CLINICO-PATHOLOGICAL STUDIES ON SPLENOMEGALY IN DOGS

SHIVASHANKARANAND GAJANAN RAUT

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THESIS

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Department of Clinical Veterinary Medicine

COLLEGE OF VETERINARY AND ANIMAL SCIENCES

MANNUTHY, THRISSUR – 680 651

KERALA, INDIA

DECLARATION

I hereby declare that this thesis, entitled **"Clinico-pathological studies on splenomegaly in dogs."** is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other university or society.

Mannuthy **SHIVASHANKARANAND GAJANAN RAUT**

22.08.2014 (12-MVM-03)

Dr. P. C. Alex
Professor and Head,
Department of Clinical Veterinary Medicine,
College of Veterinary and Animal Sciences,
Mannuthy, Thrissur-680 651

CERTIFICATE

Certified that this thesis, entitled **"Clinico-pathological studies on splenomegaly in dogs."** is a record of research work done independently by **Shivashankaranand Gajanan Raut**, under my guidance and supervision and it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to him.

Mannuthy

P. C. Alex
Chairman,
Advisory committee

CERTIFICATE

We, the undersigned members of the Advisory Committee of **Shivashankaranand Gajanan Raut**, a candidate for the degree of Master of Veterinary Science in Clinical Veterinary Medicine, agree that this thesis entitled "**Clinico-pathological studies on splenomegaly in dogs.**" may be submitted by Shivashankaranand Gajanan Raut, in partial fulfillment of the requirement for the degree.

Dr P. C. Alex
(Chairman, Advisory committee)
Professor and Head,
Department of Clinical Veterinary Medicine,
College of Veterinary and Animal Sciences, Mannuthy.

Dr. Usha Narayana Pillai
(Member)
Professor and Head,
Department of Clinical Veterinary Medicine,
College of Veterinary and Animal Sciences, Pookode.

Dr S. Ajith Kumar
(Member)
Professor and Head,
University Veterinary hospital,
College of Veterinary and Animal Sciences, Mannuthy.

Dr. C. B. Devanand
(Member)
Professor and Head,
Department of Veterinary Surgery and Radiology,
College of Veterinary and Animal Sciences, Mannuthy.

EXTERNAL EXAMINER

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Dedicated to My Late Father (Bapu)
In his memories…

INTRODUCTION

Spleen, an organ situated in the abdominal cavity forms a part of reticuloendothelial system. It filters particulate matter, namely blood borne antigens and blood cells.

It is apparently concerned with body defense. An animal is able to survive even after removal of spleen. It is often only on post-mortem examination that spleen disorders are revealed.

Spleen has multiple functions, including haematopoiesis, iron metabolism, filtration and phagocytosis, remodelling of red blood cells (RBC's), immunoglobulin M production, removal of intraerythrocytic inclusions and storage of platelets (Dellmann and Canthers, 1996).

The gross and microscopic appearance of the spleen is affected by a variety of systemic, inflammatory and haematologic disorders. Spleen is subjected to disturbances of cell growth (hyperplasia and atrophy), inflammation and neoplasia (primary and metastatic). Several of these processes, either alone or in combination may result in splenic enlargement.

The diagnosis of splenic diseases is by history taking, noting clinical signs, abdominal palpation, physical examination, haematology, radiography, ultrasonography and cytology of the splenic aspirate.

Clinical signs in dogs with splenomegaly are usually vague and nonspecific *viz*., anorexia, weightloss, weakness, abdominal distension, lethargy, vomiting, depression, polyuria and polydipsia.

The condition of the spleen can markedly influence the haemogram. The haematologic abnormalities in dogs with splenomegaly are regenerative anaemia, thrombocytopenia, presence of acanthocytes, nucleated RBCs, increased target cells, poikilocytes and metarubricytes (Couto, 1990).

Radiography is very useful in evaluating splenomegaly or splenic mass. It is usually very easy to view the spleen on survey radiographs of abdomen (Neath *et al.,* 1997).

In addition to routine clinical examination, other diagnostic procedures such as diagnostic ultrasound and ultrasound-guided biopsy could be used for the early identification of diseases of spleen. Ultrasound offers a safe and non-invasive method to detect many diseases of spleen and provides information on gross changes of spleen. Ultrasonographic changes are classified into parenchymal and vascular changes. Ultrasound might suggest the disease process, but cannot provide a histological diagnosis (Nyland and Matton, 1995). Splenic biopsy is required for confirmation of diagnosis.

Accurate diagnosis is important to determine prognosis of splenic diseases. Actual prevalence data of primary splenic diseases are scant (Spangler and culbertson, 1992).

In the light of the above views, the present study on "Clinico-pathological studies on splenomegaly in dogs" was undertaken with the following objective.

To study the clinico-pathological changes in dogs suffering from splenomegaly.

REVIEW OF LITERATURE

2.1. GROSS ANATOMY OF SPLEEN

Couto (1990) suggested that spleen was easily palpable as a flat structure oriented dorsoventrally in the left anterior abdominal quadrant of dogs.

According to Ettinger and Feldman, (2000), the spleen is located in the left anterior quadrant of the abdomen in the dorsoventral position enclosed by a fibromuscular capsule in dogs.

The spleen was an elongated, roughly dumb-bell shaped organ lying more or less vertically against the left side of the stomach but, generally, it was largely deep into the rib cage. The wide gastro-splenic ligament attached spleen to the greater curvature of the stomach (Dyce *et al*., 1996).

2.1.1. Microanatomy

Dellmann and Canthers (1996) reported that the spleen contained major structures like parenchyma, stroma and vasculature. The parenchyma included white pulp [diffuse and nodular lymphatic tissue – periarterial lymphatic sheaths (PALS)], red pulp (pulp spaces, vein and macrophages) and marginal zone located adjacent to the white pulp, which contained lymphocytes and monocytