomach, small intestine, and large intestine were positive.

Asaoka *et al* (2004) performed immunohistochemistry to demonstrate bacilli of *Clostridium perfringens* enterotoxin type A from sporadic outbreaks of fatal enteritis that occurred among free-living wild crows in an open-air park in Japan.

Miclard *et al* (2009) evaluated 52 piglets with spontaneously acquired *C. perfringens* type C enteritis and 14 control animals by immunohistochemistry. They revealed binding of *C. perfringens* beta (CPB) toxin to vascular endothelial cells in peracute to acute lesions of necrotizing enteritis. Subacute cases, in contrast, demonstrated reduced or no CPB staining at the endothelium, mainly due to widespread vascular necrosis. They concluded, that the pathogenesis of *C. perfringens* type C induced necrotizing enteritis involves binding of CPB to endothelial cells in the small intestine during the early phase of the disease.

CHAPTER – III

MATERIALS AND METHODS

3.1 Clinical study

3.1.1 Source of animals

The present study was conducted on both, live and dead cattle and buffalo suffering from gastrointestinal disorders. The study was conducted on 50 clinical cases (34 buffalo and 16 cattle) presented to the Large animal clinics, GADVASU, Ludhiana from August 2014 to February 2015. In addition, tissue samples were collected at necropsy from animals (17 buffaloes and 4 cows) that died due to gastrointestinal disorders in advanced pregnancy and were subjected to histopathology.

The clinical cases were selected on the basis of history of anorexia, reduced faecal output or loss of defecation, diarrhea, and tympany. The diagnosis was made on the basis of clinical examination, laboratory analysis, radiography, ultrasonography, rumenotomy findings and post mortem examination in case of death of the animal.

3.1.2 Signalment and anamnesis

Detailed signalment including species, breed, age and parity was recorded. A detailed history of duration of illness, feed intake, water intake, rumination status, defecation, colour of faeces, type of tympany, symptoms of regurgitation, fever and pain were recorded in every case.

3.1.3 Physical examination

The physical parameters recorded were rectal temperature (^{o}F), heart rate, respiration rate and colour of mucous membrane. Each animal was thoroughly evaluated for its general condition (alert or depressed).

3.2 Collection and analysis of samples

3.2.1 Blood Samples

3.2.1.1 Hematology

Four to five ml of blood was collected in EDTA vaccutainers from jugular vein aseptically for hematology. Hemoglobin (Hb), packed cell volume (PCV), total leukocyte count (TLC), total erythrocyte count (TEC), platelet count, mean corpuscular hemoglobin (MCH), mean corpuscular volume (MCV) and mean

corpuscular hemoglobin concentration (MCHC) were determined using ADVIA 2120 Hematology System (Siemens Healthcare Diagnostics Inc. Deerfield, IL, USA). In addition, multiple blood smears were prepared from each case and stained by Leishman stain (Jain 1986). Differential leukocyte count was done manually on stained blood smears in order to verify and recast the DLC findings after necessary corrections/ adjustments, if any. Stained blood smears were also examined for alterations in size, shape and morphology of erythrocytes, leukocytes and platelets. Neutrophils were also analyzed for alterations in kinetics and toxic changes. Absolute counts of all the leucocytes and neutrophil: lymphocyte (N: L) ratio in each case were also calculated (Jain 1986).

3.2.1.2 Clinical chemistry

For clinical chemistry, blood was collected in gel vaccutainers without anticoagulant. The vaccutainers were kept undisturbed at an angle of 30^{o}C at room temperature for 30-60 minutes and blood was allowed to clot. Serum was harvested by centrifugation at 3000 rpm for 15 minutes and stored in multiple small aliquots of 1-2 ml each at -20 ^{o}C until analysis.

Clinical chemistry analysis was carried out in VITROS DT-11 Chemistry System (Ortho-clinical Diagnostics, Johnson and Johnson, SA) using standard kits (Vitro-Ortho-Clinical Diagnostics, Mumbai). The parameters analyzed were total protein, albumin, globulin, total bilirubin, aspartate aminotransferase (AST), alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT), creatinine, blood urea nitrogen (BUN), creatine kinase (CK), lactate, sodium, potassium, chloride, calcium, phosphorus and magnesium. Results were printed on a result card having adhesive backing.

The VITROS DT Slide method was performed using the VITROS DT Slides and the VITROS Chemistry Products DT Calibrator Kit on VITROS DT 60/DT60 II Chemistry Systems. The VITROS DT Slide is a multilayered, analytical element coated on a polyester support. A drop of sample is deposited on the slide and is evenly distributed by the spreading layer to the underlying layers.

3.2.2 Peritoneal fluid sampling

In animals suspected for peritonitis, peritoneal fluid were collected in vials containing Na_2 EDTA @ 2mg/mL, by free hand centesis using 16-18 gauge, 1.5 inch

long hypodermic needle. The animal was restrained in standing position, site was surgically prepared and peritoneal fluid was collected from one or more of the sites (Radostits *et al* 2007) i.e. caudal to the xiphoid-sternum and 4-10 cm lateral to midline; or left of the midline, 3-4 cm medial and 5-7 cm cranial to the foramen for the left subcutaneous abdominal vein; or right caudoventral abdominal wall medial to the fold of flank (just anterior to the base of udder on the right side). The peritoneal fluid was evaluated for physical characteristics, cytology and clinical chemistry, viz:

a) Physical characteristics: Volume and colour.
b) Cytology: Peritoneal fluid samples were analysed for total cell count (per µL) (Benjamin 1985) and differential leukocyte count (DLC%) using same method as used for hemacytology (Jain 1986).
c) Clinical chemistry: Total protein, albumin and lactate levels were estimated as described for blood samples. In addition, Serum-ascites albumin gradient (SAAG) was also calculated using formula (Wittek *et al* 2010a):
   SAAG = (serum albumin) - (albumin level of ascitic fluid)

3.3 Histopathology

The animals dying of gastrointestinal disorders in late pregnancy were thoroughly necropsied. Tissue pieces from forestomachs, abomasum, intestine, mesenteric lymph nodes, spleen, kidney, liver, lungs and heart were collected in 10% neutral buffered formalin. After fixation, tissue samples were given overnight washings under tap water, and then dehydrated by passing through ascending grades of alcohol followed by clearing with acetone and benzene. Finally the tissues were embedded in paraffin wax (Leica Microsystem, Paraplast tissue embedding medium, 56ºC) and 4-5µ sections were cut. The sections were stained with routine hematoxylin and eosin technique (Luna 1968).

Immunohistochemistry techniques

i) Tissue sections

For immunohistochemical studies 4-5 µ thick paraffin embedded tissue sections were cut and mounted on Superfrost/Plus, positively charged microscopic slides (Fisher Scientific, USA). The slides were then kept on hot plate to melt the paraffin at 60^{o} C for 30 minutes and stored till further use.

ii) Antigen Retrieval

a) Heat induced epitope retrieval

Heat induced epitope retrieval (HIER) was employed depending upon the antibody used (Table 1). Different temperature and time settings were used in various EZ antigen retrieval solutions by EZ-Retriever ^{TM} System as per instructions in the data sheet (BioGenex Laboratories Inc., San Ramon, California, USA).

Table 1: Antigen retrieval protocols used for HIER in immunohistochemistry

S. No.	Name of AR solution used	Dilution used	Temp. & Time
1.	EZ-AR^{TM} Common Solution 5 X Concentrated	Five-fold dilution in deionized water	70^{o}C- 10min.
2.	EZ-AR^{TM} 3 Solution 10 X Concentrated	Ten-fold dilution in deionized water	95^{o}C- 10min. & 98^{o}C- 5 min.

iii) Immunohistochemical staining protocols

Single Antigen labelling

Immunohistochemical staining was performed by using advanced SS^{TM} One-Step Polymer–HRP IHC Detection System (BioGenex Laboratories Inc., San Ramon, California, USA) as per manufacturer's instructions. The sections were dewaxed and rehydrated by dipping in EZ-AR^{TM} Common Solution (BioGenex Laboratories Inc., San Ramon, California, USA), and heating at 70^{0} C for 10 minutes in EZ-Retriever ^{TM} System (BioGenex Laboratories Inc., San Ramon, California, USA) and subsequent antigen retrieval by heat (Heat induced antigen retrieval - HIER) was performed according to the antibody used (Table 2). Following HIER the sections were allowed to cool and brought to room temperature. Then three washing in PBS were given (pH 7.2-7.4) for 3 minute each. Sections were encircled with hydrophobic pen. The endogenous peroxidase was quenched with a solution of 3% H_2O_2 in methanol for 15 min at room temperature in a humid chamber, followed by washing thrice with PBS for 3 min each. The sections were then incubated with 'ready to use' power block (BioGenex Laboratories Inc., San Ramon, California, USA) to block non-specific protein binding for 15 min at room temperature in a humidified chamber. Afterwards,

the sections were incubated with 'ready to use' or reconstituted primary antibody (Table 3) for 60 minutes at room temperature in a humidified chamber. The sections were then given three washings in PBS for 3 minutes each, followed by incubation in polymer HRP (BioGenex Laboratories Inc., San Ramon, California, USA) for 30 minutes at room temperature in a humidified chamber and three washings with PBS for 3 min each. The antigen-antibody-peroxidase reaction was developed with a freshly prepared 3,3 '-diaminobenzidine (DAB) solution by mixing 2 drops of DAB with 1 ml of DAB buffer supplied by the manufacturer and adding 5 µl hydrogen peroxide. Sections were later washed in distilled water for 5 minutes and counterstained with Gill's haematoxylin (Merck, Germany) for 30 seconds and washed in running tap water for 5 minutes. Finally, the slides were dehydrated in ascending grades of alcohol, cleared in xylene, mounted in DPX and examined under microscope (BX 61, Olympus Corporation, Japan). For each antibody, a negative control was run by replacing primary antibody with PBS buffer.

Table 2: Antibodies used for Immunohistochemistry

Name of the antibody	Name of the company	Clone	Immunoglobulin Class	Dilution used	Antigen Retrieval
Rabbit anti Clostridium Perfringens	AbD setotec	Polyclonal	IgG	1:500	HIER Common & AR 3

3.4 Control group

For establishing control values for clinical cases, apparently healthy pregnant five cows and seven buffaloes were taken as control from Livestock Dairy Farm, Ludhiana.

3.5 Statistical analysis

The results obtained were subjected to statistical analysis like t-test using SPSS software version 21.0.

CHAPTER – IV

RESULTS AND DISCUSSION

The present study was conducted on clinical cases suffering from gastrointestinal disorders in advanced pregnancy (6 months or above), presented to the Large Animal Clinics, Department of Teaching Veterinary Clinical Complex, GADVASU, Ludhiana from August 2014 to February 2015. The study included 50 live animals (16 cattle and 34 buffalo) and 21 dead animals (4 cattle and 17 buffalo). History and clinical parameters were recorded and blood and peritoneal fluid were collected aseptically from the live affected animals. Tissues of different organs were collected from animals that died of such disorders at necropsy and were subjected to histopathology.

4.1 Study on live animals

4.1.1 Occurrence of GIT disorders in advanced pregnancy

Cases were divided into seven types on the basis of physical and clinical examination, radiography, ultrasonography, hematology, clinical chemistry and peritoneal fluid examination. The various types of gastrointestinal disorders (Fig. A, Table 3) were peritonitis (n=16, 32%), traumatic pericarditis (n=7, 14%), diaphragmatic hernia (n=6, 12%), omasal impaction (n=3, 6%), paralytic ileus (n=3, 6%), late pregnancy indigestion (n=7, 14%), and miscellaneous conditions (n=8, 16%). Animals without primary disorder were grouped under miscellaneous conditions. Almost similar reports of occurrence of the abdominal disorders have been reported earlier (Toor 2003, Turkar 2004, Kumar 2006, Singh 2008, Athar 2009 and Hussain 2010).

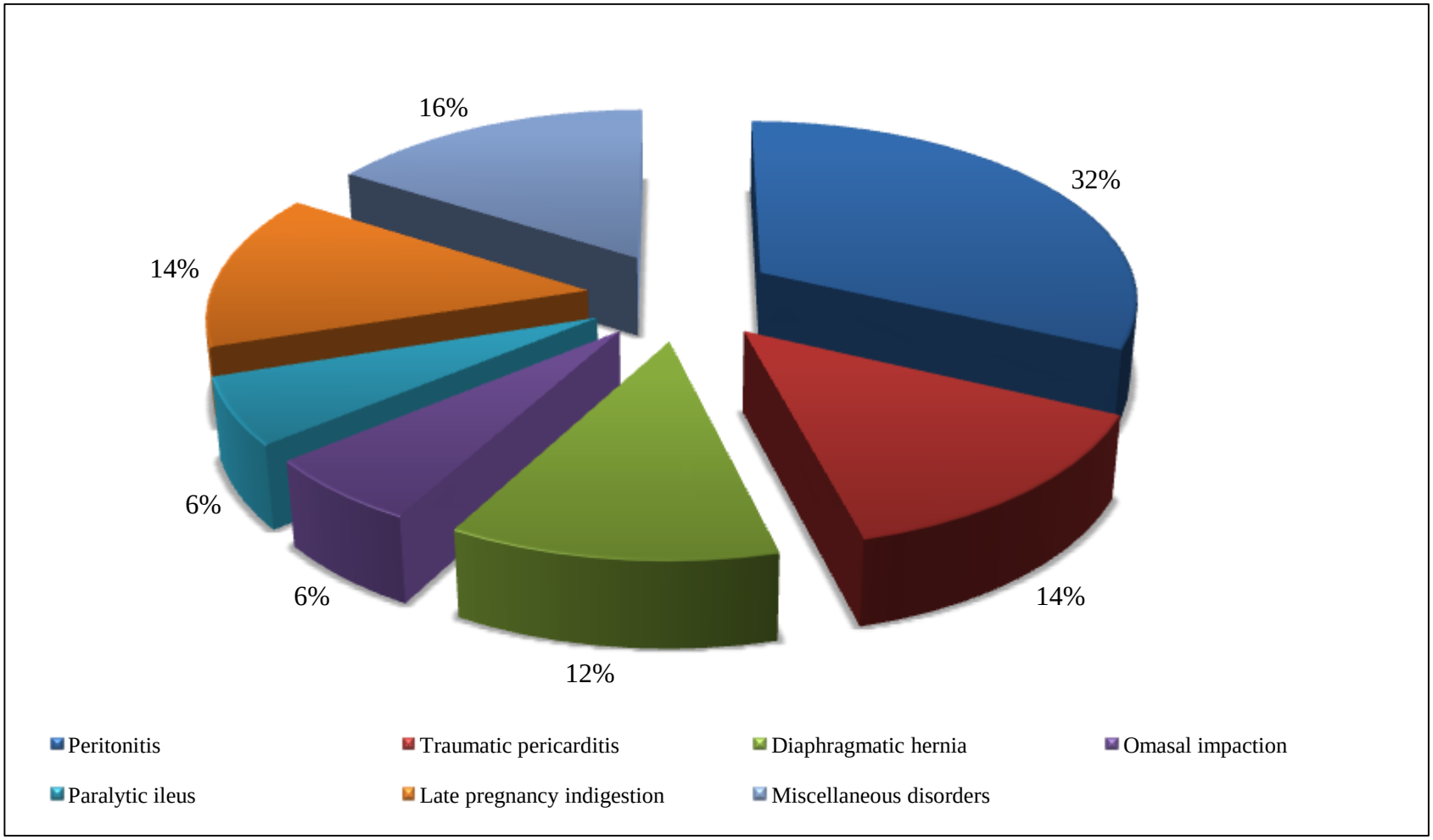

Fig. A: Distribution of gastrointestinal disorders in bovines in advanced pregnancy

Table 3: Distribution of gastrointestinal disorders associated with advanced pregnancy in bovines in relation to species, age and parity

Group	Distribution	Species		Age(yrs) (Mean±SE)	Parity							
		Cow	Buffalo		Heifer	1st	2nd	3rd	4th	5th	6th	>6th
Peritonitis	16	6(37.5)	10(62.5)	4.88 ± 0.49	0	6	3	5	0	0	1	1
Traumatic pericarditis	7	2(28.6)	5(71.4)	5.64 ± 0.8	0	0	4	1	0	1	1	0
Diaphragmatic hernia	6	1(16.7)	5(83.3)	8.33 ± 0.67	0	0	1	2	1	1	0	1
Omasal impaction	3	0	3(100)	6.67 ± 0.67	0	0	0	1	1	0	1	0
Paralytic ileus	3	1(33.3)	2(66.7)	7.67 ± 1.2	0	0	0	2	1	0	0	0
Late pregnancy indigestion	7	2(28.6)	5(71.4)	5.07 ± 0.52	0	1	1	4	0	1	0	0
Miscellaneous disorders	8	4(50.0)	4(50.0)	5.5 ± 0.57	0	1	3	2	1	1	0	0
Total	50	16(33.3)	34(66.7)	5.8 ± 0.29	0	8	12	17	4	4	3	2

4.1.2 Signalment

Higher occurrence of gastrointestinal disorders was seen in buffaloes (66.7%) as compared to cattle (33.3%). In total, 37 animals were in 1^{st}-3^{rd} lactation, 11 animals in 4^{th}-6^{th} lactation and only two animals were in $>6^{th}$ lactation. High occurrence of gastrointestinal disorders was observed in 3^{rd} lactation.

4.1.3 History

4.1.3.1 Duration of illness

The signs of TP (11.14 ± 2.35 days), DH (11 ± 2.34 days), peritonitis (9.93 ± 1.65 days) and omasal impaction (8 ± 3.51 days) developed slowly and were not appreciated by the owner considerably as compared to other disorders like paralytic ileus (3.3 ± 0.33 days), late pregnancy indigestion (4.29 ± 0.52 days) and miscellaneous (5.67 ± 0.91 days).

4.1.3.2 Feeding status

Overall, 30 animals had history of complete anorexia and 20 animals had history of reduced feed intake (Table 4). Hussain and Uppal (2014) reported complete anorexia in 28 animals out of 32 cases of peritonitis. Similarly Toor (2003) and Hussain *et al* (2013) reported anorexia in animals suffering from omasal impaction, whereas, Braun *et al* (2007) reported decrease in appetite in animals with TP. Hussain *et al* (2014b) observed partial or complete anorexia in LPI. In general water intake was reduced in 37 (74%) cases and was absent in six (12%) cases (Table 2) and was normal in seven cases (14%).

4.1.3.3 Regurgitation

Out of 50 cases, regurgitation was present in three cases only including two DH and one omasal impaction (Table 4). It could be attributed to inability of ingesta to pass further down the digestive tract due to reticular/omasal malfunction and involvement of vagus nerve (Leek 1969). The disease conditions with regurgitation in present study have been incriminated as causes of regurgitation by Smith (2002).

Table 4: Anamnesis (duration of illness, feed intake, water intake and regurgitation) of various types of gastrointestinal disorders

Group	Duration of illness (days) (Mean ± S.E)	Feed Intake		Water Intake			Regurgitation	
		Reduced	Absent	Absent	Reduced	Normal	Present	Absent
Peritonitis	9.81 ± 1.55	6	10	2	11	3	0	16
Traumatic pericarditis	11.14 ± 2.35	2	5	1	6	0	0	7
Diaphragmatic hernia	11 ± 2.34	2	4	1	4	1	3	3
Omasal impaction	8 ± 3.51	1	2	0	3	0	1	2
Paralytic ileus	3.3 ± 0.33	0	3	1	2	0	0	3
Late pregnancy indigestion	4.29 ± 0.52	4	3	1	4	2	0	7
Miscellaneous disorders	5.38 ± 0.98	5	3	0	7	1	0	8
Total	20 ± 8.2	20	30	6	37	7	4	46

4.1.3.4 History of fever and pain

Overall history of fever was observed in 23 cases and history of pain in 21 cases (Table 5). History of fever was seen in majority in cases of peritonitis (8/16), TP (6/7) and DH (3/6). History of fever in cases of peritonitis was recorded to be common sign in initial stages of peritonitis which corroborates with observations of Hosgood and Salisbury (1989).

History of pain was observed in five cases of peritonitis (5/11), five cases of TP (5/7), two cases of DH (2/6), three cases of omasal impaction (3/3), two cases of paralytic ileus (2/3), two cases of LPI (2/7) and two cases of miscellaneous conditions (2/8). This was in agreement with the observations of Cable *et al* (1998) and Smith (2002). Signs of pain and systemic reaction were reported to be more pronounced in cows compared to buffaloes having TRP and its sequele (Saleh *et al* 2008 and Abdelaal *et al* 2009).

Table 5: Anamnesis (history of fever and history of pain) of various groups of gastrointestinal disorders

Group	H/O Fever		H/O Pain	
	Yes	No	Yes	No
Peritonitis	8	8	5	11
Traumatic pericarditis	6	1	5	2
Diaphragmatic hernia	3	3	2	4
Omasal impaction	0	3	3	0
Paralytic ileus	2	1	2	1
Late pregnancy indigestion	3	4	2	5
Miscellaneous disorders	1	7	2	6
Total	23(46.0)	27(54.0)	21(42.0)	29(58.0)

4.1.3.5 Defecation status

Out of 50 animals, nine animals had history of absence of defecation, 30 animals were passing scanty faeces, six animals had history of diarrhea and five animals had normal faecal output (Table 6).

In case of peritonitis 12/16 animals had history of scanty faeces, 2 animals had diarrhea and loss of defaecation was seen in only one animal. Guard (2002) reported that in acute cases of peritonitis, faeces were present in small amounts and often dry while in chronic cases faeces tend to be diarrhoeic. Khalek El Sheikh *et al* (2012) observed chronic diarrhea in acute diffuse TRP.

In traumatic pericarditis, four out of seven animals were passing scanty faeces and two animals had history of diarrhea. In TP, main signs exhibited by animals were reduced defecation preceded or followed by brisket edema. Bexiga *et al* (2008) found either scanty or diarrheic faeces in majority of the cases suffering from pericarditis.

Majority of animals (5/6) suffering from DH passed scanty faeces and only one animal was presented with history of complete loss of defecation. Constipation and passing of small amounts of pasty faeces in cases of DH has also been reported by earlier workers (Krishnamurthy *et al* 1985 and Saini *et al* 2007).

In cases of omasal impaction, history of scanty faeces was observed in two out of three cases while complete loss of defaecation in only one case. In almost every case, the complaint by the owners was that animal was passing black colour scanty faeces. Randhawa *et al* (1996) reported the signs of scanty faeces in omasal impaction.

In cases of paralytic ileus, two animals (2/3) had history of scanty faeces and one animal had complete loss of defaecation. Smith (2009) reported that ileus is a condition of the intestinal tract that mimics complete intestinal obstruction.

In majority of animals suffering from late pregnancy indigestion, the main clinical sign observed was scanty faeces (4/7) or loss of defecation (2/7). Hussain *et al* (2014b) reported that in late pregnancy indigestion, consistent owner complaints were scanty or complete loss of defecation and anorexia or inappetance in advanced pregnancy. It could be due to hormonal changes in pregnancy.

4.1.3.6 Faecal colour:

In total 20 animals were presented with the history of passing black coloured faeces, 18 animals with the history of mucoid faeces and 12 animals with normal coloured faeces (Table 6). History of black coloured faeces was mainly observed in cases of peritonitis (7/16), TP (3/7), DH (3/6), omasal impaction (2/3) and LPI (3/7). The black or tarry colour of faeces may be attributed to bleeding from abomasal ulcers, hemorrhage due to penetrating/potential foreign bodies. Tarry coloured faeces in these conditions have been reported by Radostitis *et al* (2007).

4.1.3.7 Tympany:

Out of 50 animals, 24 animals had history of tympany (Table 6). The tympany was recurrent in 22 animals, persistent in two animals and was absent in 26 animals. In peritonitis group, recurrent tympany was present in seven animals (7/16), persistent in only one animal and was absent in half of the cases (8/16).

Most of the cases of TP (5/7) did not show any tympany while two animals had history of tympany only once. In contrast, Saleh *et al* (2008) reported ruminal bloat in TP.

There was a history of recurrent tympany in majority of the animals (5/6) suffering from DH. Interference of rumen peristalsis owing to reticular adhesions was regarded as major cause of tympany (Mally and Jayadevappa 1974). Recurrent tympany in case of DH has also been reported by Krishnamurthy *et al* (1985) and Saini *et al* (2007).

Among animals suffering from omasal impaction, two (2/3) animals had history of recurrent tympany. Nayak and Suresh Babu (1996) reported that omasal impaction, may give rise to chronic tympany.

In animals with late pregnancy indigestion, recurrent tympany was present in only two cases (2/7) and was absent in rest.

Table 6: Anamnesis (Faecal output, faecal colour and status of tympany) of various groups of gastrointestinal disorders

Group	Faecal output				Faecal colour			Status of Tympany		
	Absent	Scanty	Diarrhea	Normal	Black	Mucoid	Normal	Absent	Recurrent	Persisitent
Peritonitis	1	12	2	1	7	6	3	8	7	1
Traumatic pericarditis	0	4	2	1	3	1	3	5	2	0
Diaphragmatic hernia	1	5	0	0	3	3	0	1	5	0
Omasal impaction	1	2	0	0	2	1	0	1	2	0
Paralytic ileus	1	2	0	0	0	3	0	3	0	0
Late pregnancy indigestion	2	4	1	0	3	3	1	5	2	0
Miscellaneous disorders	1	4	1	2	2	1	5	3	4	1
Total	7	33	6	4	20	18	12	26	22	2

4.1.3.8 Pregnancy

Out of 50 animals suffering from gastrointestinal disorders, 24 (44%) animals were in eighth month, 12 (26%) in ninth month, eight (20%) in seventh month, two (4%) in sixth month and four (6%) in tenth month of pregnancy (Table 7). Incidence was more in eighth month of pregnancy. Recent parturition or advanced pregnancy has been reported to be associated with TRP, TP and DH (Prasad *et al* 1977, Deshpande *et al* 1982, Krishnamurthy *et al* 1983, Gokce *et al* 2007, Radostitis *et al* 2007 and Kumar *et al* 2012a). It could be correlated to increased intra-abdominal pressure due to gravid uterus. Turkar and Uppal (2007) reported higher incidence of omasal impaction in pregnant animals. Van Metre *et al* (1995) and Hussain *et al* (2014b) reported that late pregnancy indigestion is common in advanced stage of pregnancy.

Table 7: Distribution of gastrointestinal disorders associated with advanced pregnancy in bovines in relation to gestation period

Group	Species	Gestation period (month)				
		6^{th}	7^{th}	8^{th}	9^{th}	10^{th}
Peritonitis	Cattle	0	1	3	2	0
	Buffalo	0	2	3	3	2
Traumatic period	Cattle	0	0	1	1	0
	Buffalo	0	1	2	1	1
Diaphragmatic hernia	Cattle	0	0	1	0	0
	Buffalo	1	1	3	0	0
Omasal impaction	Cattle	0	0	0	0	0
	Buffalo	0	1	1	1	0
Paralytic ileus	Cattle	0	0	1	0	0
	Buffalo	0	0	1	1	0
Late pregnancy indigestion	Cattle	0	0	1	1	0
	Buffalo	0	1	2	1	1
Miscellaneous Disorders	Cattle	1	0	3	0	0
	Buffalo	0	1	2	1	0
Total		2	8	24	12	4

4.1.4 Physical examination

4.1.4.1 General condition

Among 50 animals, 28 animals were dull in appearance while 22 animals were alert on physical examination. Most of animals (20/50) had congested mucous membrane which could be due to the inflammatory response. Mucous membrane was anemic in 17 and normal in 13 animals (Table 8).

4.1.4.2 Ruminal motility

Ruminal motility was found to be nil in 22 animals, decreased in 18 animals and increased in five animals. It was normal in five animals (Table 8). The excessive distension by bulky ingesta may precipitate continuous activation of the epithelial receptors in forestomach lining and lead to rumen stasis, as it has a reflex inhibitory effect on primary cycle motility (Leek 1983). In peritonitis, rumen motility was absent in 10 out of 16 cases. The suspended ruminal motility may be attributed to adhesions or generalized gastrointestinal tract atony in case of peritonitis.

4.1.4.3 Temperature, heart rate and respiration rate

The mean rectal temperature at the time of presentation was almost within normal physiological limits (Table 9). Overall 29 animals had body temperature in the range of 99-102ºF, <99ºF in 2 and >102ºF in 19 animals. Fever (>102ºF) on physical examination was observed in few animals of each group viz; peritonitis (4/16), TP (4/7), DH (2/6), omasal impaction (2/3), paralytic ileus (1/3), late pregnancy indigestion (3/7) and miscellaneous (3/8). Fever has been reported to be a feature of TP, peritonitis and TRP (Radostitis *et al* 2007).

Out of 50 animals heart rate was <60 (beats per minute) in 6, 60-80 in 26 and >80 in 18 animals. The mean heart rate was higher in TP (97.4 ± 4.61 per min), late pregnancy indigestion (82 ± 5.24 per min) and peritonitis (79.6 ± 3.14 per min). Radostitis *et al* 2007 also reported increased heart rate in TP, TRP, intestinal obstruction and miscellaneous conditions. Bradycardia (<60 beats per minute) was observed in four cases of peritonitis, one case of TP and one case of late pregnancy indigestion.

Respiration rate was elevated in TP (46.3 ± 7.07), late pregnancy indigestion (45.1 ± 3.38) and paralytic ileus (42.7 ± 2.90) than rest of groups. These findings are in agreement with Radostitis *et al* (2007).

Table 8: Clinical observations (demeanour, mucous membrane and ruminal motility) in various groups of gastrointestinal disorders

Group	Demeanour		Mucous membrane			Rumen motility			
	Alert	Dull	Anaemic	Congested	Normal	Normal (3/2min)	Decreased (1-2/2min)	Increased (>3/2min)	Nil
Peritonitis	6	10	3	6	7	1	1	4	10
Traumatic pericarditis	2	5	3	2	2	0	4	0	3
Diaphragmatic hernia	2	4	3	3	0	1	3	1	1
Omasal impaction	2	1	0	2	1	0	2	0	1
Paralytic ileus	0	3	0	3	0	0	0	0	3
Late pregnancy indigestion	4	3	1	3	3	0	4	0	3
Miscellaneous disorders	6	2	3	1	4	3	4	0	1
Total	22	28	13	20	17	5	18	5	22

Table 9: Physical examination (temperature, heart rate and respiration rate) in various groups of gastrointestinal disorders

Group	Temperature (ºF)				Heart rate (per minute)				Respiration rate (per minute)
	<99ºF	99-102ºF	>102ºF	(Mean±SE)	<60	60-80	>80	(Mean±SE)	(Mean±SE)
Peritonitis	2	10	4	101.5 ± 0.33	4	7	5	79.6 ± 3.14	28.3 ± 2.69
Traumatic pericarditis	0	3	4	102.7 ± 0.53	1	2	4	97.4 ± 4.61	46.3 ± 7.07
Diaphragmatic hernia	0	4	2	101.7 ± 0.46	0	4	2	76.5 ± 4.33	40.3 ± 3.88
Omasal impaction	0	1	2	101.6 ± 0.8	0	2	1	72.7 ± 8.19	34.7 ± 4.37
Paralytic ileus	0	2	1	101.4 ± 1.2	0	2	1	72.7 ± 8.9	42.7 ± 2.90
Late pregnancy indigestion	0	4	3	101.7 ± 0.56	1	3	3	82 ± 5.24	45.1 ± 3.38
Miscellaneous disorders	0	5	3	101.8 ± 0.39	0	5	3	77.5 ± 7.19	40.3 ± 7.64
Total	2	29	19	101.9 ± 0.19	6	26	18	80.9 ± 2.15	37.8 ± 2.1

4.1.4.4 Other findings on physical examination

Majority of animals suffering from traumatic pericarditis showed brisket edema, abducted elbows, muffled heart sounds and open mouth breathing. Brisket edema was present in all cases of traumatic pericarditis, one case each of peritonitis and DH. Similar but variable clinical signs in animals suffering from pericarditis were reported by various authors (Senna *et al* 2003 and Braun 2009).

In addition to brisket edema, abdominal distension was also seen in two cases each of DH and LPI. Hussain *et al* (2014b) observed abdominal distension in cases of LPI.

4.1.5 Examiniation of blood smears

Blood smear examination revealed moderate to severe anaemia, which varied from normochromic normocytic to macrocytic hypochromic (Fig. 1) sub types. Anemia in about half of the cases was regenerative characterised by anisocytosis, polychromasia, hypochromasia and a few nucleated RBC's. Other abnormalities in the morphology of RBC's were also detected, which included presence of acanthocytes (Fig. 2), schistocytes, RBC fragments, indicating severe hepatic dysfunction/damage, crenation and crowding of RBC's, indicating hemoconcentration, marked rouleaux formation (Fig. 3) hinting at increased erythrocyte sedimentation rate and presence of heinz bodies (Fig. 4), indicating oxidative stress in affected animals.

Majority of cases revealed neutrophilia (Fig. 5 and 6) with morphological changes suggestive of regenerative to degenerative left shift (Fig. 7 and 8) and leukemoid response (Fig. 9) in some of the cases. The penetrating foreign bodies, which were responsible for majority of the cases of TRP, TP and DH, might induce inflammatory reaction at the site of penetration, resulting in increased TLC. In majority of cases the DLC was found to be better and more reliable indicator than TLC especially, in cases with normal TLC, as in some cases left shift was observed without increase in TLC. Thrombocytosis, at time massive (Fig. 10), was also observed in many cases along with presence of immature platelets, indicating septicaemia.

4.1.5 Haematological findings

4.1.5.1 Peritonitis

Mean Hb and PCV values did not differ significantly from control values in both species (Table 10). MCV was found significantly higher and MCHC was significantly lower than the control values, indicating macrocytic hypochromic anaemia (Table 11). Platelet count was significantly higher in both cattle (5.33 ± 0.23) and buffalo (3.69 ± 0.43).

Mean TLC (14719 ± 1.20/µL) and mean absolute neutrophil count (10425 ± 0.92/µL) were significantly higher than control values in buffaloes (Table 12 and 13). In cattle, they were on higher side but not statistically significant. Neutrophilic leukocytosis was observed in ten cases (7 buffaloes and 3 cattle), three cases had absolute neutrophilia (2 buffaloes and 1 cattle); two had relative neutrophilia (1 buffalo and 1 cattle) and one had leucopoenia (1 cattle). The reversal of lymphocyte to neutrophil ratio was observed in both the species (Table 13). In addition, blood smear examination revealed mild left shift in two cases (2/15), mild to moderate in four (4/15) and marked in seven cases (7/15). Severe toxic changes in neutrophils were observed in five cases (5/15). Similar haematological findings were reported by Fecteau (2005), El-Ashker *et al* (2013) and Hussain and Uppal (2014). Leukocytosis, thrombocytopenia and erythrocytopenia are secondary conditions which are observed in association with viral and bacterial septicaemias and in chronic inflammatory diseases including TRP (Coles 1986 and Ward and Ducharme 1994). The leucocytosis with neutrophilia may also be attributed to an immunological response to endotoxemia, abomasitis and peritonitis (Zadnik 2003).

4.1.5.2 Traumatic pericarditis:

Mean Hb and PCV values were significantly lower than control values in cattle (Table 10). Marked non regenerative anemia was observed in two cases. Anemia in TP could be attributed to the loss of blood during penetration of the reticulum or the chronic inflammatory process (Ocal *et al* 2008). Ghanem (2010) reported erythrocytopenia and lower Hb concentrations in the cows with TRP and TP. However, in buffaloes, the mean values of Hb and PCV were within normal physiological range. These findings were in consonance with earlier findings of Bexiga *et al* (2008). MCV was significantly higher in buffaloes while MCHC was

significantly lower in cows (Table 11). Platelets were significantly higher in both the species (Table 10). Thrombocytosis was observed in six cases (4 buffaloes and 2 cattle).

Mean TLC and mean absolute neutrophil count was on higher side in both the species; however it was statistically significant in only buffaloes (Table 12 and 13). Neutrophilic leukocytosis was observed in five cases (4 buffaloes and 1 cattle) of TP. The reversal of lymphocyte to neutrophil ratio was observed in both the species (Table 13). Braun *et al* (2007) observed leukocytosis and neutrophilia in traumatic pericarditis. Mild to moderate left shift was observed in three cases (3/7). In rest of the cases, most of the neutrophils were mature. Hemato-biochemical findings in animals suffering from pericarditis are non- specific and are indicative of any suppurative or toxemic condition of the body (Radostits *et al* 2007).

4.1.5.3 Diaphragmatic hernia

The mean value of Hb and PCV were within normal range (Table 8). Krishnamurthy *et al* (1983) and Behl *et al* (1996) have also recorded normal Hb levels in cases of DH. MCV was found significantly higher and MCHC significantly lower in both the species (Table 11).

The mean TLC did not differ significantly from control values (Table 12). Similar findings were reported by Turkar and Uppal (2006). Mean absolute neutrophil count was significantly higher than control value (Table 13). Neutrophilic leukocytosis was observed in two cases (1 cow and 1 buffalo) out of six cases of DH. In rest of the cases, relative neutrophilia was observed (4/6). The higher TLC in certain cases may be due to presence of penetrating foreign bodies in reticulum. Left shift in neutrophils was found to be mild in three cases, moderate in one case and massive in two cases. Toxic changes were seen in three cases (3/6).

4.1.5.4 Omasal impaction

The mean Hb and PCV did not differ significantly from control values (Table 10). Similar findings with respect to Hb were recorded by Turkar and Uppal (2007). MCV (56.1 ± 1.84 fL) was found significantly higher and MCHC (34.7 ± 0.61) significantly lower in both the species MCV and MCHC were significantly higher and lower than the control value respectively (Table 11).

The mean TLC and absolute neutrophil count did not differ significantly (Table 12 and 13). Neutrophilic leukocytosis was observed in one case and relative neutrophilia was observed in two cases. Neutrophilia may result from chronic irritation of forestomach wall by impacted feed materials, leaving the wall exposed to secondary infection (Hailat *et al* 1996). Leukopenia was observed in two cases. Mild left shift in neutrophils was noticed in all cases of omasal impaction.

4.1.5.5 Paralytic ileus

Only three cases of paralytic ileus were observed during study (2 buffalo and 1 cattle). PCV was on higher side in all cases (Table 10). MCV (61.6 ± 4.3) was also found to be significantly higher than control value (Table 11). Platelets (4.08 ± 0.12×10^{5}/µl) were significantly higher than control value.

Neutrophilic leukocytosis was observed in one case and relative neutrophilia was observed in two cases. Mild left shift in neutrophils was also noticed in two cases only while toxic changes were observed in only one case.

4.1.5.6 Late pregnancy indigestion

There were no significant differences in Hb concentration, PCV and TLC from control values (Table 10 and 12). Similar findings were reported by Hussain *et al* (2014) in late pregnancy indigestion. Absolute neutrophilia was observed in three cases (2 buffaloes and 1 cattle) and relative neutrophilia was observed in four cases (3 buffaloes and 1 cattle) of late pregnancy indigestion.

4.1.5.7 Miscellaneous conditions

The mean Hb, PCV and TLC did not differ significantly from control value.

4.1.6 Clinical chemistry

The mean concentration of total protein, albumin and globulin (Table 14) in control group were similar to reported by Smith (2002) and Abd Ellah *et al* (2014). Total bilirubin, AST, ALKP, GGT, BUN, creatinine and lactate mean values (Table 15 and 16) were similar to as reported by Kaneko et al (1997), Canfield *et al* (1984) and Radostitis *et al* (2007). Mean sodium, potassium, chloride, calcium, phosphorus and magnesium values in control were similar to as reported by Canfield *et al* (1984) and Smith (2002).

Table 10: Haemoglobin, PCV and platelet count in various groups of gastrointestinal disorders in advanced pregnancy in bovines

Group	Haemoglobin (g/dl) Mean ± S.E. (Range)		PCV (%) Mean ± S.E. (Range)		Platelets ($\times 10^5$/µl) Mean ± S.E. (Range)	
	Cattle	Buffalo	Cattle	Buffalo	Cattle	Buffalo
Control	10.72 ± 0.15 (10.2-11)	12.59 ± 0.78 (9-15)	30.6 ± 0.19 (29.9-31)	34.5 ± 2.09 (25.4-41.6)	3.11 ± 0.29 (2.34-3.78)	1.82 ± 0.30 (0.97-3.27)
Peritonitis	9.85 ± 0.85 (8.2-13)	11.62 ± 0.87 (5.9-16.5)	30.1 ± 2.59 (25-40)	33.6 ± 2.76 (18-46.7)	5.33 ± 0.23** (4.82-6.27)	3.75 ± 0.39** (1.78-5.74)
Traumatic pericarditis	6.15 ± 0.25** (5.9-6.4)	10.46 ± 1.21 (7.9-13.6)	18.1 ± 0.4** (17.7-18.5)	31.9 ± 4.63 (21.6-43.1)	8.68 ± 0.56* (8.12-9.23)	4.45 ± 1.42** (1.62-5.99)
Diaphragmatic hernia	11.7	11.42 ± 1.49 (6.6-15.8)	31.7	34.3 ± 3.89 (21.2-45)	4.28	3.19 ± 0.25* (2.68-3.98)
Omasal impaction	-	12.57 ± 2.8 (9-18.1)	-	37.9 ± 8.66 (26.8-55)	-	3.16 ± 0.67 (2-4.32)
Paralytic ileus	12.6	12.9 ± 0.6 (12.3-13.5)	38	39.6 ± 4.05 (35.6-43.7)	4.84	4.08 ± 0.12* (3.96-4.2)
Late pregnancy indigestion	11.75 ± 0.55 (11.2-12.3)	11.52 ± 1.41 (7.9-15.2)	34.5 ± 1.5 (33-36)	33.7 ± 3.98 (22.3-43)	3.80 ± 0.54 (3.26-4.34)	2.02 ± 0.45 (1.02-3.19)
Miscellaneous disorders	7.73 ± 1.47 (3.5-10.1)	12.71 ± 1.35 (11-15.4)	23.3 ± 4.7 (9.5-30.2)	34.7 ± 3.58 (29-45)	3.73 ± 0.98 (1.01-5.54)	1.82 ± 0.13 (1.56-2.16)

***Indicates significance level of ≤5%**

**** Indicates significance level of ≤1%**

Table 11: TEC, MCH, MCV and MCHC in various groups of gastrointestinal disorders in advanced pregnancy in cattle and buffalo

Group	TEC (×10⁶/µL) Mean ± S.E. (Range)		MCH (pg) Mean ± S.E. (Range)		MCV (fL) Mean ± S.E. (Range)		MCHC (g/dL) Mean ± S.E. (Range)	
	Cattle	Buffalo	Cattle	Buffalo	Cattle	Buffalo	Cattle	Buffalo
Control	6.39 ± 0.11 (5.99-6.62)	7.05 ± 0.58 (4.26-8.39)	16.6 ± 0.43 (15.5-17.7)	18.21 ± 0.59 (16.4-21.2)	47.6 ± 0.66 (46.1-49.9)	49.8 ± 1.95 (42.7-59.7)	35.0 ± 0.47 (33.6-35.9)	36.7 ± 0.41 (35.6-38.5)
Peritonitis	6.07 ± 0.47 (5.23-7.32)	6.83 ± 0.28 (5.36-7.77)	17.3 ± 2.29 (13.1-21.5)	18.6 ± 0.81 (16.2-23.8)	60.3 ± 4.63* (45.6-68.1)	56.0 ± 2.47* (46.2-67.6)	32.8±0.57* (31.4-34.6)	34.9 ± 0.53* (31.5-36.6)
Traumatic pericarditis	3.44 ± 0.76 (2.68-4.19)	5.28 ± 1.25 (3,96-7.77)	18.4 ± 3.7 (14.7-22.1)	19 ± 0.84 (17.5-20.4)	55 ± 11 (44-66)	54.6 ± 0.8* (53-55.4)	33.5* (8.12-9.23)	34.8 ± 1.66 (31.5-36.7)
Diaphragmatic hernia	6.76	5.81 ± 0.63 (3.39-6.89)	26.5	17.9 ± 0.48 (16.7-19.5)	72.7	60.3 ± 1.51** (54.4-62.5)	36.4	31 ± 0.67** (28.9-32.8)
Omasal impaction	-	5.62 ± 0.66 (4.65-6.89)	-	21.4 ± 1.18 (19.4-23.5)	-	56.1 ± 1.84* (52.4-58.2)	-	34.7 ± 0.61* (33.7-35.8)
Paralytic ileus	6.65	6.42 ± 0.21 (6.21-6.63)	20.1	20.1 ± 0.25 (19.8-20.3)	58	61.6 ± 4.3 (57.3-65.9)	35	32.8 ± 1.85 (30.9-34.6)
Late pregnancy indigestion	6.09 ± 0.79 (5.30-6.89)	5.80 ± 1.12 (4.12-9.07)	17.4 ± 0.55 (16.8-17.9)	19.1 ± 0.88 (16.8-21.1)	50.2 ± 2.05 (48.1-52.2)	55.7 ± 3.71 (46.1-63.5)	32.7 ± 1.5 (31.2-34.2)	34.4 ± 0.86 (32.8-36.4)
Miscellaneous disorders	5.06 ± 1.07 (1.95-6.84)	6.51 ± 0.48 (6.03-6.99)	15.8 ± 0.88 (14-17.8)	17.6 ± 0.65 (16.9-18.2)	46.5 ± 1.55 (43.5-49.4)	49.8 ± 1.65 (48.1-51.4)	33.9 ± 0.89 (32.2-36.4)	35.4 ± 0.15* (35.2-35.5)

***Indicates significance level of ≤5%**

**** Indicates significance level of ≤1%**

Table 12: Total and differential leukocyte count in various groups of gastrointestinal disorders in advanced pregnancy in bovines

Group	TLC (/µl) Mean ± S.E. (Range)		Neutrophils (%) Mean ± S.E. (Range)		Lymphocytes (%) Mean ± S.E. (Range)	
	Cattle	Buffalo	Cattle	Buffalo	Cattle	Buffalo
Control	13520 ± 4.02 (12480-14410)	11217 ± 5.97 (9310-13740)	42.4 ± 3.14 (32-50)	37.1 ± 4.19 (22-55)	56 ± 2.60 (50-64)	61.1 ± 4.19 (42-77)
Peritonitis	13767 ± 2.86 (4380-26000)	14258 ± 1.17* (10000-22580)	72.3 ± 6.38** (56-98)	73.4 ± 3.71 (56-88)	27 ± 6.08** (2-42)	26.0 ± 3.45 (12-44)
Traumatic pericarditis	20375 ± 1.23 (8070-32680)	15192 ± 1.35* (10430-18220)	78 ± 6.0* (72-84)	72.8 ± 3.88** (62-82)	22 ± 6.0 (16-28)	26.8 ± 3.61** (18-36)
Diaphragmatic hernia	14400	11232 ± 9.80 (8000-14030)	39	74.4 ± 4.26 (58-82)	60	25.6 ± 4.26* (18-42)
Omasal impaction	-	7900 ± 2.39 (4500-12500)	-	77.7 ± 1.45 (75-80)	-	22.3 ± 1.45 (20-25)
Paralytic ileus	16300	8780 ± 1.61 (7170-10390)	84	63 ± 17 (46-80)	16	37 ± 17 (20-54)
Late pregnancy indigestion	8705±1.90 (6800-10610)	9982 ± 1.39 (6500-13750)	54 ± 4 (50-58)	61.2 ± 4.59** (52-78)	44 ± 6 (38-50)	38.8 ± 4.59** (22-48)
Miscellaneous disorders	10312 ± 2.00 (4620-13940)	9273.3 ± 3.31 (5950-15900)	55 ± 7.33 (34-68)	46.7 ± 9.68 (36-66)	43.5 ± 7.68 (32-66)	53.3 ± 9.68 (34-64)

***Indicates significance level of ≤5%**

**** Indicates significance level of ≤1%**

Table 13: Absolute neutrophil and leukocyte count and neutrophil to lymphocyte ratio in various groups of gastrointestinal disorders in advanced pregnancy in bovines

Group	Absolute Neutrophils (/µL) Mean ± S.E. (Range)		Absolute Lymphocytes (/µL) Mean ± S.E. (Range)		Neutrophil to Lymphocyte ratio Mean ± S.E. (Range)	
	Cattle	Buffalo	Cattle	Buffalo	Cattle	Buffalo
Control	5754.6 ± 5.08 (3994-6880)	4101.4 ± 4.02 (2361-5440)	7561 ± 3.64 (6588-8568)	6913.9 ± 6.64 (4154-8391)	0.77 ± 0.09 (0.5-1)	0.66 ± 0.13 (0.29-1.31)
Peritonitis	10716.2 ± 3.17 (2452-25480)	10425 ± 0.92** (6664-17161)	2985.5 ± 7.49** (520-5738)	3751.1 ± 5.91** (1518-6591)	10.41 ± 7.78 (1.33-49)	3.72 ± 0.72** (1.27-7.33)
Traumatic pericarditis	16630 ± 1.08 (5810-27451)	10964 ± 9.84** (8553-14576)	3744.5 ± 1.48 (2260-5229)	4159.4 ± 7.46* (1877-6167)	3.91 ± 1.34 (2.57-5.25)	3.01 ± 0.56** (1.72-4.56)
Diaphragmatic hernia	5616	8359.4 ± 9.16** (6206-11224)	8640	2872.6 ± 4.85** (1440-4494)	0.65	3.26 ± 0.54** (1.38-4.56)
Omasal impaction	-	6200 ± 1.97 (3375-10000)	-	1699.7 ± 4.13** (1125-2500)	-	3.52 ± 0.29** (3-4)
Paralytic ileus	13692	5805 ± 2.51 (3298-8312)	2608	2975 ± 8.97 (2078-3872)	5.25	2.42 ± 1.58 (0.85-4)
Late pregnancy indigestion	4624.5 ± 6.80 (3944-5305)	6322 ± 1.33 (3510-10725)	3944 ± 1.36 (2584-5304)	3660 ± 3.57** (2990-4864)	1.27 ± 0.27 (1-1.53)	1.79 ± 0.45 (1.08-3.55)
Miscellaneous disorders	6069 ± 1.57 (1571-8364)	4968.3 ± 2.76 (2142-10494)	4080 ± 5.33** (3049-5576)	4305 ± 5.51* (3701-5406)	1.04 ± 0.45 (0.56-1.94)	1.04 ± 0.45s (0.56-1.94)

***Indicates significance level of ≤5%**

**** Indicates significance level of ≤1%**

Serum total protein, albumin and globulin levels:

There was no significant difference in the total serum protein in different groups of gastrointestinal disorder (Table 14). However, the albumin concentration was significantly lower in almost every group which may be ascribed to chronic starvation or failure of liver to synthesise adequate amounts of protein (Kaneko *et al* 1997).

The increased globulin concentration was observed in few groups. It may be due increased synthesis of globulins as a result of humoral response to antigen from enteric bacterial antigens (Parraga *et al* 1995). Hyper-globulinemia in cattle is observed in cattle suffering from TRP (Hirvonen and Pyorala 1998) and traumatic pericarditis (Fisher and Pirie 1965).

Significant decrease in albumin to globulin (A/G) ratio was observed in all groups. During chronic inflammatory disease, the A/G ratio usually decreases because of an increased globulin concentration, which may or may not be accompanied by a small decrease in albumin concentration (Ward and Ducharme 1994 and Roussel *et al* 1997).

Serum Aspartate amino transferase levels

AST was significantly higher than the control value in all groups (Table 15). The increased AST activity detected in the present study may reflect liver and/or muscle disorders in different groups (Kramer and Hoffmann 1997). It may also be high in cattle with hepatic lipidosis, passive venous congestion, and diseases that cause distension of the forestomachs and abomasum (Kramer and Hoffmann 1997, Moore 1997 and Roussel *et al* 1997). Ghanem (2010) observed significant increase in AST activity in TRP and TP. Similarly, increase in AST activity in DH, omasal impaction, paralytic ileus and LPI has been reported earlier by Turkar and Uppal (2006), Turkar and Uppal (2007), Nuss *et al* (2006) and Hussain *et al* (2014b), respectively.

Serum alkaline phosphatase levels

Similar to AST, the activity of ALKP was significantly or non-significantly higher than the control value (Table 15). The increased ALKP activity may be due to tissue damaged caused by toxic substances produced by gastrointestinal disturbances in these cases. Furthermore the increased ALKP activity may be due to varying

degrees of increased ALKP activity in hepatocytes, Kupffer's cells, connective tissue of portal areas of liver as well as in tubular epithelium and glomeruli of kidney, cardiac muscles and alveolar epithelium of lungs (Singh *et al* 1989).

Serum gamma-glutamyl transferase levels

The activity of GGT was also significantly or non-significantly higher than the control value in all groups (Table 15). Increased activity of GGT may be due to biliary obstruction, chronic active hepatitis and cholangiohepatitis (Smith 2002). Braun *et al* (2007) reported increased activities of GGT, AST and total bilirubin in TP indicate hepatic congestion and not primary liver disease.

Serum total bilirubin levels

Serum total bilirubin was significantly increased in all groups of animals (Table 15). In the present study, increased total bilirubin in association with increased ALKP and AST activity may indicate impaired hepatic function and/or hepatobiliary circulation.

Serum BUN and creatinine levels

In all groups, BUN was significantly or non-significantly higher than the control value (Table 16). The increased BUN level could be attributed to decrease in renal blood flow as a part of compensatory mechanism to maintain circulation in hypovolemia associated with dehydration (Kaneko *et al* 1997).

Creatinine concentration was also significantly or non-significantly higher than the control value (Table 16). The increased creatinine in present study may be attributed to hypovolemia and dehydration leading to azotemia. Constable *et al* (I997) and Singh *et al* (2000) also reported increased BUN and creatinine in intestinal obstructions in bovines.

Serum lactate levels:

The lactate level was significantly higher in all groups than control value (Table 16). The lactate level of blood is elevated when the rate of lactate production exceeds its use. An increase in blood lactate implies increased anaerobic metabolic activity, plus or minus a decrease in hepatic utilisation and, to a lesser extent, renal excretion. Hyperlactatemia is observed in shock and sepsis which leads to local or systemic hypoperfusion (Pang and Boysen 2007). Hughes (1999) has suggested that

Table 14: Clinical chemistry (total protein, albumin, globulin and albumin/globulin ratio) in various groups of gastrointestinal disorders in advanced pregnancy in bovines

Group	Total protein (g/dL) Mean ± S.E. (Range)		Albumin (g/dL) Mean ± S.E. (Range)		Globulin (g/dL) Mean ± S.E. (Range)		A/G Ratio Mean ± S.E. (Range)	
	Cattle	Buffalo	Cattle	Buffalo	Cattle	Buffalo	Cattle	Buffalo
Control	7.12 ± 0.24 (6.6-8)	6.97 ± 0.19 (6.2-7.6)	3.70 ± 0.1 (3.3-3.9)	3.76 ± 0.16 (3.4-4.3)	3.42 ± 0.24 (2.8-4.2)	3.07 ± 0.07 (2.8-3.3)	1.1 ± 0.09 0.9-1.36	1.12 ± 0.09 (0.72-1.4)
Peritonitis	6.58 ± 0.7 (4.8-8.5)	6.78 ± 0.6 (3.2-8.2)	2.76 ± 0.28* (2.1-3.4)	2.6 ± 0.18** (2.2-3.4)	3.48 ± 0.65 (2.1-5.8)	4.8 ± 0.39** (3.8-6)	0.73 ± 0.07** (0.47-0.87)	0.54 ± 0.06** (0.37-0.78)
Traumatic pericarditis	6.65 ± 0.45 (6.2-7.1)	6.95 ± 0.37 (5.9-7.6)	2.6 ± 0.1** (2.5-2.7)	2.95 ± 0.21* (2.4-3.4)	4.05 ± 0.5 (3.5-4.6)	4 ± 0.25* (3.5-4.6)	0.66 ± 0.12* (0.54-0.77)	0.74 ± 0.06** (0.65-0.92)
Diaphragmatic hernia	7.1	6.28 ± 0.54 (4.9-7.5)	2.6	2.6 ± 0.37* (1.7-3.5)	4.5	3.68 ± 0.18* (3.2-4)	0.58	0.69 ± 0.07** (0.53-0.88)
Omasal impaction	-	6.37 ± 0.67 (5.1-7.4)	-	3.5 ± 0.78 (2.4-5)	-	2.87 ± 0.33 (2.4-3.5)	-	1.27 ± 0.38 (0.89-2.04)
Paralytic ileus	8	7 ± 0.2 (6.8-7.2)	3.5	3.3 ± 0.1* (3.2-3.4)	4.5	3.7 ± 0.1* (3.6-3.8)	0.78	0.89 ± 0.01* (0.88-0.89)
Late pregnancy indigestion	7.2 ± 0.3 (6.9-7.5)	7.2 ± 0.77 (5.9-10.2)	3.6 ± 0.1 (3.5-3.7)	3.14 ± 0.21* (2.7-3.9)	3.6 ± 0.4 (3.2-4)	4.06 ± 0.58 (3.2-6.3)	1.02 ± 0.14 (0.88-1.16)	0.80 ± 0.06** (0.62-0.94)
Miscellaneous disorders	6.85 ± 0.47 (6.2-8.2)	7.23 ± 0.27 (6.7-7.9)	3.03 ± 0.17* (2.7-3.4)	3.25 ± 0.21 (2.8-3.8)	3.83 ± 0.39 (3.5-5)	3.98 ± 0.08** (3.8-4.1)	0.81 ± 0.07* (0.64-1)	0.82 ± 0.04** (0.72-0.93)

***Indicates significance level of ≤5%**

**** Indicates significance level of ≤1%**

Table 15: Clinical chemistry (AST, ALP, GGT and total bilirubin) in various groups of gastrointestinal disorders in advanced pregnancy in bovines

Group	AST (U/L) Mean ± S.E. (Range)		ALKP (U/L) Mean ± S.E. (Range)		GGT (U/L) Mean ± S.E. (Range)		Total bilirubin (mg/dL) Mean ± S.E. (Range)	
	Cattle	Buffalo	Cattle	Buffalo	Cattle	Buffalo	Cattle	Buffalo
Control	69 ± 4.13 (60-80)	99 ± 3.07 (87-110)	69 ± 1.58 (65-73)	103.6 ± 11.3 (69-155)	32 ± 4.55 (20-47)	25 ± 1.53 (18-30)	0.26 ± 0.07 (0.1-0.4)	0.27 ± 0.05 (0.2-0.5)
Peritonitis	210 ± 44.7* (98-326)	217.3 ± 17.8** (173-316)	68.8 ± 17.7 (32-123)	79.4 ± 9.2 (34-105)	57.6 ± 9.33* (40-85)	39.9 ± 6.43* (20-78)	0.82 ± 0.20* (0.4-1.4)	0.69 ± 0.15* (0.3-1.5)
Traumatic pericarditis	179. ± 5.5**s (174-185)	273.8 ± 52.1* (178-398)	55 ± 10.0 (45-65)	91 ± 25.8* (51-165)	51.5 ± 2.5* (49-54)	89.8 ± 14.1* (56-125)	1.25 ± 0.05** (1.2-1.3)	0.9 ± 0.18* (0.5-1.3)
Diaphragmatic hernia	168	637 ± 25.9** (599-713)	28	73.3 ± 7.56* (55-90)	61	55.5 ± 9.73* (38-83)	0.6	1.53 ± 0.33* (0.9-2.4)
Omasal impaction	-	254.3 ± 27.8* (199-286)	-	105.7 ± 8.09 (92-120)	-	77 ± 6.66** (66-89)	-	0.4 ± 0.1 (0.3-0.6)
Paralytic ileus	134	303 ± 19.5* (283-322)	173	45 ± 10* (35-55)	41	50.5 ± 1.5** (49-52)	0.9	0.95 ± 0.25** (0.7-1.2)
Late pregnancy indigestion	125 ± 38 (87-163)	204 ± 34.5* (132-324)	47 ± 5.0 (42-52)	40.8 ± 3.15** (33-48)	51 ± 17.5 (34-69)	33 ± 5.37 (22-47)	0.53 ± 0.03* (0.5-0.55)	0.96 ± 0.2* (0.5-1.7)
Miscellaneous disorders	126.3 ± 34.5 (73-225)	242 ± 43.0 (156-288)	42.8 ± 10.7 (25-74)	36.3 ± 8.45** (23-52)	84.3 ± 39.1 (27-159)	33 ± 12.5 (19-58)	0.5 ± 0.07* (0.4-0.7)	0.95 ± 0.13** (0.7-1.3)

***Indicates significance level of ≤5%**

**** Indicates significance level of ≤1%**

Table 16: Clinical chemistry (BUN, creatinine, lactate and CK) in various groups of gastrointestinal disorders in advanced pregnancy in bovines

Group	BUN (mg/dL) Mean ± S.E. (Range)		Creatinine (mg/dL) Mean ± S.E. (Range)		Lactate (mmol/L) Mean ± S.E. (Range)		CK (U/L) Mean ± S.E. (Range)	
	Cattle	**Buffalo**	**Cattle**	**Buffalo**	**Cattle**	**Buffalo**	**Cattle**	**Buffalo**
Control	13 ± 0.95 (10-15)	18.3 ± 2.02 (15-30)	1.3 ± 0.1 (1-1.6)	2.06 ± 0.17 (1.6-3)	3.44 ± 0.14 (3.1-3.8)	4.73 ± 0.43 (3.5-6.9)	184.6 ± 18.4 (148-252)	155.6 ± 9.46 (128-201)
Peritonitis	27.2 ± 11.9 (8-73)	15.6 ± 1.59 (9-20)	1.78 ± 0.50 (0.8-3.2)	1.73 ± 0.15 (1.2-2.3)	3.8 ± 0.98 (1.5-6.5)	8.41 ± 1.14** (5.8-15.3)	1222.2±3.58* (532-2178)	1260.9 ± 2.05** (572-2145)
Traumatic pericarditis	13.5 ± 0.5 (13-14)	51.3 ± 9.57* (34-78)	1.15 ± 0.15 (1-1.3)	5.58 ± 1.08* (4.1-8.7)	9.7 ± 0.5* (9.2-10.2)	9.08 ± 1.13* (6.8-11.8)	338.5 ± 26.5* (312-365)	2140 ± 3.33** (1564-2775)
Diaphragmatic hernia	12	27.3 ± 2.06* (22-27)	1	2.4 ± 0.72 (1.3-4.5)	5.3	8.23 ± 0.91* (6.2-10)	330	1286.5 ± 1.41** (888-1546)
Omasal impaction	-	31.7 ± 3.48* (26-38)	-	3.33 ± 0.35* (2.8-4.0)	-	7.33 ± 0.66* (6.2-8.5)	-	473.7 ± 54.0* (378-565)
Paralytic ileus	42	43.5 ± 2.5** (41-46)	2.2	3.35± 0.15** (3.2-3.5)	2.4	9.15 ± 0.65* (8.5-9.8)	122	638 ± 49.0* (589-687)
Late pregnancy indigestion	15.5 ± 0.5 (15-16)	26.6 ± 7.17 (15-54)	1.05 ± 0.35 (0.7-1.4)	2.16 ± 0.26 (1.4-3.0)	5.25 ± 1.45 (3.8-6.7)	9.94 ± 1.74* (5.6-15.3)	548 ± 3.44 (204-892)	958.5 ± 1.76* (612-1449)
Miscellaneous disorders	20.5±6.65 (7-37)	29.8 ± 7.49 (14-47)	1.5 ± 0.35 (0.9-2.5)	3.01 ± 0.26* (2.7-3.8)	6.15 ± 3.17 (2.1-15.6)	6.93 ± 1.73 (4.2-11.4)	452 ± 65.9* (324-543)	966.3 ± 1.63* (769-1289)

***Indicates significance level of ≤5%**

**** Indicates significance level of ≤1%**

the degree of hyperlactatemia is directly associated with the severity of hypoperfusion and mild hypoperfusion was typically associated with lactate values of 3 to 5 mmol/L, moderate with values of 5 to 7 mmol/L, and severe with values above 7 mmol/L. According to Allen and Holm (2008) tissue hypoxia responsible for increased lactate levels, may have several causes such as hypoperfusion (decreased cardiac output or hypovolemia), anaemia (decreased arterial blood oxygen content) or oedema (decrease tissue ability to mobilise oxygen). Futhermore Vary *et al* (1988) reported that not only hypovolemia or oxygen debt but also metabolic conditions such as hepatic malfunction with decreased lactate uptake or diabetic ketoacidosis may induce hyperlactatemia.

***Serum creatine phosphokinase and lactate dehydrogenase levels**:*

Serum enzymatic activity of CK and LDH were significantly higher than the control value in all groups (Table 16 and 18). Increased CK activity in serum generally indicates skeletal and cardiac muscles involvement (Smith 2002).

Inflammation and damage to the myocardium in various gastrointestinal disorders was later confirmed by histopathological examination. On the other hand, it has been demonstrated that the LDH level increases after injury to the liver, skeletal muscle, cardiac muscle, and kidney (Meyer and Harvey 2004); therefore, the significant increase of this enzyme may suggest that these organs were affected.

Serum calcium, phosphorus and magnesium levels:

Mean serum calcium level was significantly lower than the control value in some groups (Table 17). Hypocalcemia may be due to less assimilation of feed materials (Sethuraman and Rathore 1979). Daniel (1983) reported that both rumen and abomasal motilities were similarly reduced in hypocalcemia due to general effect of depression of levels of ionised calcium on smooth muscle contractability.

The mean phosphorus and magnesium concentration among most of the groups did not vary significantly from the control value.

Serum sodium, chloride and potassium levels:

The sodium concentration was almost within normal range in all groups except omasal impaction group (Table 18). These findings were in contrast with Toor

(2003), Brar (2004) and Kumar (2006) who all reported mean plasma sodium levels to be below normal values in animals suffering from forestomach disorders. Chloride and potassium was within normal range in all groups except paralytic ileus group. Hypokalemia could be attributed to intracellular shift of potassium subsequent to metabolic alkalosis or may be due to anorexia and urinary loss of potassium (Ward *et al* 1993). Hypokalemia could also be due to fasting and adaptive response to continued hypoadrenocortical activity in chronic stress (Kaneko *et al* 1997). The low chloride level has also been related to anorexia, dehydration and decreased rumeno-reticular motility (Behl *et al* 1996). Chloride is a major extracellular anion which maintains water and osmotic pressure and regulates acid-base balance in conjunction with sodium.

4.1.6 Peritoneal fluid analysis

Peritonitis in cattle and buffaloes was precisely diagnosed on the basis of peritoneal fluid examination, especially its cytology. Peritoneal fluid analysis has been considered as supplementary aid to the hematological and clinical examination in the diagnosis of bovine abdominal disorders. Assessing the volume, cellularity and protein concentration of peritoneal fluid can give an indication of inflammatory changes in the peritoneal cavity (Oehme 1969 and Oehme and Noordsy 1970).

In present study, peritoneal fluid was collected from 24 cases. Out of these cases, evidence of peritoneal inflammation was found in 19 cases. Peritoneal fluid was obtained from 12 cases of peritonitis, four cases of traumatic pericarditis, three cases of DH, one case of paralytic ileus, two cases of late pregnancy indigestion and two cases of miscellaneous conditions. The five peritoneal fluid samples (1 DH, 1 paralytic ileus, 2 each of late pregnancy indigestion and miscellaneous conditions) with no abnormality, on cytological examination, were regarded as unaffected or normal. The normal peritoneal fluid samples were colourless and watery in consistency and did not clot on standing, without the addition of anticoagulant. The total protein content, albumin and lactate ranged from 1-2.7 g/dL, 0.5-2 g/dL and 0.5-3.9 mmol/L, respectively. The total cell count of normal peritoneal fluid samples ranged from 65-800/µL and polymorph to mononuclear ratio was almost 1:1.

Table 17: Clinical chemistry (calcium, phosphorus and magnesium) in various groups of gastrointestinal disorders in advanced pregnancy in bovines

Group	Calcium (mg/dL) Mean ± S.E. (Range)		Phosphorus (mg/dL) Mean ± S.E. (Range)		Magnesium (mg/dL) Mean ± S.E. (Range)	
	Cattle	Buffalo	Cattle	Buffalo	Cattle	Buffalo
Control	8.04 ± 0.22 (7.6-8.9)	8.31 ± 0.21 (7.8-9.5)	5.5 ± 0.26 (5.1-6.5)	6.04 ± 0.38 (4.5-7.5)	2 ± 0.14 (1.6-2.4)	2.03 ± 0.12 (1.6-2.4)
Peritonitis	6.64 ± 0.92 (3.9-8.6)	7.3 ± 0.45 (5.1-8.5)	5.14 ± 1.73 (2.3-11.7)	4.78 ± 0.61 (1.8-6.7)	2.04 ± 0.10 (1.8-2.4)	2.26 ± 0.12 (1.8-2.8)
Traumatic pericarditis	7.15 ± 0.05* (7.1-7.2)	6.13 ± 0.54* (5.3-7.7)	6.6 ± 0.6 (6-7.2)	5.45 ± 0.73 (4.2-6.9)	2.65 ± 0.25 (2.4-2.9)	3.63 ± 0.41* (2.8-4.7)
Diaphragmatic hernia	5.7	7.33 ± 0.69 (5.9-8.8)	4.6	5.85 ± 1.65 (2.5-9.3)	2.3	2.1 ± 0.13 (1.8-2.4)
Omasal impaction	-	6.1 ± 0.55* (5-6.7)	-	4.63 ± 0.75 (3.4-6)	-	2.13 ± 0.18 (1.8-2.4)
Paralytic ileus	7.2	7.3 ± 0.1** (7.2-7.4)	4.2	6.05 ± 1.85 (4.2-7.9)	2	2.23 ± 0.28 (1.9-2.8)
Late pregnancy indigestion	9.15 ± 0.05** (9.1-9.2)	7.9 ± 0.92 (5.3-10.1)	3.85 ± 0.65 (3.2-4.5)	4.88 ± 1.11 (2.1-8.7)	2.15 ± 0.15 (2-2.3)	2.08 ± 0.13 (1.8-2.4)
Miscellaneous disorders	7.15 ± 0.55 (5.8-8.1)	7.33 ± 1.57 (2.8-9.9)	3.83 ± 0.58* (2.5-5.1)	4.55 ± 0.83 (2.6-6.6)	2 ± 0.22 (1.6-2.6)	2.1 ± 0.13 (1.8-2.4)

***Indicates significance level of ≤5%**

**** Indicates significance level of ≤1%**

Table 18: Clinical chemistry (sodium, potassium, chloride and LDH) in various groups of gastrointestinal disorders in advanced pregnancy in bovines

Group	Sodium (mmol/L) Mean ± S.E. (Range)		Potassium (mmol/L) Mean ± S.E. (Range)		Chloride (mmol/L) Mean ± S.E. (Range)		LDH (U/L) Mean ± S.E. (Range)	
	Cattle	Buffalo	Cattle	Buffalo	Cattle	Buffalo	Cattle	Buffalo
Control	145.8±1.46 (143-151)	149.4±4.21 (140-174)	4.18±0.12 (3.8-4.5)	4.39±0.22 (3.4-5.2)	104.6±0.68 (103-107)	102.3±3.46 (93-122)	2132.2±1.53 (1789-2700)	1713.8±57.9 (1460-1863)
Peritonitis	162.5±14.2 (146-205)	153.8±1.19 (149-161)	4.44±0.36 (3.7-5.7)	4.36±0.14 (3.8-4.8)	112.3±9.29 (101-140)	102.1±0.85 (100-107)	2464.8±8.54 (879-4845)	1666±1.05 (1154-2172)
Traumatic pericarditis	145.5±2.5 (143-148)	141.8±5.22 (128-153)	4.2±0.5 (3.7-4.7)	4.9±0.4 (4.2-5.9)	100.5±7.5 (93-108)	91.5±6.66 (72-102)	1048.5±59.5** (989-1108)	2403±1.89* (2156-2956)
Diaphragmatic hernia	155	149±4.67 (138-159)	4.7	3.95±0.79 (1.9-5.5)	112	94±3.69 (85-103)	3861	3369.2±47.7** (3242-3467)
Omasal impaction	-	162±1.52* (160-165)	-	4±0.36 (3.3-4.5)	-	112.3±1.76* (109-115)	-	1870±69.7 (1745-1986)
Paralytic ileus	150	143.5±0.5 (143-144)	3.5	3.15±0.05** (3.1-3.2)	98	89±1.0** (88-90)	1421	1442.5±3.0 (1142-1743)
Late pregnancy indigestion	155.5±2.5 (153-158)	143.4±4.25 (128-152)	4.05±0.35** (3.7-4.4)	4.12±0.94 (2.2-7.7)	107.5±1.5 (106-109)	94.2±5.72 (75-104)	2041.5±4.13 (1629-2454)	1546.5±87.7 (1418-1797)
Miscellaneous disorders	147.5±1.32 (144-150)	148±3.56 (142-158)	4.28±0.17 (3.8-4.6)	3.48±0.48 (2.7-4.7)	100.8±0.85 * (99-103)	96.8±4.64 (85-106)	1910.3±1.71 (1736-2253)	1442±1.03 (1289-1639)

***Indicates significance level of ≤5%**

**** Indicates significance level of ≤1%**

These samples had no abnormal finding on cytological examination. The present findings are in concurrence with Trent (2004) who observed that normal peritoneal fluid was clear to slightly turbid or light yellow in colour, with specific gravity less than 1.016 and total protein less than 3g/dl. He observed the total cell count less than 1000/µL, with mature neutrophils and mononuclear cells in fairly equal numbers. Similarly, Radostitis *et al* (2007) reported that normal peritoneal fluid in cows appeared amber in colour with specific gravity 1.005-1.015, total protein 0.1-3.1 g/dL and total cell count 300-5300/µL with 1:1 ratio of polymorphs to mononuclear cells.

In cases of peritonitis, concentration of total protein, albumin and lactate in peritoneal fluid ranged from 2.9-6.7 g/dL, 1.6-3.2 g/dL and 1.8-10.5 mmol/L. The total cell count of normal peritoneal fluid samples ranged from 1200-80000/µL.

4.1.6.1 Physical characteristics

In cases of peritonitis, colour of peritoneal fluid was pus like in five animals, reddish in four animals, yellowish in two and black in one animal. The volume of peritoneal fluid obtained was upto 5 ml in seven animals, less than 3 ml in four animals and more than 6 ml in one animal (Table 19).

In cases of traumatic pericarditis, colour of peritoneal fluid was reddish in two animals, pus like in one animal and yellowish in one animal. The volume obtained was less than 3 ml in one case and upto 5 ml in three cases (Table 19).

In cases of DH, peritoneal fluid was straw coloured in one animal and yellow coloured in two animals. The volume was less than 3 ml in one case and upto 5 ml in two cases. Dirksen (1979) reported that peritoneal fluid in diseased animals was reddish, slightly cloudy, and odourless to foul smelling, which concurred well with the present study (Table 19). It should be noted that failure of collection of the peritoneal fluid does not rule out peritonitis because in cattle, it is characterized by marked fibrinous response and localization of the lesion and the amount of exudative fluid available at the site may be minimal (Radostitis *et al* 2000).

4.1.6.2 Peritoneal fluid chemistry

4.1.6.2.1 Total protein

The total protein concentration of peritoneal fluid in cases of peritonitis, traumatic pericarditis and diaphragmatic hernia was 4.22 ± 0.42 g/dL, 3.7 ± 0.84 g/dL and 3.15 ± 0.25 g/dL respectively (Table 20). These findings were similar to those of

Table 19: Physical characteristics (colour and volume) of peritoneal fluid in different types of gastrointestinal disorders in bovines

Group	Peritoneal fluid colour					Peritoneal fluid volume			
	Straw/ colourless	Yellowish	Reddish	Pus like	Black	Less than 3 ml	Upto 5 ml	6-10 ml	>10ml
Peritonitis	0	2	4	5	1	4	7	1	0
Traumatic pericarditis	2	0	2	0	0	1	3	0	0
Diaphragmatic hernia	1	2	0	0	0	1	2	0	0
Omasal impaction	0	0	0	0	0	0	0	0	0
Paralytic ileus	1	0	0	0	0	0	1	0	0
Late pregnancy indigestion	1	1	0	0	0	0	2	0	0
Miscellaneous disorders	2	0	0	0	0	0	1	1	0
Total	7	5	6	5	1	6	16	2	0

Wilson *et al* (1985) and Radostitis *et al* (2000) who reported total protein value of >3g/L in peritonitis cases.

4.1.6.2.2 Albumin

The albumin concentration of peritoneal fluid in cases of peritonitis, traumatic pericarditis and diaphragmatic hernia was 2.03 ± 0.18 g/dL, 1.7 ± 0.43 g/dL and 1.45 ± 0.05 g/dL, respectively (Table 20).

4.1.6.2.3 Lactate

The concentration of lactate in peritoneal fluid in cases of peritonitis, traumatic pericarditis and diaphragmatic hernia was 5.86 ± 0.76 mmol/L, 2.55 ± 0.46 mmol/L and 5.2 ± 2.4 mmol/L, respectively (Table 20). The concentration of lactate in the peritoneal fluid was not significantly different from that in the serum. In the present study, there was positive correlation between peritoneal fluid lactate and serum lactate but it was not significant statistically. It has been reported that intestinal ischaemic conditions result in an increase in the concentration of lactate in peritoneal fluid, followed by increase in its plasma concentration (Moore *et al* 1977 and Latson *et al* 2005).

Table 20: Clinical chemistry (total protein, albumin, SAAG ratio and lactate) of peritoneal fluid in various types of gastrointestinal disorders in bovines

Group	Total protein (g/dL) Mean ± S.E (Range)	Albumin (g/dL) Mean ± S.E (Range)	SAAG (g/dL)	Lactate (mmol/L) Mean ± S.E (Range)
Normal	0.1–3.1 (Radostitis *et al* 2007)	0.27–2.39 (Wittek *et al* 2010a)	1.23 (Wittek *et al* 2010a)	0.19-1.31 (Wittek *et al* 2010a)
Peritonitis	4.22 ± 0.42 (2.6-6.7)	2.03 ± 0.18 (1.3-3.2)	0.83 ± 0.15 (0.1-1.6)	5.86 ± 0.76 (2.9-10.5)
Traumatic pericarditis	3.85 ± 0.79 (2.9-6.2)	1.7 ± 0.43 (1.2-3)	1.2 ± 0.29 (0.4-1.7)	2.55 ± 0.46 (1.2-3.3)
Diaphragmatic hernia	3.16 ± 0.15 (2.9-3.4)	1.5 ± 0.06 (1.4-1.6)	0.65 ± 0.35 (0.3-1)	5.7 ± 1.47 (2.8-7.6)

4.1.6.2.4 Serum-ascites albumin gradient

The mean value of SAAG was found to be 0.83 ± 0.15 g/dL in cases of peritonitis, 1.2 ± 0.29 g/dL in cases of traumatic pericarditis and 0.65 ± 0.35 g/dL in cases of DH (Table 20). Wittek *et al* (2010a) reported that cut-off value for SAAG in cows is 12.3 g/L, which was slightly higher than the cut-off values of 11 g/L for humans. In humans SAAG has high diagnostic importance and is indicative of inflammatory conditions in the peritoneal cavity. However, Wittek *et al* (2010b) reported that SAAG is not superior in detecting peritonitis in cows compared with total protein concentration alone.

4.1.6.3 Total and differential cell count

The total cell count of peritoneal fluid in cases of peritonitis ranged from 1600 to 80000/µL with a mean of 18627 ± 8.08/µL. The neutrophil, lymphocyte and esinophil counts were 72 ± 5.66%, 24.5 ± 5.0% and 7.67 ± 6.17%, respectively (Table 21).

In cases of traumatic pericarditis, the total cell count of peritoneal fluid ranged from 1200 to 9500/µL with a mean of 3650 ± 1.96/µL. The neutrophil and lymphocyte counts were 79.5 ± 3.86% and 20.5 ± 3.86 %, respectively (Table 21).

In cases of DH, the total cell count of peritoneal fluid ranged from 1800 to 2500/µL with a mean of 2150 ± 3.5/µL. The neutrophil and lymphocyte counts were 66 ± 4% and 39 ± 9%, respectively (Table 21).

Table 21: Total and differential leukocyte count of peritoneal fluid in various types of gastrointestinal disorders in cattle and buffalo

Group	Total Leukocyte Count Mean ± S.E (Range)	Neutrophils (%) Mean ± S.E (Range)	Lymphocytes (%) Mean ± S.E (Range)	Eosinophils (%) Mean ± S.E (Range)
Normal (Radostitis *et al* 2007)	300-5300	Polymorphonuclear and mononuclear cells, ratio 1:1		
Peritonitis	18627 ± 8.08 (1600-80000)	72 ± 5.66 (46-96)	24.5 ± 5.0 (4-47)	7.67 ± 6.17 (1-20)
Traumatic pericarditis	3650 ± 1.96 (1200-9500)	79.5 ± 3.86 (72-90)	20.5 ± 3.86 (10-28)	0
DH	2166.7 ± 2.03 (1800-2500)	69.3 ± 4.06 (62-76)	30.7 ± 4.06 (24-38)	0

4.1.6.4 Peritoneal fluid cytology

Peritoneal fluid cytology revealed variable numbers of degenerated neutrophils, aggregate of macrophages and mesothelial cells, bacteria, plant fibre, gut contents and protozoa (Fig. 11). On microscopic examination of peritoneal fluid, leakage of gut contents into peritoneal cavity was characterised by presence of squamous epithelial cells, plant fibre, fungi (Fig. 12) or protozoa like organisms. Peritonitis was classified as septic and non-septic peritonitis, based on the presence or absence of bacteria. The bacteria were seen either free or phagocytosed by neutrophils or macrophages (Fig. 13 and 14). Based upon the predominance of cells and other constituents, the peritonitis was further classified into fibrinous, fibrinopurulent, frank suppurative and adhesive peritonitis. In fibrinous peritonitis, there was lot of fibrin strands with few polymorphs and mononuclear cells (Fig. 15). In fibrinopurulent peritonitis, there was fibrin strands with lot of degenerated neutrophils (Fig. 16). In suppurative peritonitis, there was massive number of neutrophils with only few mononuclear cells (Fig. 17). In adhesive peritonitis, there was presence of clumps of degenerated neutrophils, macrophages, increased number of mesothelial/reactive mesothelial cells and fibroblast (Fig. 18). Cytological abnormalities of peritoneal fluid according to Ziemer (1989) were increased number of neutrophils with degenerative or toxic changes, as also observed in the present study. According to Kumar (2009) wide N:L, degenerated neutrophils with engulfed bacteria and overwhelming bacterial number indicated peritonitis.

Among the cases of peritonitis, septic peritonitis was seen in seven cases out of 12. Severe sepsis was observed in five cases and was characterised by massive neutrophilia and markedly degenerated neutrophils with engulfed bacteria along with high count of activated macrophages and mesothelial cells. Fibrinous peritonitis was observed in three, fibrinopurulent in four, frank suppurative peritonitis in two and adhesive peritonitis in three cases. Leakage of gut contents was observed in four cases of peritonitis.

In traumatic pericarditis, two cases of adhesive peritonitis and one case each of fibrinous and fibrinopurulent peritonitis were observed. Bacteria were seen in two cases. Leakage of gut contents was seen in only one case.

In DH, adhesive peritonitis was observed in two cases. Bacteria were seen in only one case.

4.2 Study on dead animals

Tissue samples were collected at necropsy from animals that died due to gastrointestinal disorders in advanced pregnancy and were subjected to histopathology. Samples were collected from 17 buffaloes and 4 cows (Table 22). Based upon gross and histopathological findings, these were divided into following types.

Table 22: Based upon gross and histopathological findings, different types of gastrointestinal disorders associated with advanced pregnancy in bovines

Group	Distribution	Species		Age (years) (Mean±SE)
		Cow	Buffalo	
Peritonitis	6	1	5	6.42 ± 0.76
Traumatic Pericarditis	4	-	4	6.25 ± 0.63
Omasal impaction	3	2	1	5.67 ± 0.33
Diaphragmatic hernia	2	0	2	9 ± 3
Intestinal obstruction	5	1	4	6.6 ± 0.4
Enzootic leukosis	1	-	1	5
Total	21	4	17	6.5 ± 0.38

Table 23: Distribution of different types of gastrointestinal disorders observed at necropsy in relation to gestation period

Group	Species	Gestation (month)				
		6th	**7th**	**8th**	**9th**	**10th**
Peritonitis	Cattle	0	1	0	0	0
	Buffalo	0	1	3	0	1
Traumatic pericarditis	Cattle	0	0	0	0	0
	Buffalo	0	0	2	1	1
Omasal impaction	Cattle	0	1	1	0	0
	Buffalo	0	0	1	0	0
Diaphragmatic hernia	Cattle	0	0	0	0	0
	Buffalo	0	1	0	1	0
Intestinal obstruction	Cattle	0	0	0	0	0
	Buffalo	1	2	1	0	1
Enzootic leukosis	Cattle	0	0	0	0	0
	Buffalo	0	1	0	0	0
Total		1	7	8	2	3

Table 24a: Gross findings in different types of gastrointestinal disorders associated with advanced pregnancy in bovines

Group	Peritonitis		Impacted forestomach		Impacted intestine		Abomasal ulcers		Intestinal hemorrhage	
	Present	**Absent**	**Present**	**Absent**	**Present**	**Absent**	**Present**	**Absent**	**Present**	**Absent**
Peritonitis	6	0	1	5	0	6	3	3	2	4
Traumatic Pericarditis	1	3	3	1	0	4	2	2	0	4
Omasal impaction	1	2	3	0	0	3	0	3	0	3
Diaphragmatic hernia	2	0	0	2	0	2	1	1	0	2
Intestinal obstruction	2	3	2	3	2	3	3	2	3	2
Enzootic leukosis	0	1	0	1	0	1	1	0	1	0
Total	12	9	11	10	2	19	10	11	6	15

Table 24b: Gross findings in different types of gastrointestinal disorders associated with advanced pregnancy in bovines

Group	Epicardial hemorrhage		Sub-endocardial hemorrhage		Pericarditis		Foreign body		Pneumonia		Aspiration	
	Present	Absent	Present	Absent	Present	Absent	Present	Absent	Present	Absent	Present	Absent
Peritonitis	5	1	4	2	0	6	0	6	1	5	0	6
Traumatic Pericarditis	0	4	4	0	4	0	2	2	2	2	0	4
Omasal impaction	0	3	0	3	1	2	0	3	2	1	1	2
Diaphragmatic hernia	0	2	1	1	0	2	0	2	2	0	2	0
Intestinal obstruction	5	0	1	4	0	5	0	5	1	4	1	4
Enzootic leukosis	1	0	0	1	0	1	0	1	0	1	0	1
Total	11	10	10	11	5	16	2	19	8	13	4	17

4.2.1 Peritonitis

Gross findings

Lesions varied from focal to diffuse including fibrinous to fibrinopurulent peritonitis (Fig. 19). There were four cases of fibrinous peritonitis and two cases of fibrinopurulent peritonitis. Extensive fibrinous adhesions involving the forestomachs, abomasum, small and large intestines, spleen, liver, bladder, reproductive tract and pelvic cavity were present. Loops of intestine and omenta were commonly stuck together by thick layers of fibrin. Foul-smelling turbid fluid containing clots of fibrin were usually present in the peritoneal cavity. In one case, along with fibrinous peritonitis massive haemorrhages involving serosa of rumen, reticulum, omasum and abomasum were seen (Fig. 20). A reticulophrenic adhesion was observed in two cases (Fig. 21). It has been reported that internal adhesions of the abdominal cavity resulting from peritonitis are one of the main sources of death in dairy cattle (Sikkink *et al* 2009). Signs of toxaemia/septicaemia i.e. epicardial and myocardial haemorrhages, congestion of visceral organs were present in all cases (Fig. 22). Abomasal ulcers were present in three cases (Fig. 23). Peritonitis due to rupture of intestine was observed in another case. Omasal impaction was also noticed in one case (Table 24a and 24b). Peritonitis results in toxemia, alimentary tract stasis, dehydration and shock (Radostitis *et al* 2007)

Histopathological findings

Forestomachs:

Histopathological examination of the rumen, reticulum and omasum revealed necrosis and sloughing of epithelium along with fibroplasia (Fig. 24 and 25). There was infiltration of inflammatory cells in reticular epithelium involving lamina propria indicating chronic reticulitis. Muscular layer of reticulum and omasum showed degeneration and atrophy, indicative of chronic atony. Serosal layer of reticulum and omasum revealed chronic active peritonitis with hyperplasia of mesothelial cells.

Abomasum:

Histopathological examination of the abomasum revealed congestion, superficial gastritis and sloughing of mucosa. In chronic cases, there was superficial necrosis and thickening of lamina propria/submucosa. In one case of peritonitis, there appeared to be regurgitation of bile into abomasum (Fig. 26). Type-1 ulcers were also

observed in three cases (Fig. 27). Whitlock (1980) classified erosions and non-perforating ulcers as type-1 ulcers.

Intestine

Histopathological examination of the intestine revealed chronic enteritis. There was ischaemic necrosis of intestinal mucosa with sloughing of mucosa and partial to complete denudation of villi along with massive congestion of serosal vasculature (Fig. 28). Intestinal necrosis was a consistent finding and at times the necrosis was massive resulting in stuffing of intestinal lumen with necrotic debris. Similar findings were reported by Kumar (2009) and Hussain (2010)

Peritoneum

Sections of omentum/peritoneum revealed hyperplastic to dysplastic changes of mesothelial cells along with chronic active inflammation (Fig. 29). There was also evidence of leakage of gut contents in the peritoneal cavity as remnant of plant material.

Liver

Liver revealed mild to severe changes ranging from congestion, haemorrhage focal to multi-focal area of degeneration/necrosis. In most cases mild to moderate fatty changes and centrilobular necrosis were evident (Fig. 30). Atrophy of hepatic cords, mild cholangiohepatitis, chronic perihepatitis and multiple necrotic foci with mixed or mononuclear cell infiltration in periportal area and elsewhere were also observed. Early fibrosis to frank cirrhosis was also observed in all cases of peritonitis. Fibrinopurulent perihepatitis was also noted in two cases (Fig. 31).

Kidneys

Microscopic changes in kidneys were thickening of capsule, perirenal inflammation, and degeneration of tubular epithelium, glomerulonephritis and interstitial nephritis (Fig. 32). In some cases, chronic glomerulitis was observed with narrowing of Bowman's space. Proliferative glomerulonephritis and calcification were also observed in two cases.

Lungs

Mild to severe congestion, atelectiasis, emphysema, peribronchial/ peribronchiolar fibrosis and alveolar edema were observed in animals that died due to peritonitis.

Lymph nodes

Lymph nodes revealed moderate to severe depletion of lymphoid tissue, congestion, haemorrhage and dilatation of lymph vessels (Fig. 33). Necrosis and cystic dilatation of lymphoid follicles was observed in some cases. Perilymphadenitis was also observed in cases of diffuse peritonitis.

Spleen

Spleen revealed increase in red pulp and decrease in white pulp and depletion of PALS, indicating rarefaction of lymphoid tissue. Chronic splenitis characterised by increase in capsular thickness, increase in hemosiderin laden macrophages and atrophy were also observed in a few cases. Fibrinopurulent to chronic perisplenitis was also observed (Fig. 34).

Heart

Heart revealed epicardial and myocardial haemorrhages as well as atrophy and disarray of cardiac myocytes. In addition fibrinous epicarditis was observed in two cases.

4.2.2 Traumatic pericarditis

Gross findings

Traumatic pericarditis was diagnosed at postmortem in four (4/21) animals. Two cases each of fibrinopurulent and suppurative pericarditis were observed. There was thickening of pericardium and pericardial sac was filled with pus containing flakes of fibrin and the serous surface was covered with heavy deposits of fibrin (Fig. 35). Metal wire was identified as the cause in only one animal. In the other three animals no foreign body could be found, owing to extensive inflammation and adhesions. Additional lesions of pleurisy and pneumonia were present in all cases of traumatic pericarditis. There were generalised signs of chronic active peritonitis in one case only. Signs of septicaemia were present in all cases (Table 24a and 24b).

Histopathological findings

Forestomachs

Ruminal epithelium showed necrosis and sloughing of epithelium. Chronic active reticulitis was evident in one case. Reticular and omasal muscular layer showed degenerative changes and fibroplasia.

Abomasum

Mild superficial gastritis with goblet cell hyperplasia and underlying glandular hyperplasia was evident in every case of traumatic pericarditis (Fig. 36 and 37). Superficial necrosis of mucosa with type-1 ulcers was observed in two cases of traumatic pericarditis.

Intestine

Histopathological examination of the intestine revealed chronic enteritis with extension of inflammation to lamina propria. Ischaemic necrosis of mucosa along with massive congestion of serosal vasculature was evident. There was sloughing of mucosa and partial to complete denudation of intestinal villi.

Liver

Liver revealed chronic venous congestion with atrophy of hepatocytes and dilataion of sinusoids (Fig. 38). Mild to moderate fatty changes and early cirrhosis was observed. Chronic active perihepatitis with thickening of capsule, mild to moderate periportal hepatitis with mixed or mononuclear cell infiltration, cholangiohaepatitis and bile duct hyperplasia were also observed. Kumar *et al* (2012b) observed sinusoidal dilatation in all animals with pericarditis along with chronic cholangiohepatitis, amyloidosis or chronic sepsis, purulent hepatitis, chronic active hepatitis and fatty liver.

Kidneys

Microscopic changes in kidney were degeneration of tubular epithelium, glomerulonephritis and interstitial nephritis. Nephrosis and calcification was observed in one case.

Lungs

Pulmonary hypertension, congestion from mild to severe, atelectiasis, emphysema and alveolar edema were observed. Thickening of interalveolar septa and pleura along with interstitial pneumonia was also observed.

Lymph nodes

Lymph nodes revealed moderate to severe depletion of lymphoid tissue. Multiple abscessation of lymph node was also observed in one case (Fig. 39).

Spleen

Spleen revealed increase in red pulp and decrease in white pulp, indicating rarefaction of lymphoid tissue. Haemorrhages were present in capsule and trabeculae of spleen.

Heart

Histopathological examination of heart revealed severe fibrinopurulent pericarditis with thickening of the pericardium due to accumulation of fibrin and pus cells in between the pericardium and myocardium. Multiple necrotic foci with bacterial colonies in pericardium were also observed (Fig. 40 and 41). There was disarray and atrophy of cardiac cells with interfasicular fibrosis. Epicardial haemorrhages were evident.

4.2.3 Omasal impaction

4.2.3.1 Gross findings

At necropsy, the impacted omasum was grossly distended with dry coarse particles and patches of necrosis were present on the omasal leaves (Fig. 42). These post mortem lesions were consistent to those mentioned by Radostits *et al* (2007). Aspiration was present in one case of omasal impaction (Table 22 and 23).

4.2.3.2 Histopathological alterations

Forestomach

Sloughing of mucosa was observed in all compartments of forestomach i.e. rumen, reticulum and omasum. There was chronic reticulitis and omasitis characterised by infiltration of mononuclear cells in lamina propria and fibroplasia in between muscles. Omasal epithelium showed hyperplastic to dysplastic changes (Fig. 43 and 44). Neovascularization and proliferation of granulation tissue in omasum was also evident in some of the cases, indicating chronicity of impaction (Fig. 45).

Abomasum

Mild superficial gastritis with goblet cell hyperplasia and underlying glandular hyperplasia was observed. Superficial necrosis of mucosa with Grade-1 ulcers was observed.

Intestine

Chronic enteritis with mononuclear cells infiltration, superficial to deep exfoliation/sloughing of mucosa and partial to complete denudation of villi was observed. Hyperplasia of mesothelial cells of serosal layer was also observed.

Liver

Liver revealed congestion, severe ballooning degeneration, mild to moderate centrilobular necrosis and early cirrhosis with stasis of bile. Chronic perihepatitis with thickening of capsule was also observed.

Kidneys

Microscopic changes in kidney were thickening of capsule, degeneration of tubular epithelium and interstitial nephritis.

Lungs

Histopathology of lungs revealed congestion from mild to severe, atelectiasis, emphysema and alveolar edema. Thickening of interalveolar septa and pleura along with chronic interstitial pneumonia was observed.

Aspiration was observed in only one case of omasal impaction. Bronchiolar epithelium was sloughed and plant material was present in bronchiole. Congestion, haemorrhage and suppurative pneumonia were also observed. Trachea revealed severe haemorrhagic tracheitis with complete loss of epithelium.

Lymph nodes

Lymph nodes revealed chronic lymphadenitis with macrophage proliferation and fibrosis. Lymphoid hyperplasia of paracortical area was also observed.

Spleen

Spleen revealed increase in red pulp and decrease in white pulp. Thickening of capsule with hyperplasia of smooth muscles was observed. Mesothelial lining was lumpy bumpy in appearance because of hyperplasia of mesothelial lining.

Heart

Histopathology of heart revealed myocardial haemorrhage, vacuolar and granular degeneration of cardiac cells.

4.2.4 Diaphragmatic hernia

4.2.4.1 Gross lesions/necropsy findings

In all cases, a portion of the reticulum was protruded into the pleural cavity and was very tightly adhered to the hernial ring (Fig. 46). The omasum and abomasum are relatively empty but the rumen was overfilled with frothy, porridge-like material which contains very little fiber. Aspiration was evident in all cases of DH (Fig. 47).

4.2.4.2 Histopathological alterations

Forestomach

Sloughing of mucosa with chronic inflammation was observed in all compartments of forestomach. There was chronic reticulitis with thromboembolism and fibroplasia besides extensive sloughing of epithelium (Fig. 48). There was superficial necrosis of reticular epithelium with formation of microabscesses in the wall (Fig. 49 and 50).

Abomasum

Superficial gastritis with goblet cell hyperplasia was observed in abomasum.

Intestine:

There was ischaemic necrosis of intestinal mucosa with complete denudation of villi. Necrosis was massive resulting in stuffing of intestinal lumen with necrotic debris.

Liver

Liver revealed congestion, atrophy of hepatic cords, mild cholangiohepatitis and chronic perihepatitis with thickening of capsule.

Kidneys

Degeneration of tubular epithelium, interstitial nephritis and thickening of capsule were present.

Lungs

Histopathology of lungs in cases of DH revealed severe congestion, alveolar edema, haemorrhage, atelectiasis and emphysema. Aspiration was evident in all cases of DH (Fig. 51). Trachea revealed severe haemorrhagic inflammation with complete loss of epithelium.

Lymph nodes

Lymph nodes revealed chronic lymphadenitis and mild to moderate depletion of lymphoid tissue. Thickening of capsule and trabeculae was observed.

Spleen

Spleen revealed haemorrhage, increase in red pulp and decrease in white pulp.

Heart

Histopathology of heart revealed epicardial and myocardial haemorrhages.

4.2.5 Intestinal obstruction:

4.2.5.1 Gross lesions/necropsy findings

At necropsy, two cases of mechanical obstruction (duodenal faecoliths), one case of malposition of intestine (volvulus) and two cases of functional obstruction (2 cases of paralytic ileus) were observed.

In two animals, there was presence of faecoliths in the duodenum. In one case, faecolith had blocked bile outflow leading to distension of gall bladder. Signs of toxaemia were present in both the cases.

In case of volvulus, there was serosal and mesenteric hemorrhages of varying degrees were present. Intestinal contents contained gas, ingesta and various amounts of blood.

In cases of paralytic ileus, the intestine was distended with a mixture of gas and fluid, and the wall was flaccid with no evidence of peritonitis. Both the cases were clinically diagnosed as paralytic ileus.

4.2.5.2 Histopathological alterations

Forestomach

Fibroplasia and chronic inflammatory changes were observed in all the forestomach compartments.

Abomasum

Superficial gastritis with goblet cell hyperplasia was observed in abomasum.

Intestine

In cases of paralytic ileus, there was marked chronic enteritis characterized by loss of villous epithelium and infiltration of mononuclear cells (Fig. 52). Ischaemic necrosis of intestinal mucosa was there.

In case of volvulus, intestine showed massive haemorrhage and venous congestion (Fig. 53).

In case of duodenal obstruction, there was chronic enteritis at the site of faecolith with complete denudation of villi and partial to complete destruction of crypts (Fig. 54 and 55). Serosal side was congested and thickened.

Liver:

In paralytic ileus, liver revealed congestion, atrophy of hepatic cords and mild cholangiohepatitis.

In volvulus, histopathology of liver revealed massive diffuse degeneration with fatty changes in hepatocytes, atrophy of hepatic cords and cholangiohepatitis (Fig. 56).

In cases of duodenal obstruction, liver showed chronic venous congestion with sinusoidal dilatation, atrophy of hepatic cords, retention of bile and multifocal infiltration by mononuclear cells (Fig. 57). Bile was stagnated in bile canaliculi and bile ducts (Fig. 58). Hepatocellular necrosis, cholangiohepatitis and bile imbibition in hepatocytes was also there.

Kidneys

Degeneration of tubular epithelium, proliferative glomerulitis and interstitial nephritis was present in all cases of intestinal obstruction.

Lungs

Histopathology of lungs revealed congestion from mild to severe, atelectiasis, emphysema and alveolar edema. Aspiration was seen in all cases of diaphragmatic hernia. There was severe congestion with presence of bacterial colonies in lungs. Trachea revealed severe haemorrhagic tracheitis with complete loss of epithelium.

Lymph nodes

Lymph nodes revealed chronic lymphadenitis and mild to moderate depletion of lymphoid tissue. Thickening of capsule and trabeculae was observed.

Spleen

Spleen revealed haemorrhage, increase in red pulp and decrease in white pulp.

Heart

Histopathology of heart revealed epicardial and myocardial haemorrhages.

4.2.6 Enzootic leukosis

4.2.6.1 Gross lesions/necropsy findings

Large growth was present in mediastinum involving all the mediastinal lymph nodes. Other lymph nodes were also enlarged. There was haemorrhagic abomasitis and enteritis (Table 22 and 23).

4.2.6.2 Histopathological alterations

Forestomach

Fibroplasia and chronic inflammation was observed in all forestomach compartments. Sloughing of epithelial mucosa was also there.

Abomasum

Abomasum revealed mild superficial gastritis with congestion.

Intestine

Intestine showed ischaemic necrosis with marked infiltration by neoplastic cells in lamina propria submucosa (Fig. 59 and 60).

Liver:

Liver revealed mild to severe microscopic changes ranging from multi-focal area of congestion, focal degenerative changes, mild fatty changes and centrilobular necrosis. Moderate to marked cholangiohepatitis and hyperplasia of kupfer cells was also seen. There was formation of various size granulomas in some places resembling typhoid granuloma (Fig. 61).

Kidneys

There was mild to moderate tubular degeneration and necrosis with tubular lumen containing casts and RBC's. There was also chronic proliferative glomerulitis with widening of Bowman's space.

Lymph nodes

There was massive infiltration of neoplastic lymphoid cells in the lymph nodes. There was thickening and fibrosis of medullary cords.

Spleen

Marked thickening of capsule and trabeculae was observed. There was infiltration of neoplastic lymphoid cells in spleen replacing most of the splenic parenchyma along with chronic splenitis (Fig. 62).

Heart

Histopathology of heart revealed epicardial and myocardial haemorrhages.

4.2.5 Immunohistochemistry

Immunoreactivity to C*lostridium perfringens*: Immunohistochemical staining was performed by using advanced SS^{TM} One- Step Polymer–HRP IHC Detection System for *Clostridium perfringens*. Out of 21 cases, 11 cases were found immunopositive. Immunoreactivity was demonstrated over the intestinal epithelium mainly in the superficial and deeper mucosa, however, the reactivity was more in cases, which showed marked necrotic enteritis. In addition, the reactivity of Clostridium was also found in the deep microvasculature of intestine in a few cases. Bacciarini *et al* (2003) conducted a retrospective study to assess the presence of the *C. perfringens* β2-toxin in tissues of the equine gastrointestinal tract. They produced mono-specific polyclonal antibodies against recombinant β2-toxin in rabbits and used them to demonstrate the β2 -toxin in sections of the gastrointestinal tract by immunohistochemical methods. Sections from 69 horses were stained and β2-toxin was detected immunohisto-chemically in 40 animals. Sections from the stomach, small intestine, and large intestine were positive for *C perfringens*.

Asaoka *et al* (2004) performed immunohistochemistry to demonstrate bacilli of *Clostridium perfringens* enterotoxin type A from sporadic outbreaks of fatal enteritis that occurred among free-living wild crows in an open-air park in Japan.

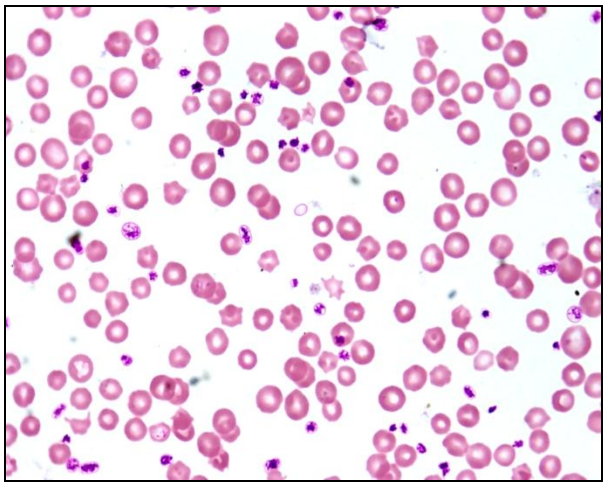

Fig. 1: Macrocytic hypochromic anemia and thrombocytosis (Leishman stain, original magnification x 1000 X)

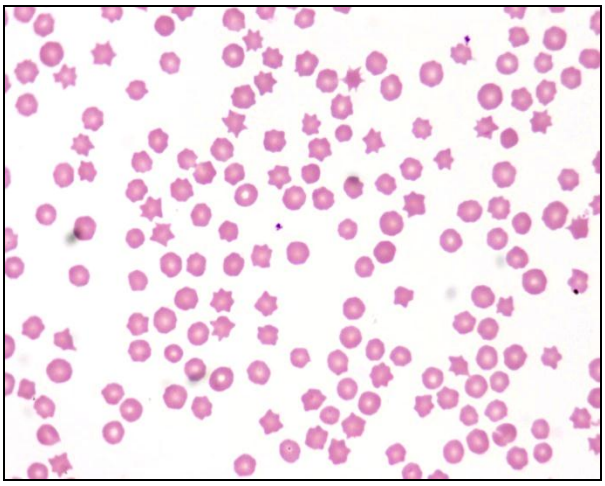

Fig. 2: Acanthocytes (Leishman stain, original magnification x 1000 X)

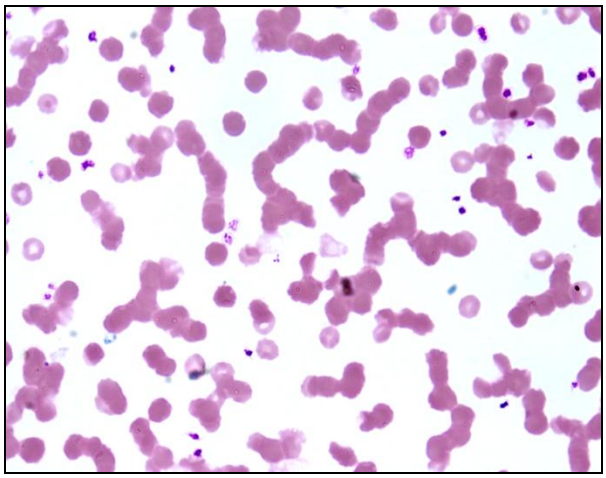

Fig. 3: Dehydration and marked rouleaux formation (Leishman stain, original magnification x 1000 X)

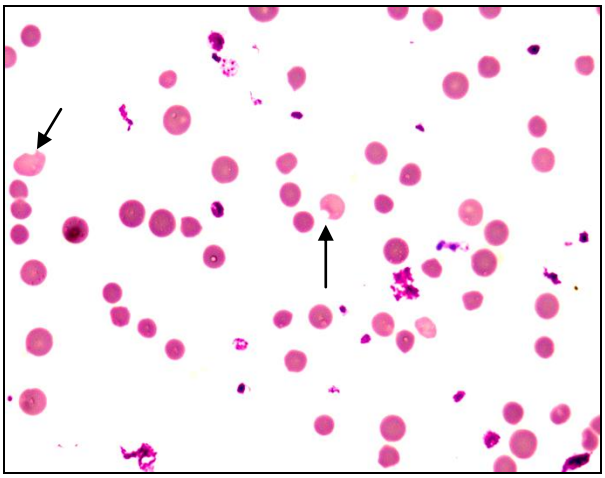

Fig. 4: Heinz body formation (Leishman stain, original magnification x 1000 X)

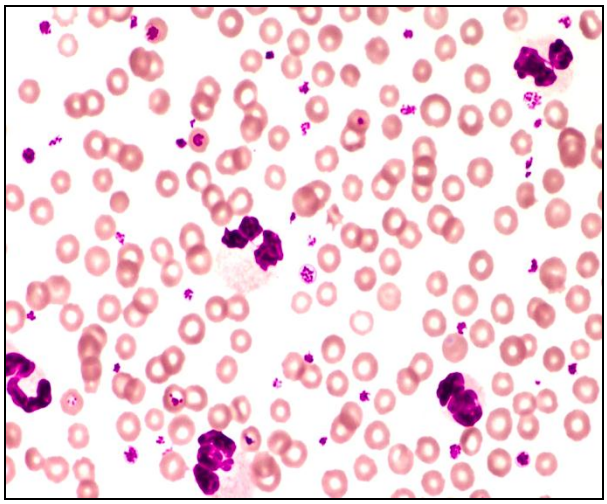

Fig. 5: Neutrophilia (Leishman stain, original magnification x 1000 X)

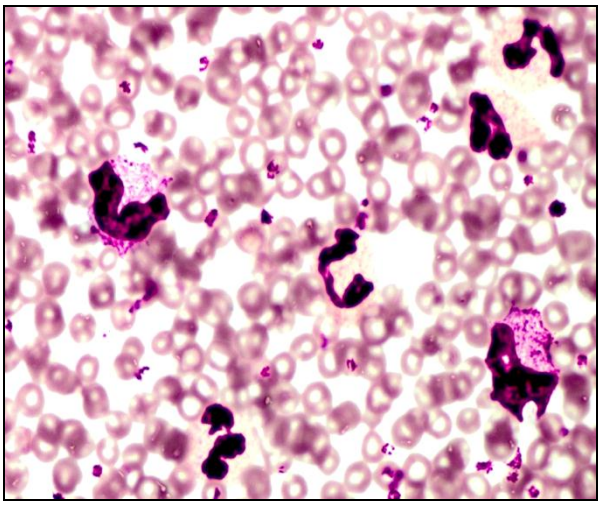

Fig. 6: Toxic neutrophils and nuclear dyscrasia (Leishman stain, original magnification x 1000 X)

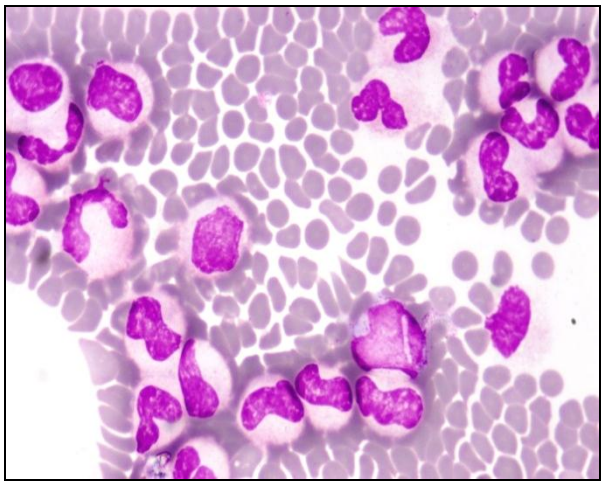

Fig. 7: Significant left shift (Leishman stain, original magnification x 1000 X)

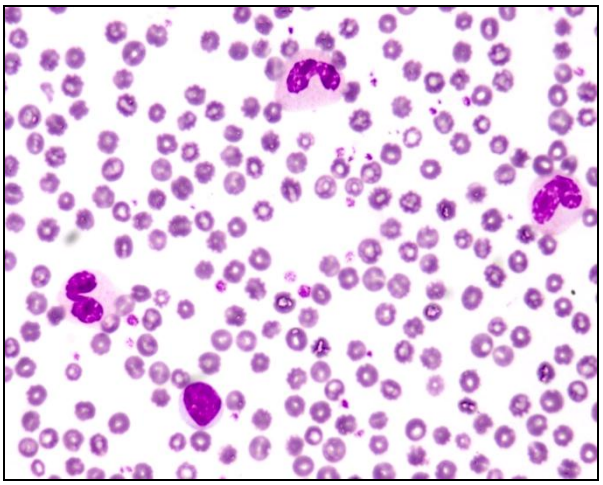

Fig. 8: Moderate left shift (Leishman stain, original magnification x 1000 X)

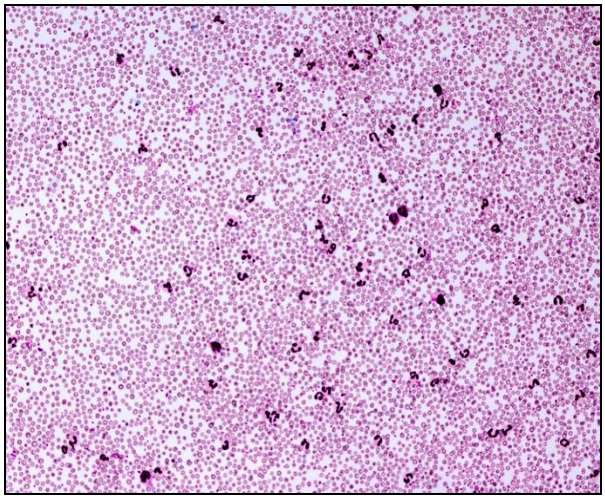

Fig. 9: Leukemoid reaction (Leishman stain, original magnification x 200 X)

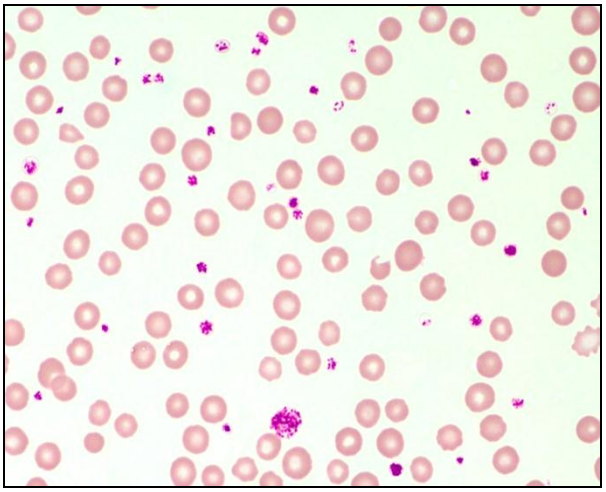

Fig. 10: Activated platelets (Leishman stain, original magnification x 1000 X)

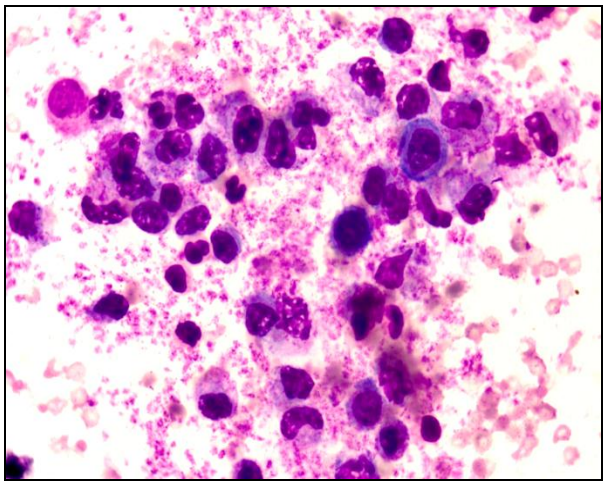

Fig. 11: Mixture of degenerated neutrophils, macrophages and mesothelial cells along with large number of platelets and RBC's indicating chronic active peritonitis overlapped by fresh bleeding (Leishman stain, original magnification x 1000 X)

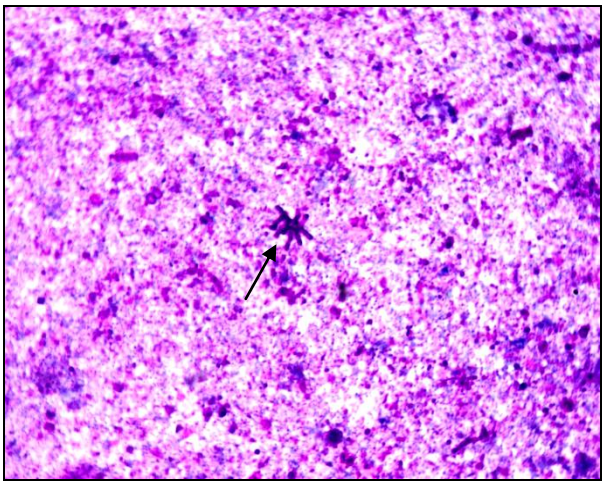

Fig. 12: Overwhelming peritonitis due to leakage of gut contents with presence of fungus in center and numerous bacteria (Leishman stain, original magnification x 1000 X)

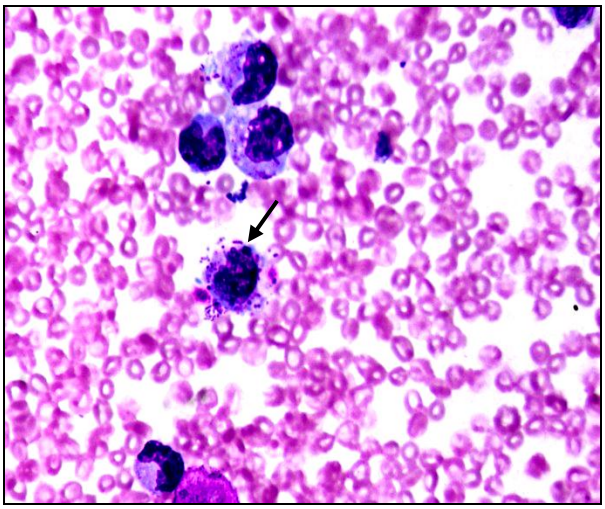

Fig. 13: Activated macrophages showing engulfed bacteria indicating septic peritonitis (Leishman stain, original magnification x 1000 X)

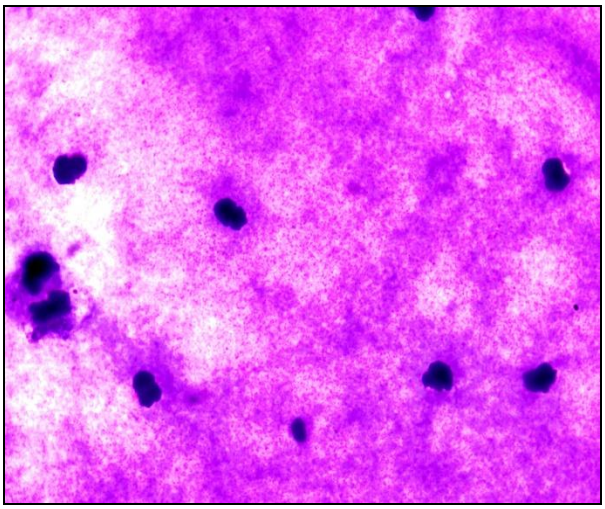

Fig. 14: Overwhelming septic peritonitis with high protein content and markedly degenerated neutrophils (Leishman stain, original magnification x 1000 X)

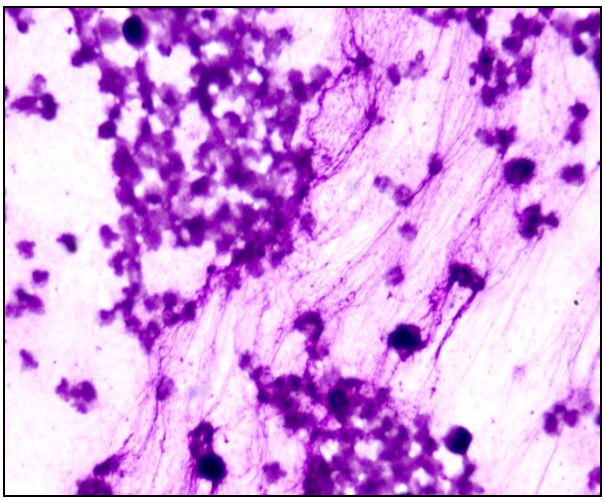

Fig. 15: Fibrinous peritonitis (Leishman stain, original magnification x 1000 X)

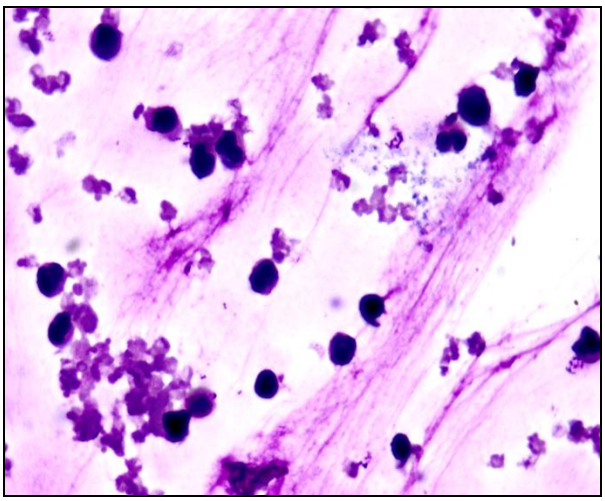

Fig. 16: Fibrinopurulent peritonitis with presence of degenerated neutrophils and fibrin strands (Leishman stain, original magnification x 1000 X)

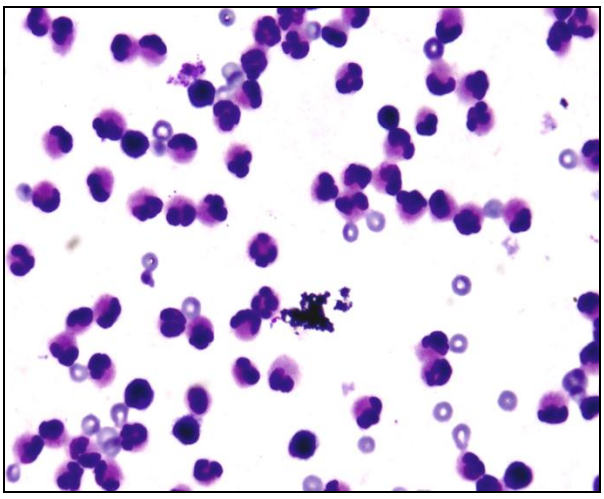

Fig. 17: Suppurative peritonitis characterized by moderately degenerated neutrophils, along with some RBC's

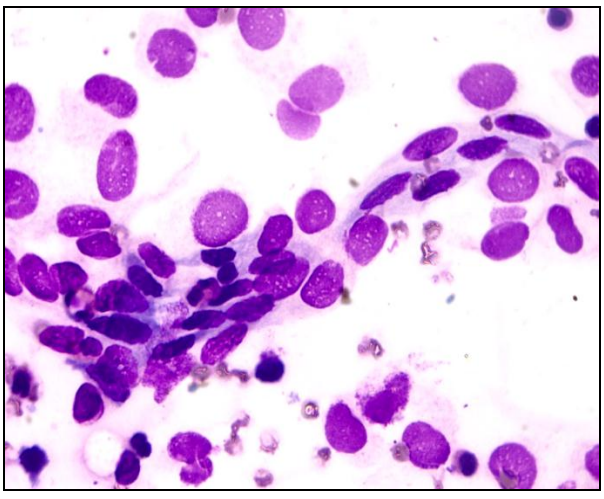

Fig. 18: Chronic active peritonitis characterized by large number of macrophages and active fibroblasts

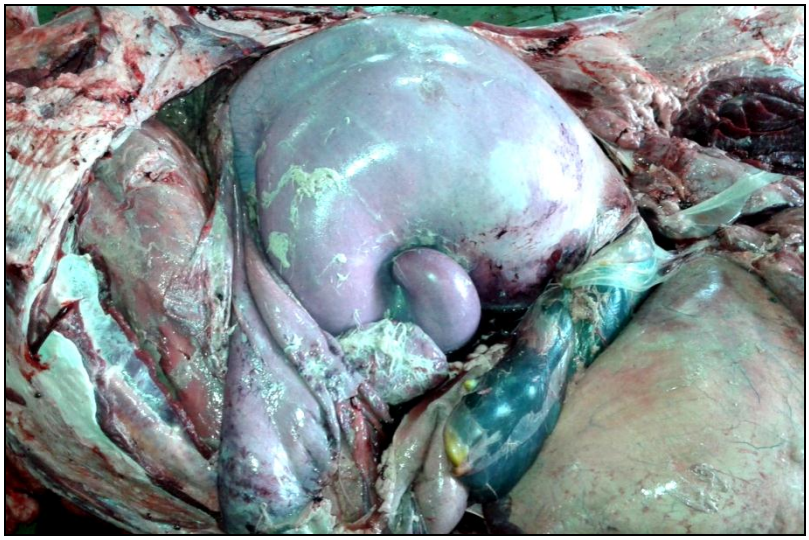

Fig. 19: Peritoneum showing lot of fibrin strands and adhesions and some exudate

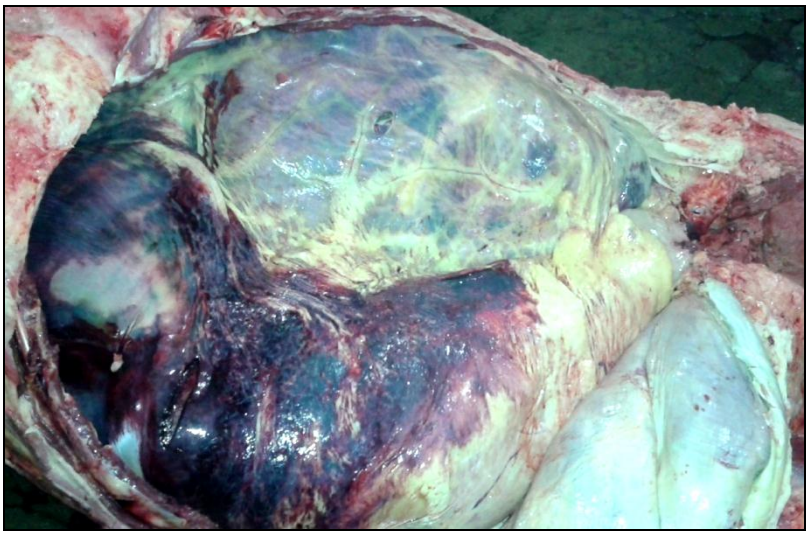

Fig. 20: Marked omental hemorrhage

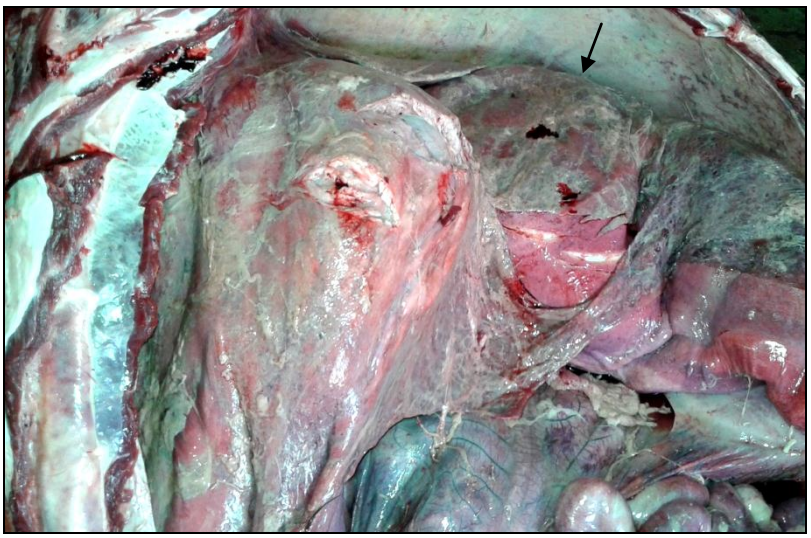

Fig. 21: Adhesive peritonitis characterized by adhesions between various abdominal organs and diaphragm along with hemorrhages

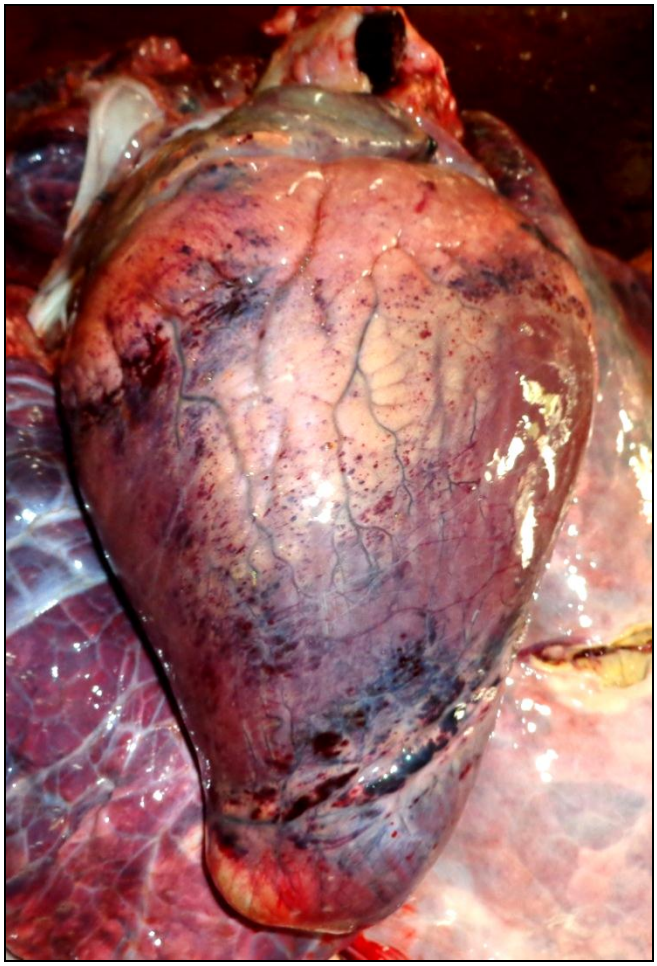

Fig. 22: Heart showing epicardial hemorrhages and fibrinous exudation

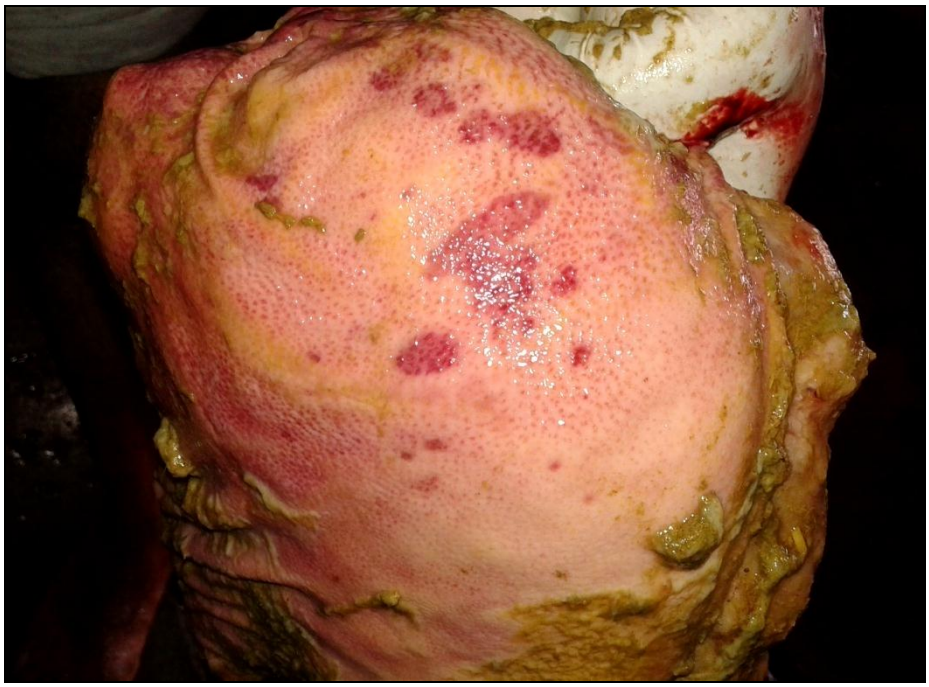

Fig. 23: Abomasal hemorrhages and erosions

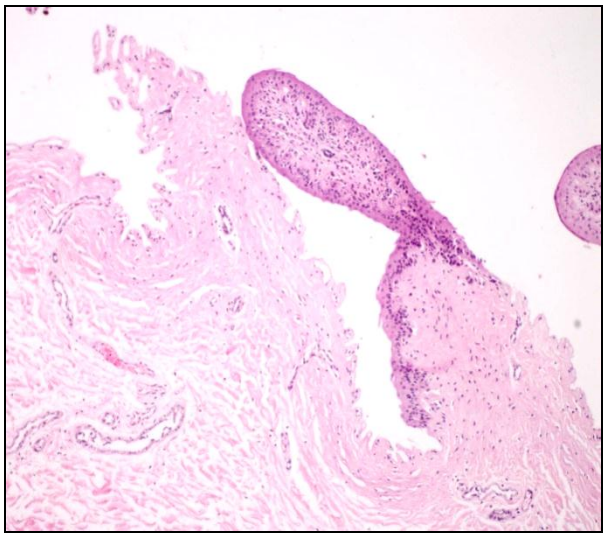

Fig. 24: Massive sloughing of ruminal mucosa (H&E, original magnification x 100 X)

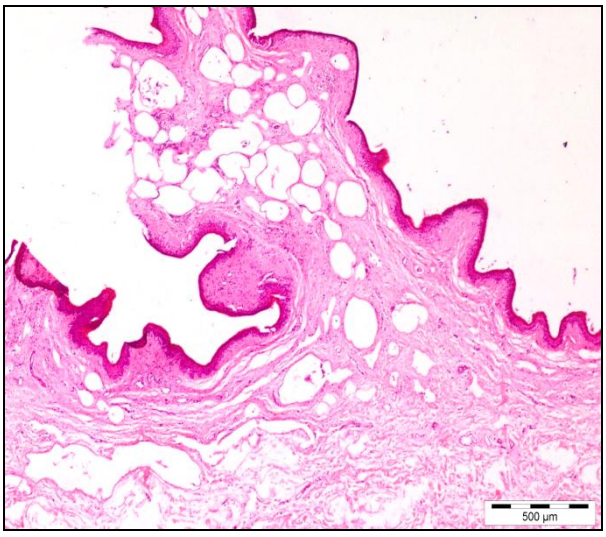

Fig. 25: Sloughing of reticular epithelium and marked dilatation of lymphatics (H&E, original magnification x 100 X)

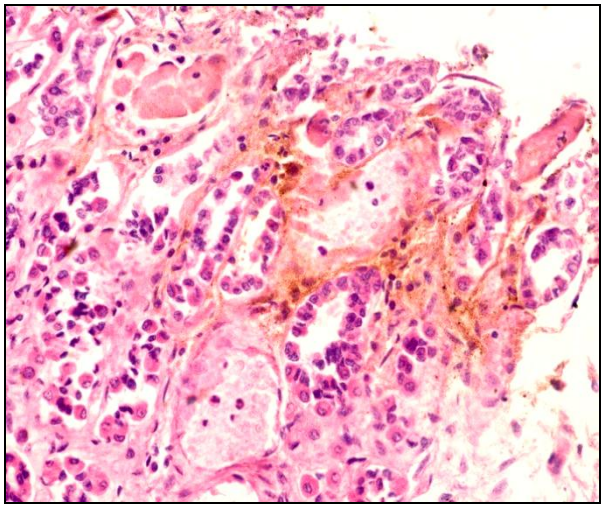

Fig. 26: Bile regurgitation in abomasum (H&E, original magnification x 400 X)

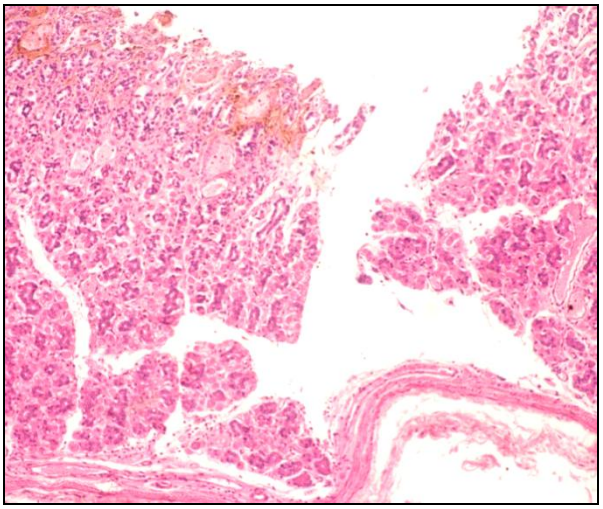

Fig. 27: Type-1 ulcer in abomasum (H&E, original magnification x 100 X)

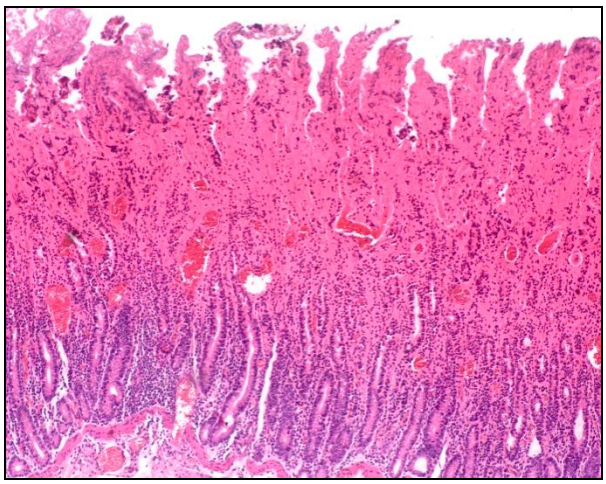

Fig. 28: Section of intestine showing ischaemic necrosis (H&E, original magnification x 100 X)

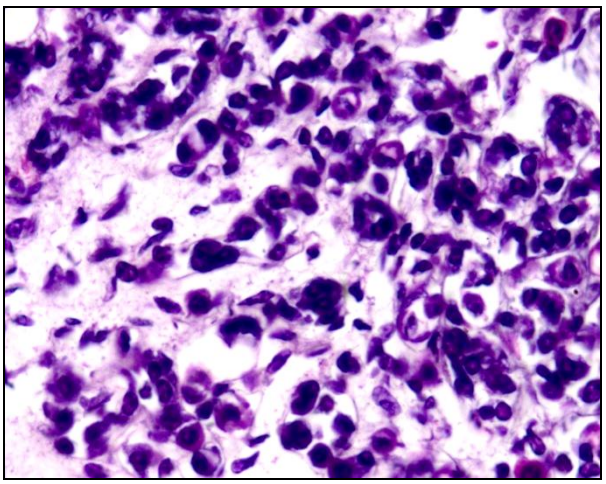

Fig. 29: Marked fibroplasia and mesothelial cell hyperplasia in peritoneum (H&E, original magnification x 400 X)

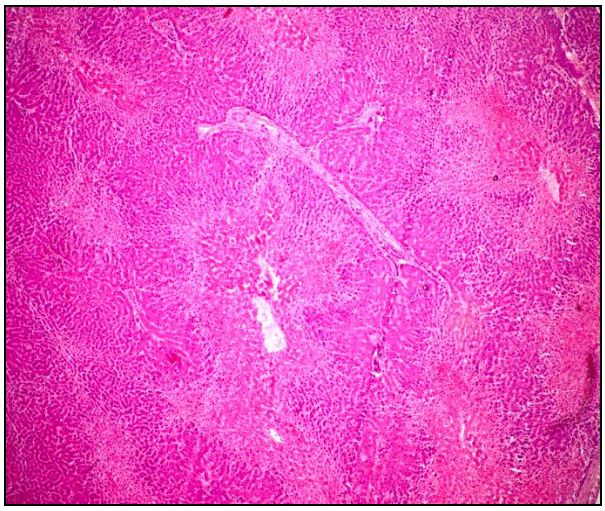

Fig. 30: Centrilobular necrosis and early cirrhosis (H&E, original magnification x 40 X)

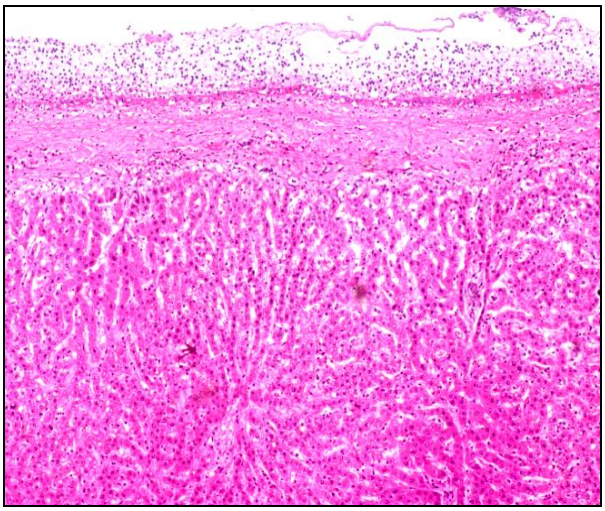

Fig. 31: Fibrinopurulent perihepatitis (H&E, original magnification x 100 X)

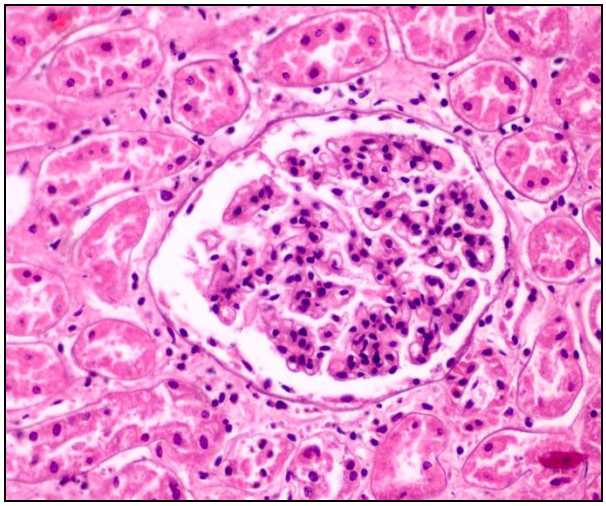

Fig. 32: Proliferative glomerulonephritis, tubular degeneration and nephrosis (H&E, original magnification x 400 X)

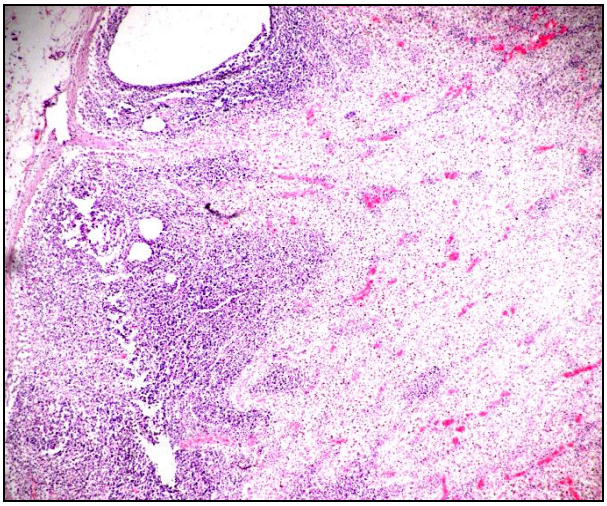

Fig. 33: Lymph node showing congestion and lymphoid cell depletion (H&E, original magnification x 40 X)

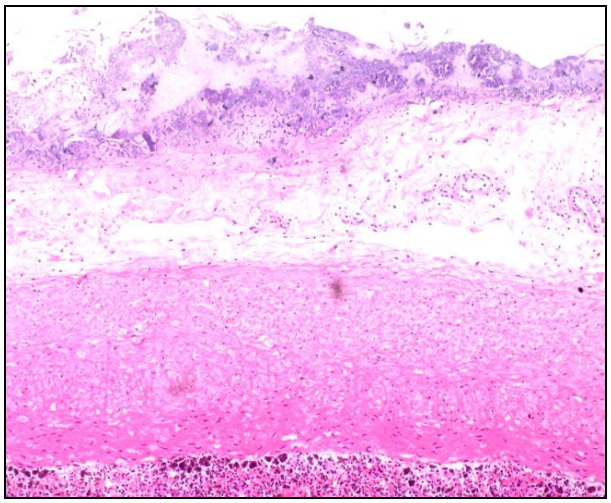

Fig. 34: Fibrinopurulent perisplentitis (H&E, original magnification x 100 X)

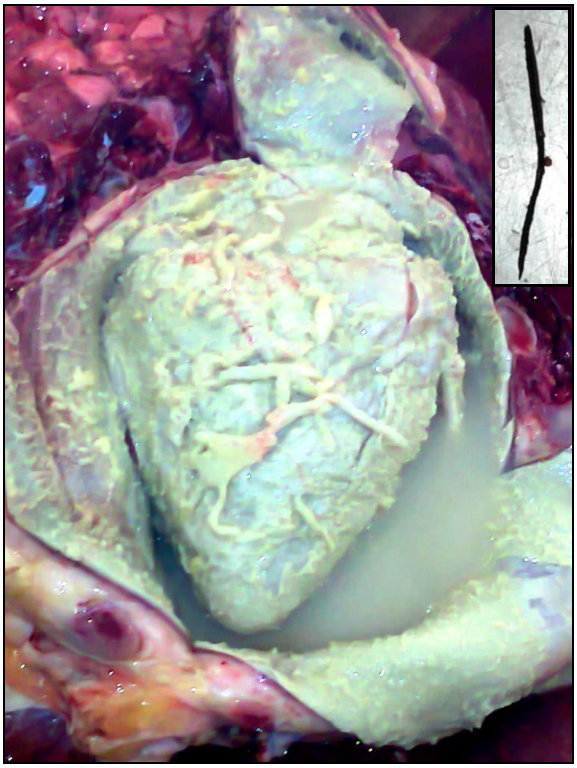

Fig. 35: Suppurative pericarditis with inset showing recovered foreign body

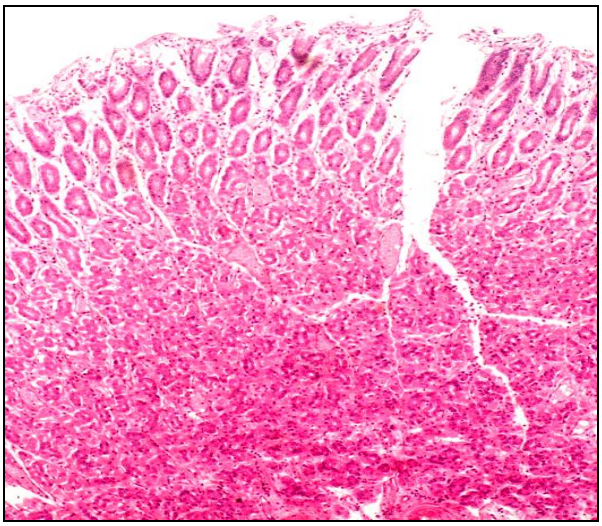

Fig. 36: Chronic gastritis with glandular hyperplasia (H&E, original magnification x 100 X)

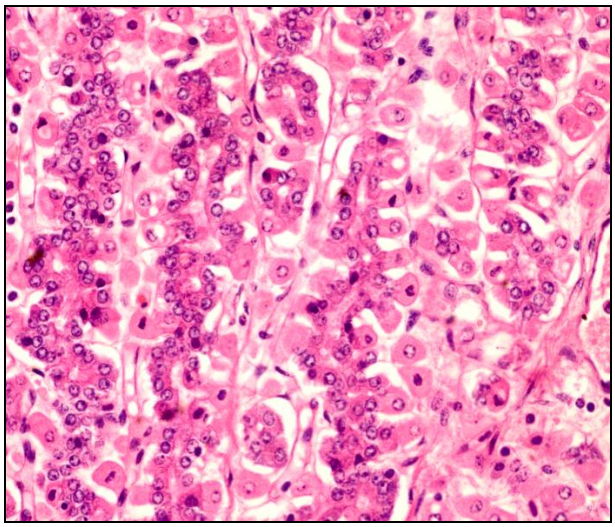

Fig. 37: Higher magnification of previous photo showing pronounced glandular hyperplasia (H&E, original magnification x 400 X)

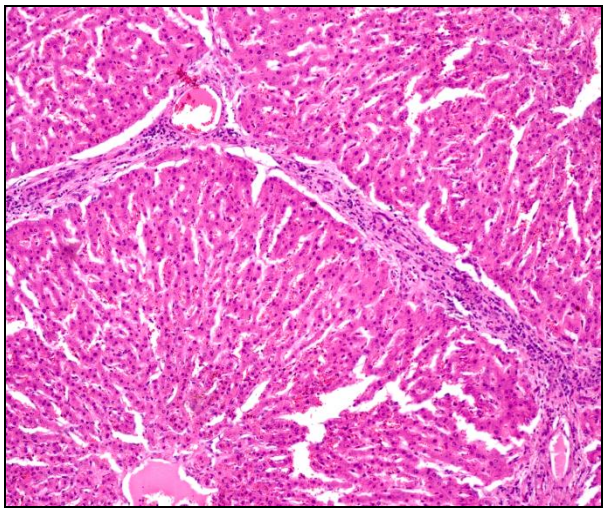

Fig. 38: TP case showing early cirrhosis and pseudolobulation besides chronic venous congestion (H&E, original magnification x 100 X)

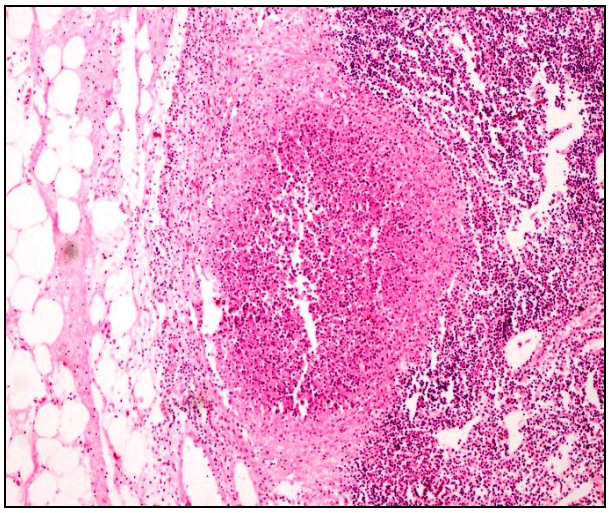

Fig. 39: A large abscess in a mesenteric lymph node in a case of TP (H&E, original magnification x 100 X)

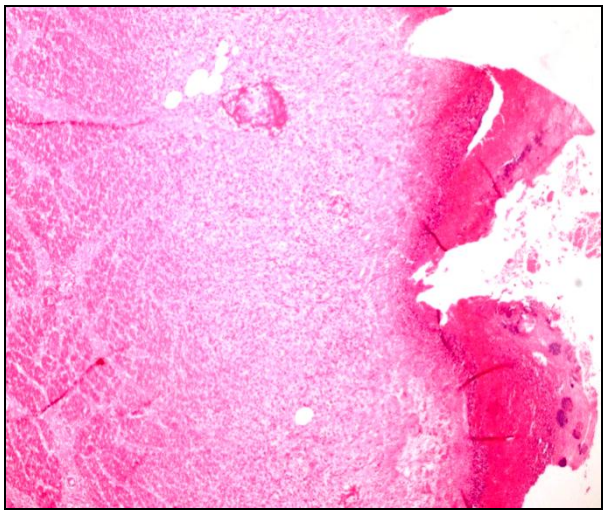

Fig. 40: TP case with massive epicarditis (superficially there is presence of bacterial colonies, massive necrosis, and fibrinopurulent exudation with granulation tissue at the base) (H&E, original magnification x 100 X)

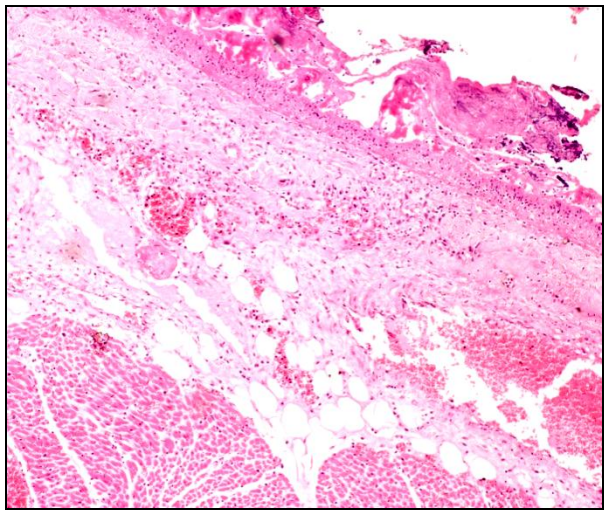

Fig. 41: Another case of TP showing moderate epicarditis (H&E, original magnification x 100 X)

Fig. 42: Omasal impaction

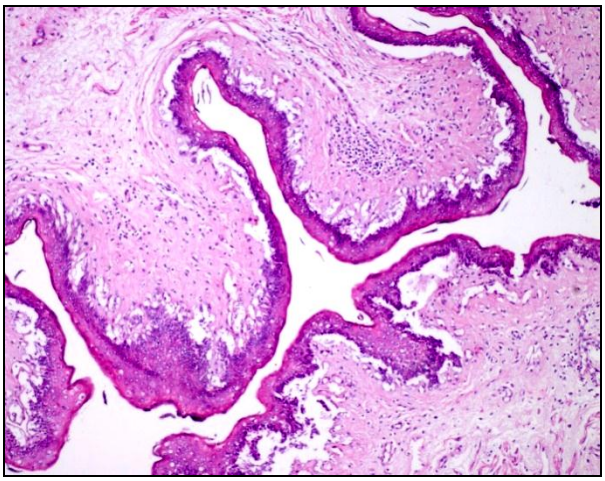

Fig. 43: Omasal impaction revealing early denudation of mucosa and hyperplasia of basal epithelial cells along with chronic inflammation in submucosa (H&E, original magnification x 100 X)

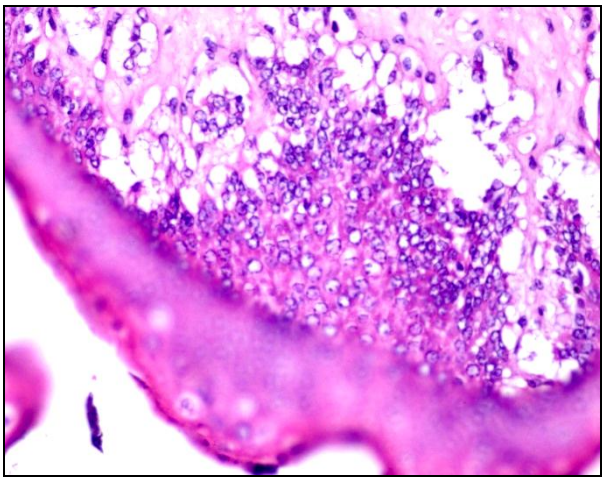

Fig. 44: Higher magnification of previous photo showing hyperplasia and dysplasia of the basal epithelium (H&E, original magnification x 400 X)

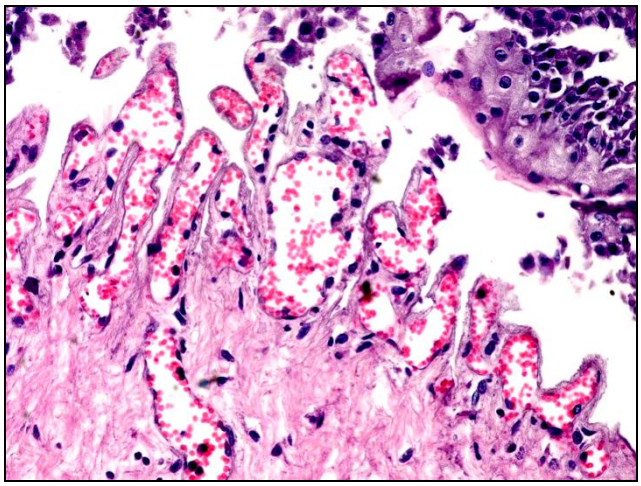

Fig. 45: Prolonged omasal impaction with marked epithelial sloughing, chronic inflammation and neovascularization associated with it (H&E, original magnification x 400 X)

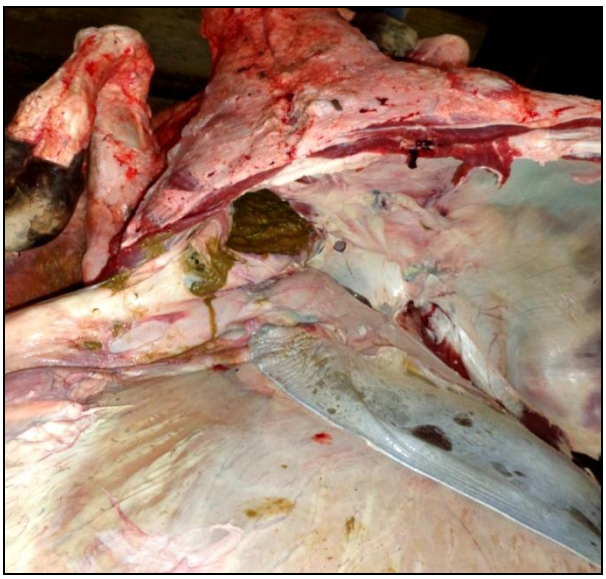

Fig. 46: DH showing herniation site

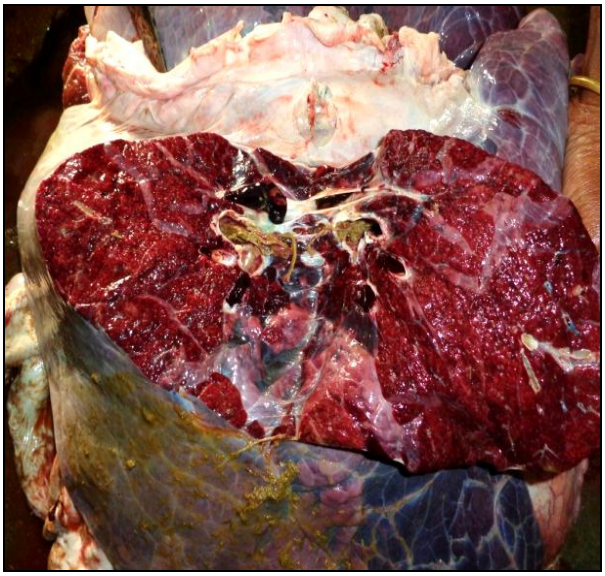

Fig. 47: Marked aspiration of GIT contents in the lung

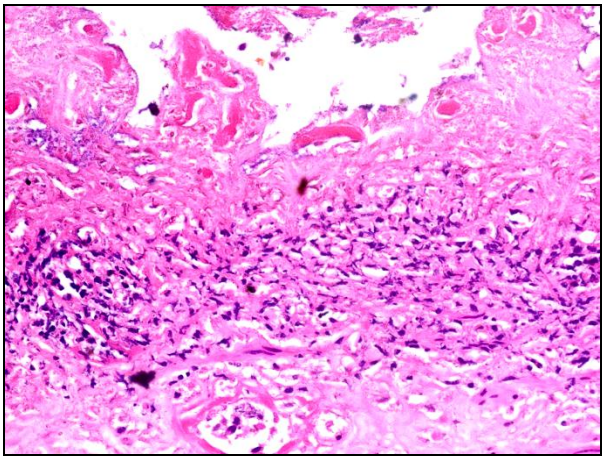

Fig. 48: Chronic reticulitis with thromboembolism and fibroplasia besides extensive sloughing of epithelium (H&E, original magnification x 400 X)

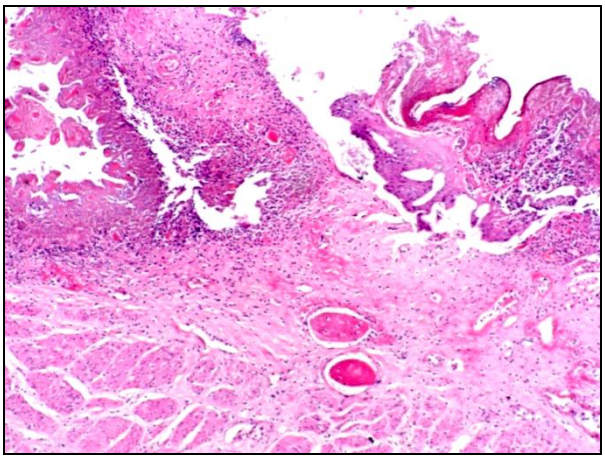

Fig. 49: Reticulum showing thromboembolism, sloughing of epithelium and chronic active inflammation with focal microabscesses (H&E, original magnification x 100 X)

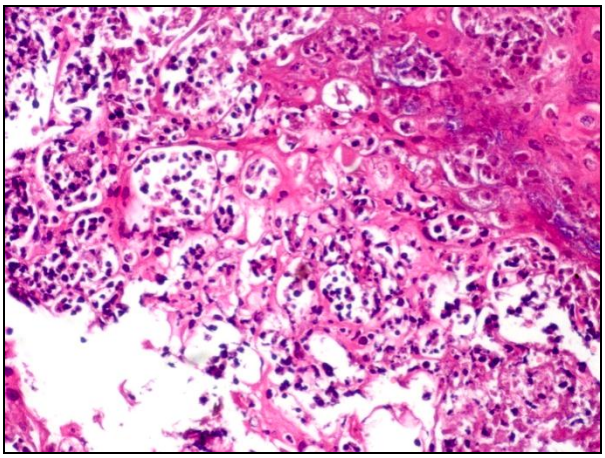

Fig. 50: Higher magnification of previous showing several coalescing microabscesses (H&E, original magnification x 400 X)

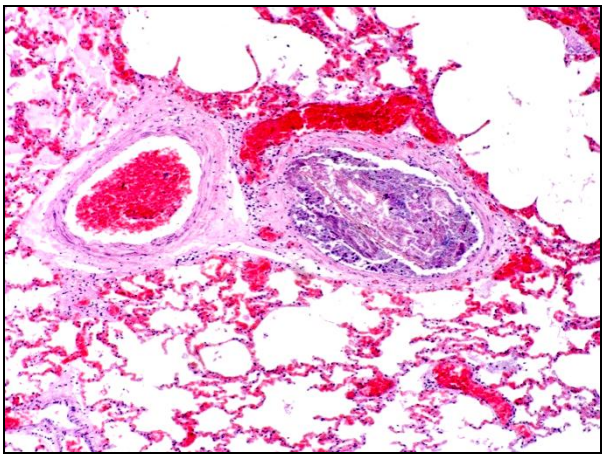

Fig. 51: Histopathology of the case showing aspiration grossly, revealing microscopic evidence of aspiration in the bronchiole (H&E, original magnification x 100 X)

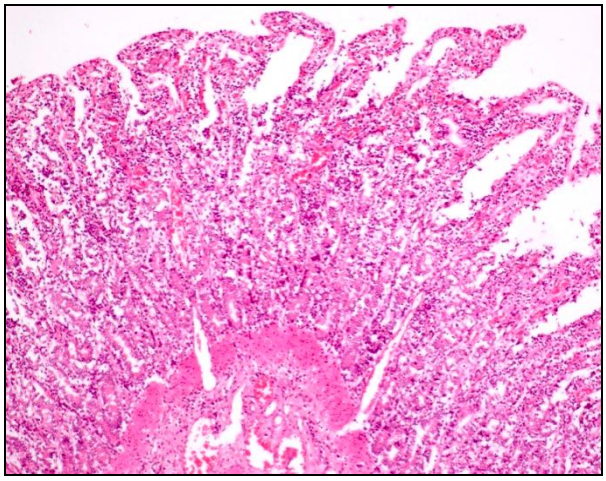

Fig. 52: Marked chronic enteritis characterized by loss of villous epithelium and infiltration of mononuclear cells in case of paralytic ileus (H&E, original magnification x 100 X)

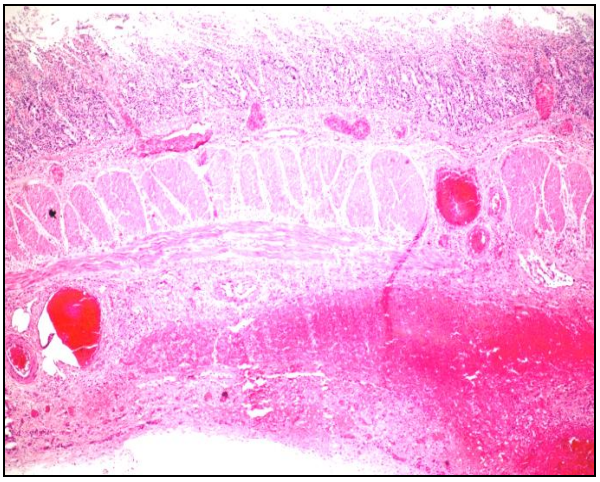

Fig. 53: Section of intestine from volvulus case with massive hemorrhage and venous congestion (H&E, original magnification x 100 X)

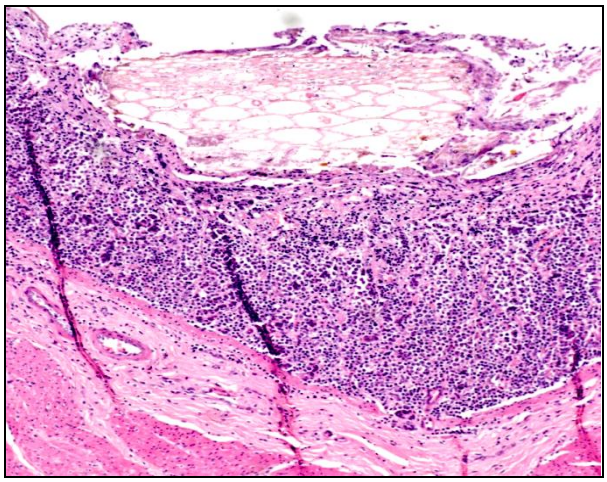

Fig. 54: Duodenal obstruction showing total loss of mucosa, presence of large amount of plant material in the necrotic mucosa and massive infiltration of chronic inflammatory cells (H&E, original magnification x 100 X)

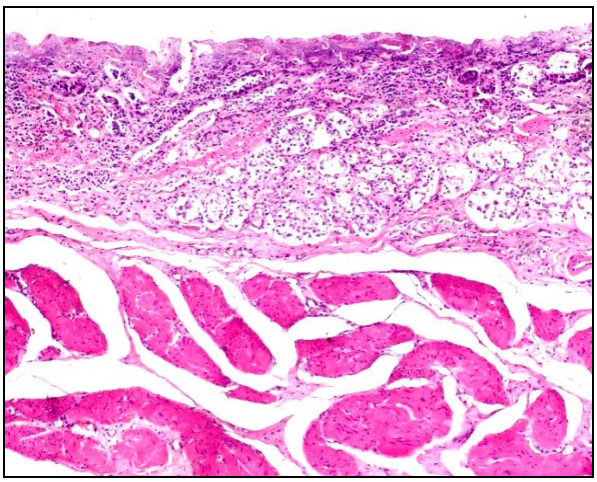

Fig. 55: Duodenal obstruction with total sloughing of mucosa and massive necrosis of underlying crypt epithelium along with chronic inflammation (H&E, original magnification x 40 X)

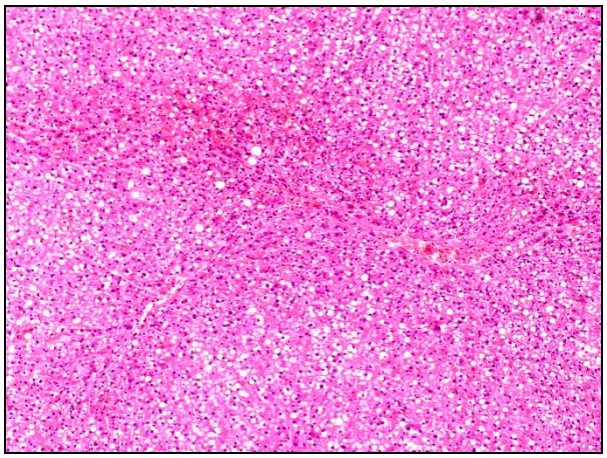

Fig. 56: Severe diffuse fatty change in liver with sinusoidal congestion in the case diagnosed as volvulus (H&E, original magnification x 100 X)

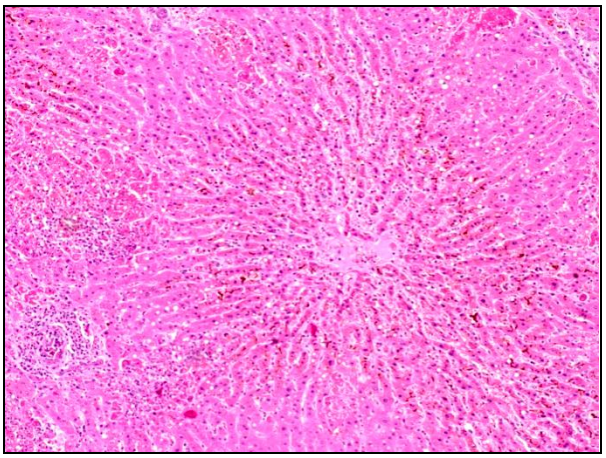

Fig. 57: Chronic venous congestion with sinusoidal dilatation, atrophy of hepatic cords, retention of bile and multifocal infiltration by mononuclear cells in a case of duodenal obstruction (H&E, original magnification x 100 X)

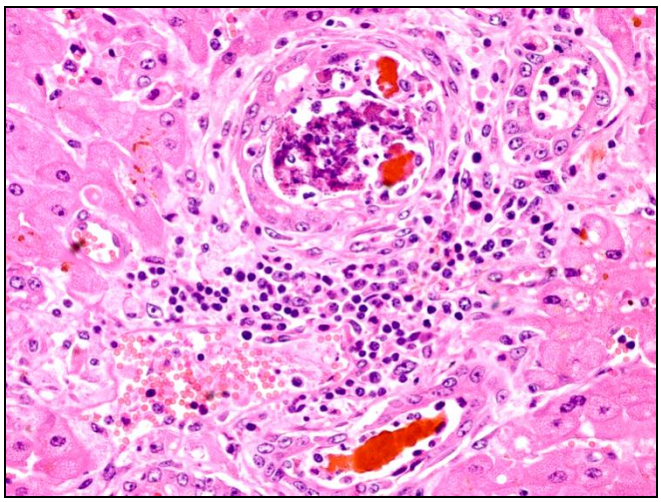

Fig. 58: Section of liver showing cholangiohepatitis with retention of bile (H&E, original magnification x 400 X)

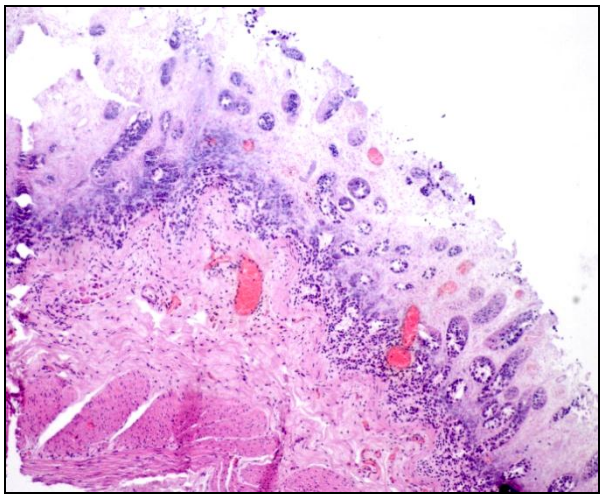

Fig. 59: Intestine showing ischaemic necrosis and marked infiltration by neoplastic cells in lamina propria submucosa (H&E, original magnification x 100 X)

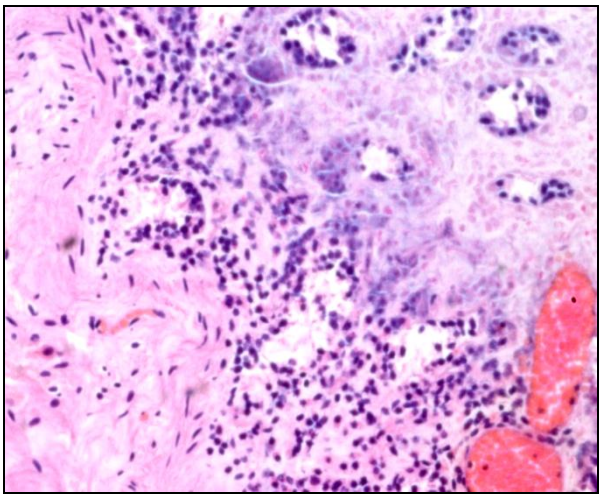

Fig. 60: Higher magnification of previous figure showing pleomorphic lymphoid cells more clearly (H&E, original magnification x 400 X)

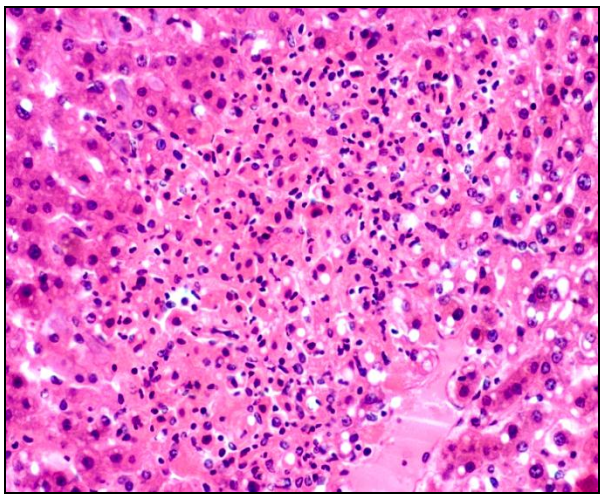

Fig. 61: Enzootic leukosis case showing a granuloma in the liver parenchyma along with fatty change (H&E, original magnification x 400 X)

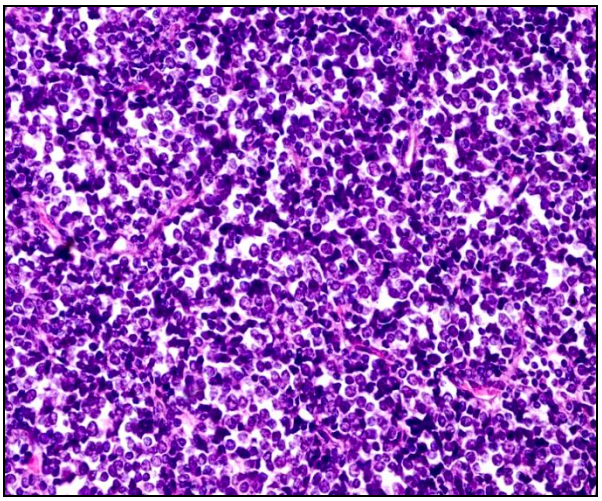

Fig. 62: Spleen from lymphoid leukosis case showing marked diffuse infiltration by neoplastic lymphoid cells (H&E, original magnification x 400 X)

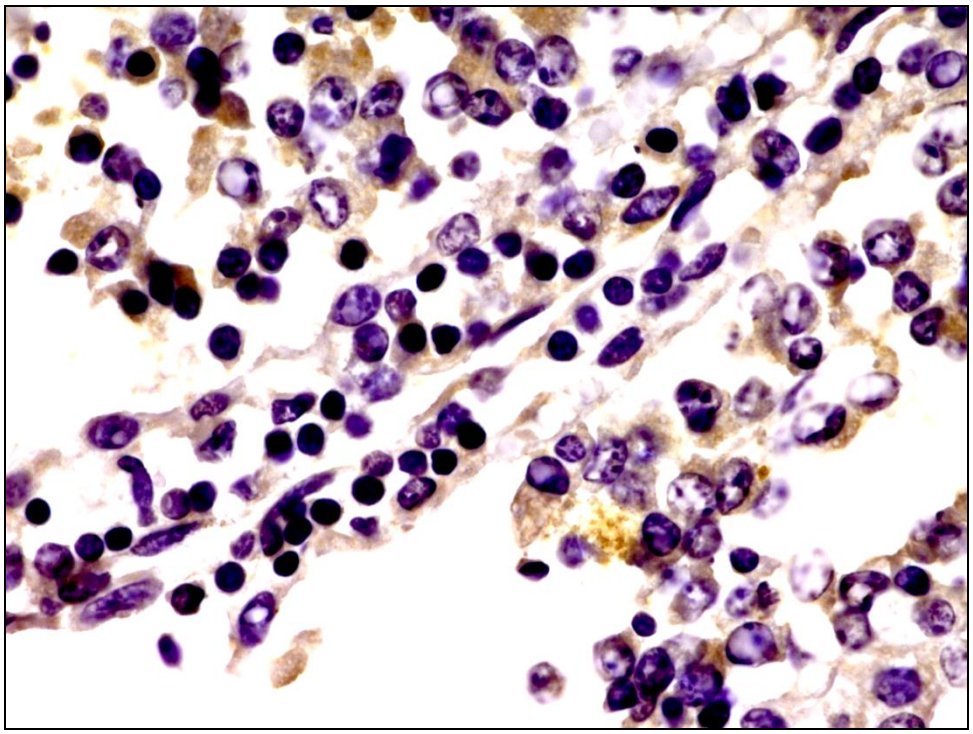

Fig. 63: Section of intestine showing mild to moderate *C. Perfringens* immunoreactivity. One step polymer HRP detection system, counter stained by Gill's hematoxylin. Original magnification x 1000 X

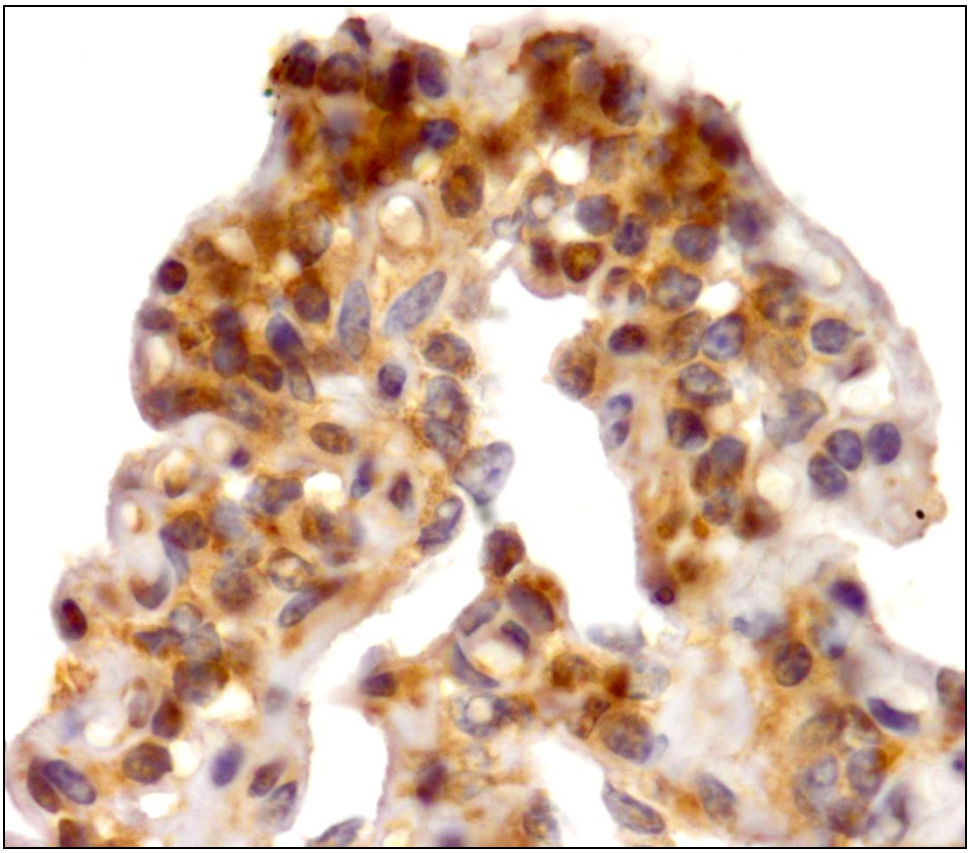

Fig. 64: Section of intestine showing severe *C. perfringens* immunoreactivity. One step polymer HRP detection system, counter stained by Gill's hematoxylin. Original magnification x 1000 X

CHAPTER V

SUMMARY AND CONCLUSIONS

The present study was conducted on clinical cases of gastrointestinal disorders affecting bovines in advanced pregnancy and to correlate the pathological alterations with specific disorders. It was comprised of two parts i.e. study on live animals and study on dead animals. For live animal study, 50 clinical cases (34 buffalo and 16 cattle) presented to the Large Animal Clinics, GADVASU, Ludhiana were used. In addition, tissue samples were collected at necropsy from animals (17 buffaloes and 4 cows) that died during the period due to gastrointestinal disorders in advanced pregnancy.

Cases were divided into seven types on the basis of physical and clinical examination, radiography, ultrasonography, hematology, clinical chemistry and peritoneal fluid examination. The various types of gastrointestinal disorders in advanced pregnancy were DH (6, 12%), peritonitis (16, 32%), omasal impaction (3, 6%), paralytic ileus (3, 6%), late pregnancy indigestion (7, 14%), traumatic pericarditis (7, 14%) and miscellaneous conditions (8, 16%). Among various gastrointestinal problems, the occurrence of peritonitis was found to be highest. Higher occurrence of gastrointestinal disorders was noted in the eighth month of gestation and 3rd parity. Out of 50, 30 animals had history of complete anorexia and 20 animals had history of reduced feed intake. Out of 50 animals, 9 animals had history of absence of defecation, 30 animals were passing scanty faeces, 6 animals had history of diarrhea and 5 animals had normal faecal output. Fever (>102ºF) was observed only in few animals.

Haematology revealed significant changes in almost every group. Mean haemoglobin and PCV was within normal range in each group, except in cases of traumatic pericarditis. TLC was significantly increased in peritonitis and traumatic pericarditis cases. In majority of the cases, DLC was found to be better and more reliable indicator than TLC, especially in cases with normal TLC, as in some cases left shift was observed without increase in TLC. N:L was significantly increased in cases of peritonitis, traumatic pericarditis, DH and omasal impaction. Blood smear

examination revealed abnormalities in the morphology of RBC, which included presence of acanthocytes, schistocytes, crenation and crowding of RBC's and marked rouleaux formation.

Clinical chemistry revealed significant decrease in albumin concentration and increase in globulin concentration, indicating chronic liver damage. Total bilirubin, GGT and lactate level were found significantly higher in almost all the groups.

The peritoneal fluid was collected from 24 cases. Out of 24 cases, evidence of peritoneal inflammation was observed in 19 cases. Peritoneal fluid analysis revealed increase in total and differential leukocyte count and clinical chemistry parameters. All these parameters were found higher than the normal range, in cases of peritonitis. The mean SAAG value was found lower than the cut-off value in peritonitis cases. Peritoneal fluid cytology revealed variable numbers of degenerated neutrophils, macrophages and mesothelial cells, bacteria, plant fibre, gut contents and protozoa. Peritonitis was classified as septic and non-septic peritonitis, based on the presence or absence of bacteria. Based upon the predominance of cells and other constituents, the peritonitis was further classified into fibrinous, fibrinopurulent, frank suppurative and adhesive peritonitis.

The dead animal study included 17 buffaloes and 4 cows. Based upon gross and histopathological findings, the cases were divided into six types viz peritonitis (6/21), traumatic pericarditis (4/21), omasal impaction (3/21), DH (2/21), intestinal obstruction (5/21) and enzootic leucosis (1/21).

The peritonitis included four cases of fibrinous peritonitis and two cases of fibrinopurulent peritonitis. Histopathological examination of the rumen, reticulum and omasum in cases of peritonitis revealed necrosis and sloughing of epithelium along with fibroplasia. Abomasum revealed congestion, superficial gastritis, sloughing and ulceration. Bile regurgitation was also observed in one case. Histopathological examination of the intestine revealed chronic enteritis, ischaemic necrosis, sloughing of mucosa and partial to complete denudation of villi along with massive congestion of serosal vasculature. Sections of omentum/peritoneum revealed hyperplastic to dysplastic changes of mesothelial cells along with chronic active inflammation. Liver

revealed congestion, haemorrhage, degeneration and necrosis. In addition, atrophy of hepatic cords, mild cholangiohepatitis, chronic perihepatitis and early fibrosis to frank cirrhosis was also observed. Fibrinopurulent perihepatitis was also noted in two cases. Microscopic changes in kidneys revealed degeneration of tubular epithelium, glomerulonephritis and interstitial nephritis. Lymph nodes revealed depletion of lymphoid tissue, congestion, haemorrhage and dilatation of lymph vessels. Spleen revealed increase in red pulp and decrease in white pulp and depletion of PALS, indicating rarefaction of lymphoid tissue.

In cases of traumatic pericarditis, fibrinopurulent exudate was predominant. The pericardial sac was filled with pus, containing flakes of fibrin and the serous surface was covered with heavy deposits of fibrin. Microscopically, forestomach revealed necrosis and sloughing of epithelium. Liver revealed chronic venous congestion with atrophy of hepatocytes, dilataion of sinusoids mild to moderate fatty change and early cirrhosis. Kidney revealed degeneration of tubular epithelium, glomerulonephritis and interstitial nephritis and heart revealed severe fibrinopurulent pericarditis with multiple necrotic foci and bacterial colonies. In addition, there was disarray and atrophy of cardiac cells with interfasicular fibrosis.

In omasal impaction, the omasum was grossly distended with dry feed particles with necrosis were present on the omasal leaves. Histopathological examination of omasum revealed chronic inflammatory changes with neovascularisation and proliferation of granulation tissue. Liver revealed congestion, severe ballooning degeneration, mild to moderate centrilobular necrosis, early cirrhosis and stasis of bile. Lungs revealed congestion, haemorrhage, suppurative pneumonia and sloughing of bronchiolar epithelium.

In diaphragmatic hernia, a portion of the reticulum was herniated into the pleural cavity. Aspiration was evident in all cases of DH. Histopathology of reticulum revealed formation of microabscesses, superficial necrosis of epithelium and microthrombi formation in blood vessels. Liver revealed congestion, atrophy of hepatic cords, mild cholangiohepatitis and chronic perihepatitis with thickening of capsule.

At necropsy, one case of malposition of intestine and two cases each of mechanical obstruction (2 cases of faecolith) and functional obstruction (2 cases of paralytic ileus) of intestine were observed. In all the cases of intestinal obstruction, sloughing of mucosa of all forestomach compartments was observed. In cases of paralytic ileus, there was chronic inflammation of intestine with complete denudation of villi. In case of volvulus, there was haemorrhagic enteritis with complete loss of epithelium. In case of duodenal obstruction, there was chronic enteritis at the site of faecolith with complete denudation of villi and partial to complete destruction of crypts. In case of duodenal obstruction, liver was icteric. Microscopically, there was multifocal hepatocellular necrosis and cholangiohepatitis. Kidneys revealed degeneration of tubular epithelium, proliferative glomerulitis and interstitial nephritis.

In the solitary case of enzootic leukosis, there was massive enlargement of mediastinal and other lymph nodes. There was haemorrhagic abomasitis and enteritis. Microscopically, the abomasum revealed mild superficial gastritis and congestion and intestine showed chronic enteritis with infiltration of lymphoid cells in the submucosa of intestine. In liver, there were granulomas resembling typhoid granuloma. Other changes observed were fatty changes, centrilobular necrosis, moderate to marked cholangiohepatitis and hyperplasia of kupfer cells. In lymph nodes, there was massive infiltration of neoplastic lymphoid cells with thickening and fibrosis of medullary cords. In the spleen, there was infiltration of neoplastic lymphoid cells replacing most of the parenchyma along with chronic splenitis.

Immunohistochemistry done on the intestine of 21 cases affected with GIT disorders elucidated the presence of *Clostridium perferingens* in 11 cases showing necrotic enteritis.

CONCLUSIONS

1. Various gastrointestinal disorders recorded in present study were- peritonitis, traumatic pericarditis, diaphragmatic hernia, late pregnancy indigestion, omasal impaction and paralytic ileus.
2. Higher occurrence of gastrointestinal disorders was noted in the eighth month of gestation and 3rd parity.

3. Among all GIT disorders, traumatic pericarditis showed maximum hematological alterations characterized by moderate anemia, marked neutrophilic leukocytosis and thrombocytosis.
4. Hematology seems to play an important role in diagnosis of traumatic pericarditis as more significant alterations in haemoglobin, TLC, DLC and platelets were noted in this condition.
5. Hematology seems to play a great role in diagnosis and prognosis, in inflammatory conditions of GIT including peritonitis, TP and DH and more so in TP.
6. In addition, large number of acanthocytes and schistocytes in both cattle and buffalo were directly correlated with advanced liver damage, in the peripheral blood.
7. A/G ratio, GGT and total bilirubin seemed to be a sensitive indicator of inflammatory condition of GIT in advanced pregnancy.
8. Peritoneal fluid chemistry showed significant increase in total protein and lactate but decrease in SAAG ratio indicating severe inflammation.
9. However, peritoneal fluid in conjunction with peritoneal fluid chemistry was able to differentiate various types of peritonitis viz. fibrinous, fibrinopurulent, suppurative and adhesive peritonitis.
10. The occurrence of late pregnancy GIT disorders confirmed at postmortem was broadly in concurrence with that recorded in live animals.
11. A significant finding of present study was marked liver damage in nearly all the cases of GIT disorders of live and dead animals possibly perpetuated by advanced pregnancy.

REFERENCES

Abd Ellah M R, Hamed M I and Derar R I. 2014. Serum biochemical and hematological reference values for lactating buffaloes. *Comparative Clinical Pathology* **23**(5): 1179-88.

Abdelaal A and Floeck M. 2015. Clinical and sonographical findings in buffaloes (*Bubalus bubalis*) with traumatic reticuloperitonitis. *Veterinarski Arhiv* **85** (1): 1-9.

Abdelaal A M, Floeck M, Maghawry S E and Baumgartner W. 2009. Clinical and ultrasonographic differences between cattle and buffaloes with various sequelae of traumatic reticuloperitonitis. *Veterinari Medicina* **54**: 399-406.

Abdelaal A, Gouda S, Ismail A and Gomaa M. 2014. Reticular diaphragmatic hernia in Egyptian buffaloes: clinical, hemato-biochemical and ultrasonographic findings. *Pakistan Veterinary Journal* **34**(4): 541-44.

Akkoç A. 2007.Traumatic Reticulopericarditis in a Saanen goat. *Turkish Journal of Veterinary and Animal Sciences* **31**(4): 283-28.

Allen S E and Holm E. 2008. Lactate: physiology and clinical utility. *Veterinary Emergency* and *Critical Care* **18**: 123-32.

Anderson D E, Cornwell D, Anderson L S, St-Jean G, Desrochers A. 1995. Comparative analyses of peritoneal fluid from calves and adult cattle. *American Journal of Veterinary Research* **56**(8): 973-6.

Aref N M and Abdel-Hakiem M A H. 2013. Clinical and diagnostic methods for evaluation of sharp foreign body syndrome in buffaloes. *Veterinary World* **6**(9): 586-91.

Asaoka Y, Yanai T, Hirayama H, Une Y, Saito E, Sakai H, Goryo M, Fukushi H and Masegi T. 2004. Fatal necrotic enteritis associated with *Clostridium perfringens* in wild crows. *Avian Pathology* **33**(1): 19-24.

Ashcroft R A. 1983. Abomasal impaction of cattle in Saskatchewan. *Canadian Veterinary Journal* **24**: 375-80.

Athar H, 2009. Use of ultrasonography for the diagnosis of thoracoabdominal disorders in bovines. Thesis submitted to Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, India.

Athar H, Mohindroo J, Singh K, Kumar A and Raghunath M. 2010a. Comparison of radiography and ultrasonography for diagnosis of diaphragmatic hernia in bovines. *Veterinary Medicine International* **2010**: 1-7.

Athar H, Mohindroo J, Singh K, Kumar A and Randhawa C S. 2010b. Clinical, haematobiochemical, radiographic and ultrasonographic features of traumatic

reticuloperitonitis in bovines. *Indian Journal of Animal Sciences* **80**(7): 608–12.

Athar H, Parrah J D, Moulvi B A, Singh M and Dedmari F H. 2012. Pericarditis in bovines- A review. *International Journal of Advanced Veterinary Science and Technology* **1**(1): 19-27.

Bacciarini L N, Boerlin P, Straub R, Frey J and Grone A. 2003. Immunohistochemical localization of *Clostridium perfringens* β2-toxin in the gastrointestinal tract of horses. *Veterinary Pathology* **40**:376–81.

Baron T H, Ramirez B and Richter J E. 1993. Gastrointestinal motility disorders during pregnancy. *Annals of Internal Medicine* **118**(5): 366-75.

Beechler B R, Jolles A E and Ezenwa V O. 2009. Evaluation of hematologic values in free-ranging African buffalo (*Syncerus caffer*). *Journal of Wildlife Diseases* **45**(1): 57-66.

Behl S M, Singh J and Krishnamurthy D. 1996. Functional disorders of stomach (vagus indigestion) in bovine: A review. *Indian Journal of Veterinary Surgery* **17**: 71-80.

Bellavance A, Bonneville-Hébert A, Desrochers A and Fecteau G. 2010. Surgical correction of a diaphragmatic hernia in a newborn calf. *Canadian Veterinary Journal* **51**: 767-69.

Benjamin M M, 1985. *Outline of Veterinary Clinical Pathology*, Kalyani Publisher, New Delhi, India. pp 60-63, 71-75.

Bexiga R, Mateus A, Philbey A W, Ellis K, Barrett D C and Mellor D J. 2008. Clinicopathological presentation of cardiac disease in cattle and its impact on decision making. *Veterinary Record* **162**: 575-80.

Brar G S. 2004. 'Clinical studies on surgically treated reticulo-diaphragmatic and omasal disorder in bovines.' M.V.Sc. Thesis, Punjab Agricultural University, Ludhiana, India.

Braun U, Haussammann and Forrer R. 1989. Reflux of bile acids from the duodenum into the rumen of cows with a reduced intestinal passage. *Veterinary Record* **126**: 373-76.

Braun U, Iselin U, Lischer C and Fluri E. 1998. Ultrasonographic finding in five cows before and after treatment of reticular abscess. *Veterinary Record* **142**: 184-89.

Braun U, Lejeune B, Schweizer G, Puorger M and Ehrensperger F. 2007. Clinical findings in 28 cattle with traumatic pericarditis. *Veterinary Record* **161**: 558-63.

Braun U, Steiner A and Götz M. 1993. Clinical signs, diagnosis and treatment of duodenal ileus in cattle. *Schweizer Archiv fur Tierheilkunde* **135**: 345-55.

Braun U, Steiner A and Kaegi B. 1990. Clinical, haematological and biochemical findings and the results of treatment in cattle with acute functional pyloric stenosis. *Veterinary Record* **126**(5): 107-10.

Braun U. 2006. Therapeutic decision in acute abdominal disorders of the cow. Proceedings of the 24th World Buiatrics Congress, 15-19 October. Nice, France.

Braun U. 2009. Traumatic pericarditis in cattle: Clinical, radiographic and ultrasonographic findings. *The Veterinary Journal* **182**: 176-86.

Cable C S, Rebhun W C, Fubini S L, Erb H N, Ducharme N L. 1998. Concurrent abomasal displacement and perforating ulceration in cattle: 21 cases (1985-1996). *Journal of American Veterinary Medical Association* **212**: 1442-45.

Canfield P J, Best F G, Fairburn A J, Purdie J and Gilham M. 1984. Normal haematological and biochemical values for the swamp buffalo (*Bubalus bubalis*) in Australia. *Australian Veterinary Journal* **61**(3): 89-93.

Cebra C K, Cebra M L and Garry F B. 1996. Gravel obstruction in the abomasum or duodenum of two cows. *Journal of American Veterinary Medical Association* **209**(7): 1294-96.

Chanie M and Tesfaye D. 2012. Clinico-pathological findings of metalic and non-metallic foreign bodies in dairy cattle: a review. *Academic Journal of Animal Diseases* **1**(3): 13-20.

Chvojka J, Sykora R, Krouzecky A, Radej J, Varnerova V, Karvunidis T, Hes O, Novak I, Radermacher P and Matejovic M. 2008. Renal haemodynamic, microcirculatory, metabolic and histopathological responses to peritonitis-induced septic shock in pigs. *Critical Care* **12**(6): 164.

Coles E H. 1986. Erythrocytes, leukocytes, hemostasis and coagulation of blood. In: Coles E H (ed.), *Veterinary Clinical Pathology*, 4th edn, W.B. Saunders, Philadelphia, pp. 12-22, 48-56, 98-1113.

Constable P D, St Jean G, Hull B L, Rings D M, Morin D E and Nelson D R. 1997. Intussusception in cattle: 336 cases (1964-1993). *Journal of American Veterinary Medical Association* **210**: 531-36.

Daniel R C W. 1983. Motility of the rumen and abomasum during hypocalcaemia. *Canadian Journal of Comparative Medicine* **47**: 276-80.

Deshpande K S, Krishnamurthy D, Nigam J M and Sharma D N. 1981. Patho-anatomy of herniation of the reticulum through the diaphragm in the bovine. *Canadian Veterinary Journal* **22**: 234-36.

Deshpande K S, Krishnamurthy D, Peshin P K, Chandna I S and Nigam J M. 1982. Diaphragmatic hernia in bovines. *Indian Veterinary Journal* **59**: 642-46.

Desrochers A and St-Jean G. 1995. Cecal impaction in a cow. *Canadian Veterinary Journal* **36**: 430-31.

Dhablania D C, Tyagi R P S and Nigam J M. 1971. A study on diaphragmatic hernia in bovines. *Indian Veterinary Journal* **48**: 91-98.

Dharmaceelan S, Rajendran N, Nanjappan K, Subramanian M and Balasubramaniam G A. 2012. Incidence of bovine gastrointestinal obstruction in a Teaching Veterinary Hospital of Tamilnadu, India. *Indian Journal of Veterinary Sciences* **1**(3): 112-14.

Dirksen G. 1979. Digestive system Special examination. In: *Clinical Examination of Cattle*. 2nd edn. pp. 184-254 [Rosenberger G (ed.)] Verlag Paul, Parley Berlin and Hamburg, Germany.

Divers T J and Peek S F. 2008. Rebhun's diseases of dairy cattle. Saunders Elsevier, St. Louis, Missouri, pp. 141-47.

Doll K. 1991. Hematologic and clinico-chemical research findings in calves and young cattle with ileus. *Tierarztliche Praxis* **19**(1): 44-47.

Dubensky R A and White M E. 1983. The sensitivity, specificity and predictive value of total plasma protein in the diagnosis of traumatic reticuloperitonitis. *Canadian Journal of Comparative Medicine* **47**: 241-44.

El-Ashker M, Salama M and El-Boshy M. 2013. Traumatic Reticuloperitonitis in water buffalo (*Bubalus bubalis*): Clinical findings and the associated inflammatory response. *Journal of Veterinary Medicine* **2013**:1-6.

Fecteau G. 2005. Management of peritonitis in cattle. *Veterinary Clinics of North America: Food Animal Practice* **21**: 155-71.

Fisher E W and Pirie H M. 1965. Traumatic pericarditis in cattle: a clinical, physiological and pathological study. *British Veterinary Journal* **121**: 552-67.

Fubini S L and Ducharme N G. 2004. *Farm Animal Surgery*. Saunders Elsevier, Philadelphia.

Garry F B. 2002. Indigestion in ruminants. In: Smith B P (ed). *Large Animal Internal Medicine*. 3rd edn. pp 748. Mosby Inc, Philadelphia, USA.

Ghanem M M. 2010. A comparative study on traumatic reticuloperitonitis and traumatic pericarditis in Egyptian cattle. *Turkish Journal of Veterinary and Animal Sciences* **34**(2): 143-53.

Gokce H I, Gokce G and Cihan M. 2007. Alterations in coagulation profiles and biochemical and haematological parameters in cattle with traumatic reticuloperitonitis. *Veterinary Research Communications* **31**: 529-37.

Grosche A, Fürll M and Wittek T. 2012. Peritoneal fluid analysis in dairy cows with left displaced abomasum and abomasal volvulus. *Veterinary Record* **170**(16): 413.

Guard C. 2002. Traumatic reticuloperitonitis (Hardware; Traumatic Reticulitis; TRP). In: Smith B P (ed). *Large Animal Internal Medicine*. 3rd Edn. pp 747-53. Mosby Inc. St. Louis Missouri USA.

Hailat N, Nouh S, AI-Darraji A, Lafi S, Al-Ani F and Al-Majali A. 1996. Prevalence and pathology of foreign bodies (plastics) in Awassi Sheep in Jordan. *Small Ruminant Research* **24**: 43-48.

Hanks D R, Judkins M B, McCracken B A, Holcombe D W, Krysl L J and Park K K. 1993. Effects of pregnancy on digesta kinetics and ruminal fermentation in beef cows. *Journal of Animal Science* **71**: 2809-14.

Hirsch V M and Townsend H G G. 1982. Peritoneal fluid analysis in the diagnosis of abdominal disorders in cattle: a retrospective study. *Canadian veterinary Journal* **23**: 348-54.

Hirvonen J and Pyoral S. 1998. Acute-phase response in dairy cows with surgically-treated abdominal disorders. *The Veterinary Journal* **155**: 53-61.

Hosgood G L and Salisbury S K. 1989. Pathophysiology and pathogenesis of generalized peritonitis. *Problems in Veterinary Medicine* **1**: 159-67.

Hughes D. 1999. Lactate measurement: Diagnostic, therapeutic and prognostic implications. In: Bonagura J (ed): *Kirk's Current Veterinary Therapy XIII: Small Animal Practice*. Philadelphia, WB Saunders, pp. 112-16.

Hussain A S. 2010. 'Studies on prevalence, clinico-hemato-biochemical alterations and therapy of gastrointestinal impaction in dairy animals'. M.V.Sc. Thesis, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, India.

Hussain S A and Uppal S K. 2014. Haemato-biochemical changes and peritoneal fluid cytology in clinical cases of bovine peritonitis. *Indian Journal of Animal Research* **48**(2): 188-93.

Hussain S A, Uppal S K, Bhutia C N, Hassan N, Dar L M. 2012. Clinical findings and haemato-biochemical alterations in caecal dilatation of buffaloes (Bubalus bubalis). *International Journal of Livestock Research* **2**(3): 127-32.

Hussain S A, Uppal S K, Randhawa C S and Sood N K. 2011. Frank exudative peritonitis due to perforated abomasal ulceration in a cross bred cow. *International Journal for Agro Veterinary and Medical Sciences* **5**(5): 447-51.

Hussain S A, Uppal S K, Randhawa C S and Sood N K. 2014a. Bovine intestinal obstruction: blood gas analysis, serum C-reactive protein and clinical, haematological and biochemical alterations. *Journal of Applied Animal Research* **43**(2): 224-30.

Hussain S A, Uppal S K, Randhawa C, Sood N K and Mahajan S K. 2013. Clinical characteristics, hematology, and biochemical analytes of primary omasal

impaction in bovines. *Turkish Journal of Veterinary and Animal Sciences* **37**: 329-36.

Hussain S A, Uppal S K, Sood N K and Mahajan S K. 2014b. Clinico hemato biochemical findings, clinical management, and production performance of bovines with late pregnancy indigestion (type IV vagal indigestion). *Veterinary Medicine International* **2014**: 1-6.

Imran S, Tyagi S P, Kumar A and Sharma S. 2011. Ultrasonographic imaging of normal and impacted omasum in indiancrossbred cows. *Veterinary Medicine International* **2011**: 1-5.

Jain N C. 1986. *Schalm's Veterinary Hematology*. 4th edn. Lea and Febiger, Philadelphia, USA.

Jensen H E, Olsen S N and Aalbek B. 1994. Gastrointestinal aspergillosis and zygomycosis of cattle. *Veterinary Pathology* **31**: 28-36.

Johnson R and Jamison K. 1984. Amyloidosis in six dairy cows. *Journal of the American Veterinary Medical Association* **185**: 1538-43.

Kahnamoii R, Hashtarodi G and Hassaanpour. 2013. Evaluation of hepatic serumal enzyme activity in dairy cattle with cecum dilatation and twisting. *European Journal of Zoological* **2**(5): 75-78.

Kaneko J J. 1997. Serum proteins and dysproteinemias. In: Kaneko J J, Harvey J W and Bruss M L (Eds). *Clinical biochemistry of domestic Animals*, 6th ed. Academic Press, London., pp 117-138.

Khalek El Sheikh A E, Selim H and Monem Ahmed A E. 2012. Studies on chronic diarrhoea associated with acute traumatic reticuloperitionitis in cows and buffaloes. *New York Science Journal* **5**(11): 11-14.

Koller U, Lischer C, Geyer H, Dressel C and Braun U. 2001. Strangulation of the duodenum by the uterus during late pregnancy in two cows. *The Veterinary Journal* **162**: 33-37.

Kramer J W and Hoffmann W E. 1997. Clinical enzymology. In: Kaneko J J, Harvey J W and Bruss M L (eds), *Clinical Biochemistry of Domestic Animals*, 5th edn, (Academic Press, San Diego), pp. 315-17.

Krishnamurthy D, Nigam J M, Deshpande K S, Peshin P K, Singh S C and Sharma D N. 1983. Bovine diaphragmatic hernia: An analysis of 40 clinical cases. *Indian Veterinary Journal* **60**: 1011-16.

Krishnamurthy D, Nigam J M, Peshin P K, Sharma D N and Tyagi R P S. 1985. Monograph on diaphragmatic hernia in bovines. Haryana Agricultural University, Hissar, India.

Kumar A, Saini N S, Mohindroo J, Sangwan V, Mahajan S, Raghunath M and Singh N. 2012a. Long term outcomes of survivors of diaphragmatic herniorrhaphy in

crossbred cows and buffaloes. *Indian Journal of Animal Sciences* **82**(9): 971–75.

Kumar A, Saini N S, Mohindroo J and Sood N K. 2012b. Ultrasonographic features of normal heart and liver in relation to diagnose pericarditis in bovine. *Indian Journal of Animal Sciences* **82**(12): 1489-94.

Kumar A. 2009. 'Diagnostic and therapeutic approach to functional forestomach disorders in cattle buffaloes'. Ph.D. Thesis, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, India.

Kumar M. 2006. 'Studies on the diagnosis of reticular disorders in bovines'. M.V.Sc. Thesis, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, India.

Latson K M, Nieto J E, Beldomenico P M and Snyder J R. 2005. Evaluation of peritoneal fluid lactate as a marker of intestinal ischaemia in equine colic. *Equine Veterinary Journal* **37**: 342-46.

Leek B F. 1969. Reticulo-ruminal function and dysfunction. *Veterinary Record* **84**: 238-43.

Leek B F. 1983. Clinical diseases of the rumen: A physiologist's view. *Veterinary Record* **113**: 10-14.

Luna L G. 1968. *Manual of histologic methods of the Armed forces Institute of Pathology*. 3rd Edn. McGraw -Hill, New York.

Madan J, Gupta M, Rose M K, Sindhu S and Kar D. 2013. Metabolic alterations in buffaloes suffering from digestive disorders. *Haryana Veterinary Journal* **52**: 71-72.

Maden M, Ozturk A S, Bulbul A, Avci G E, and Yazar E. 2012. Acute-phase proteins, oxidative stress, and enzyme activities of blood serum and peritoneal fluid in cattle with abomasal displacement. *Journal of Veterinary Internal Medicine* **26**: 1470-75.

Makhdoomi D M, Singh A P, Singh M P, Krishnamurthy D and Peshin P K. 2002. Simple intestinal obstruction in buffalo calves-A clinical and haematobiochemical study. *Indian Journal of Animal Sciences* **72**(3): 248-50.

Mally K V and Jayadevappa S M. 1974. Diaphragmatic hernia in bovines. *Mysore Journal of Agriculture and Science* **8**: 252-64.

Meyer D J and Harvey J W. 2004. *Veterinary Laboratory Medicine*. 3rd edn., Elsevier Inc., Philadelphia, USA; 169-196.

Miclard J, Jaggi M, Sutter E, Wyder M, Grabscheid B and Posthaus H. 2009. *Clostridium perfringens* beta-toxin targets endothelial cells in necrotizing enteritis in piglets. *Veterinary Microbiology* **137**: 320-25.

Mohamed T. 2010. Clinicopathological and ultrasonographic findings in 40 water buffaloes (*Bubalus bubalis*) with traumatic pericarditis. *Veterinary Record* **167**(21): 819-24.

Mohan R, Saini N S and Singh S S. 2006. Changes in different body fluids during simple intestinal obstruction in buffaloes. *Indian Journal of Animal Sciences* **76**(12): 1001-3.

Mohindroo J, Kumar M, Kumar A and Singh S S. 2007. Ultrasonographic diagnosis of reticular diaphragmatic hernias in buffaloes. *Veterinary Record* **161**: 757-58.

Moore F. 1997. Interpreting serum chemistry profiles in dairy cows. *Veterinary Medicine* **92**: 903-12.

Moore J N, Traver D S, Turner M F, White F J, Huesgen J G and Butera T S. 1977. Lactic acid concentration in peritoneal fluid of normal and diseased horses. *Research in Veterinary Science* **23**: 117-18.

Muggli E, Lesser M, Braun U and Nuss K. 2014. Herniation of the gravid uterus through a mesoduodenal defect and concurrent omental hernia in a cow. *Veterinary Surgery* **43**(1): 91-94.

Nayak S and Suresh Babu S N. 1996. Partial reticulo-omasal orifice obstruction by a perforated phytobezoar in a crossbred cow. *Indian Veterinary Journal* **73**: 983-84.

Netto A C, Gomide M W, Cattelan J W, Marques C, Momo C. 2008. Diaphragmatic hernia associated with traumatic reticuloperitonitis in a jersey cow. *ARS Veterinaria* **24**(2): 72-76.

Nuss K, Lejeune B, Lischer C and Braun U. 2006. Ileal impaction in 22 cows. *The Veterinary Journal* **171**: 456-61.

Ocal N, Gokce G, Gucu A I, Uzlu E, Yagci B B and Ural K. 2008. Pica as a predisposing factor for traumatic reticuloperitonitis in dairy cattle: serum mineral concentrations and hematological findings. *Journal of Animal and Veterinary Advances* **7**: 651-56.

Oehme F W and Moorsdy J L. 1970. Examination of peritoneal fluid in differential diagnosis of bovine disease. *Journal of American Veterinary Medical Association* **65**: 54-59.

Oehme F W. 1969. Cytologic examination of the peritoneal fluid in the diagnosis of cattle disease. *Journal of American Veterinary Medical Association* **155**: 1923– 1927.

Palmer J E and Whitlock R H. 1984. Perforating abomasal ulcers in adult dairy cattle. *Journal of American Veterinary Medical Association* **184**: 171-74.

Pang D S and Boysen S. 2007. Lactate in Veterinary Critical Care: Pathophysiology and Management. *Journal of the American Animal Hospital Association* **43**: 270-79.

Pardon B, Vertenten G, Cornillie P, Schauvliege S, Gasthuys F, Loon G V and Deprez P. 2012. Left abomasal displacement between the uterus and rumen during bovine twin pregnancy. *Journal of Veterinary Sciences* **13**(4): 437-40.

Parraga M E, Carlson G P and Thurmond M. 1995. Serum protein concentrations in horses with severe liver disease: a retrospective study and review of the literature. *Journal of Veterinary Internal Medicine* **9**(3): 154-61.

Pradhan M S, Waghaye U G and Jadhave S G. 2008. Paralytic ileus in a cattle. *Veterinary World* **1**(6): 180.

Prasad B, Singh J, Khanna A K, Khianey N K and Kohli R N. 1979. Abomasal involvement in bovine diaphragmatic hernia and surgical management *Canadian Veterinary Journal* **20**: 26-27.

Prasad B, Singh J, Kumar R, Sharma S N, Rathor S S and Kohli R N. 1977. Surgical repair of DH in buffaloes- An analysis of 42 cases. *Indian Veterinary Journal* **54**: 656-60.

Quiroz-Rocha G F, LeBlanc S J, Duffield T F, Wood D, Leslie K E and Jacobs R M. 2009. Reference limits for biochemical and hematological analytes of dairy cows one week before and one week after parturition. *Canadian Veterinary Journal* **50**: 383-88.

Radostits O M, Gay C C, Hinchcliff K W and Constable P D. 2007. *Veterinary Medicine: A Textbook of the Diseases of Cattle, Horses, Sheep, Pigs, and Goats*. 10th edn., Saunders Elsevier, Philadelphia.

Radostits O M, Gray C C, Blood D C and Hinchcliff K W. 2000. *Veterinary Medicine A Textbook of the Diseases of Cattle, Sheep, Pigs, Goats and Horses*. 9th edn, pp. 250-55, 303-13. W B Saunders Company Ltd, Philadelphia.

Ramakrishna O. 1993. Cardiovascular system. In: Tyagi R P S and Singh J (eds) *Ruminant Surgery*. 1st edn. (Eds), pp. 255-62, CBS Publishers and Distributors, New Delhi, India.

Randhawa C S, Bansal B K, Singh K B and Nauriyal D C. 1996. A clinical report on omasal impaction in crossbred cows. *Indian Veterinary Journal* **73**: 205-06.

Roth L and King J M. 1991. Traumatic reticulitis in cattle: a review of 60 fatal cases. *Journal of Veterinary Diagnostic Investigation* **3**: 52-54.

Roussel A J, Whitney M S and Cole D. 1997. Interpreting a bovine serum chemistry profile: Part 1. *Veterinary Medicine* **92**: 551-58.

Roy S, Roy M and Mishra S. 2010. Hematological and biochemical profile during gestation period in Sahiwal cows. *Veterinary World* **3**(1): 26-28.

Saini N S, Kumar A, Mahajan S K and Sood A C. 2007.The use of ultrasonography, radiography, and surgery in the successful recovery from diaphragmatic hernia in a cow. *Canadian Veterinary Journal* **48**: 575-78.

Saleh M A, Rateb H Z and Misk N A. 2008. Comparison of blood serum proteins in water buffaloes with traumatic reticuloperi- tonitis and sequellae. *Research in Veterinary Science* **85**(2): 208-13.

Senna N A, El-Ghoul W S, Saleh I and Berbish E A. 2003. Clinicopathological, ultrasonographic and pericardiocentesis findings in cattle and buffaloes with traumatic reticulo-peritonitis and pericarditis. *Veterinary Medical Journal Giza* **51**: 381-401.

Sethuraman V and Rathore S S. 1979. Clinical, haematological and biochemical studies in secondary indigestion in bovines due to traumatic reticulitis and diaphragmatic hernia. *Indian Journal of Animal Science* **49**: 703-06.

Shahin H and Ghane M. 2010. Traumatic Reticuloperitonitis in Cattle of Khorramabad (Center of Lorestan Provenience, West of Iran). *Global Veterinaria* **5**(2): 135-39.

Sikkink C J J M, Reijnen M M P J, Laverman P, Oyen W J G and Goor H V. 2009. Tc-99 m-PEG- Liposomes target both adhesions and abscesses and their reduction by hyaluronate in rats with fecal peritonitis. *Journal of Surgical Research* **154**: 246-51.

Simkins K M and Nagele M J. 1997. Omasal and abomasal impaction in beef suckler cows. *Veterinary record* **141**(18): 466-68.

Singh G. 2004. 'Clinical studies on surgically treated reticulo-diaphragmatic and omasal disorder in bovines'. M.V.Sc. Thesis, Punjab Agricultural University, Ludhiana, India.

Singh H, Tyagi R P S, Singh K, Tayal R, Kumar A and Peshin P K. 2000. Effects of strangulated ileal obstruction on clinico-hematological, acid-base, blood gases and biochemical status in crossbred cow calves (*Bos indicus*). *Indian Journal of Veterinary Surgery* **21**(1): 1-6.

Singh N, Kumari R and Akbar M A. 1989. Concentration of biogenic amines in the rumen liquor and blood of healthy buffaloes suffering from indigestion. *Indian Veterinary Medicine Journal* **13**: 84-87.

Singh S. 2008. 'Ultrasonographic evaluation of bovine reticulum, omasum and abomasum in health and disease'. M.V.Sc. Thesis. Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana.

Smith B P. 2002. *Diseases of alimentary tract. Large Animal Internal Medicine*. 3rd Edn. Pp 722-68 Mosby St. Louis Missouri USA.

Smith B P. 2009. *Diseases of alimentary tract. Large Animal Internal Medicine*. 4th Edn. Pp 849-50 Mosby St. Louis Missouri USA.

Sojka J E, White M R, Widmer W R and VanAlstine W G. 1990. An unusual case of traumatic pericarditis in a cow. *Journal of Veterinary Diagnostic Investigation* **2**: 139-42.

Tabrizi B A, Hasanpour A and Mousavi G. 2007. A survey on the biochemical parameters in serum of the Azarbaijan buffaloes with TRP. *Italian Journal of Animal Sciences* **6**(2): 1022-24.

Tharwat M, Ahmed A F and El-Tookhy. 2012. Chronic peritonitis in buffaloes and cattle: Clinical, hematological, ultrasonographical findings and treatment. *Journal of Animal and Veterinary Advances* **11**(15): 2775-81.

Tharwat M. 2011. Traumatic pericarditis in cattle: sonographic, echocardiographic and pathologic findings. *Journal of Agricultural and Veterinary Sciences* **4**(1): 45-59.

Toor A S and Saini N S. 2008. Diagnostic and prognostic indicators of omasal impaction in buffaloes (*Bubalus bubalis*). *Veterinary Record* **162**(9):275-77.

Toor A S. 2003. 'Clinical studies on the surgically treated ruminoreticular, omasal and abomasal disorders in bovines'. M.V.Sc. thesis, Punjab Agricultural University, Ludhiana, India.

Trent A M. 2004. Surgery and the peritoneum. In: Fubini S L and Ducharme N G (ed). *Farm Animal Surgery* pp 271-74. Saunders Elsevier, Philadelphia.

Turkar S and Uppal S K. 2006. Studies on haemato-biochemical profile of diaphragmatic hernia in buffaloes. *Indian Journal of Veterinary Pathology* **30**(1): 64-65.

Turkar S and Uppal S K. 2007. Blood biochemical and ruminal liquor profile in buffaloes (*Bubalus bubalis*) showing omasal impaction. *Veterinary Research Communications* **31**: 967-75.

Turkar S. 2004. 'Studies on impaction of forestomach in buffaloes (*Bubalus bubalis*).' M.V.Sc. thesis, Punjab Agricultural University, Ludhiana, India.

Van Metre D C, Fecteau G, House J K and George L W. 1995. Indigestion of late pregnancy in cow. *Journal of the American Veterinary Medical Association* **206**: 625- 27.

Vary T C, Siegel J H, and Rivkind A. 1988. Clinical and therapeutic significance of metabolic patterns of lactic acidosis. *Perspectives of Critical Care* **1**: 85-132.

Vogel S R, Nichols S, Buczinski S, Desrochers A, Babkine M, Veillette M, Francoz D, Doré E, Fecteau G, Bélanger A M, Badillo M. 2012. Duodenal obstruction caused by duodenal sigmoid flexure volvulus in dairy cattle: 29 cases (2006-2010). *Journal of the American Veterinary Medical Association* **241**(5): 621-25.

Ward J L and Ducharme N G. 1994. Traumatic reticoluperitonitis in dairy cattle. *Journal of the American Veterinary Medical Association* **204**: 874-77.

Ward J L, Smith D F, Fubini S L and Gröhn Y T. 1993. Comparison of 0.9, 3.6, and 7.2% NaCl for correction of experimentally induced hypochloremic, hypokalemic metabolic alkalosis in sheep. *American Journal of Veterinary Research* **54**(7): 1160-69.

Whitlock R H. 1980. Bovine stomach diseases. In: Anderson NV (ed) *Veterinary Gastroenterology*. Lea and Febiger, Philadelphia.

Wilson, A. D., Hirsch, V. M. and Osporne, A. D. 1985. Abdomeno-centesis in cattle. Technique and criteria for diagnosis of peritonitis. *Canadian Veterinary Journal* **26**: 74-80.

Wittek T, Fürll M and Grosche A. 2012. Peritoneal inflammatory response to surgical correction of left displaced abomasum using different techniques. *Veterinary Record* **171**: 594.

Wittek T, Grosche A and Locher L. 2010a. Biochemical constituents of peritoneal fluid in cows. *Veterinary record* **166**: 15-19.

Wittek T, Grosche A, Locher L F and Fürll M. 2010b. Diagnostic accuracy of d-dimer and other peritoneal fluid analysis measurements in dairy cows with peritonitis. *Journal of Veterinary Internal Medicine* **24**: 1211-17.

Zadnik T. 2003. A comparative study of the hematobiochemical parameters between clinically healthy cows and cows with displacement of the abomasum. *Acta Veterinaria Beograd* **53**(5-6): 297-309.

Zadnik T. 2010. A retrospective study of peritoneal fluids in cows with abdominal disorders. *Veterinary Glasnik* **64**(3-4): 187-95.

Ziemer E L. 1989. Cytological analysis of large animal body fluids. *Veterinary Medicine* **6**: 574-83.

VITA

Name of the student	:	Swati Sharma
Father's name	:	Mr. Tilak Raj Sharma
Mother's name	:	Mrs. Sudesh Sharma
Nationality	:	Indian
Date of birth	:	20.01.1990
Permanent home address	:	VPO Dheera, Tehsil Palampur District Kangra – 176101 Himachal Pradesh

EDUCATIONAL QUALIFICATIONS

Bachelor degree	:	B.V.Sc. & A. H.
University	**:**	Chaudhary Sarwan Kumar Himachal Pradesh Krishi Vishvavidyalaya, Palampur
Year of award	:	2013
OGPA	:	7.27/10.00
Master's degree	:	M.V.Sc.
OCPA	:	8.44/10.00
Award/Fellowship /Scholarship	:	University Merit Scholarship during Master's Degree Programme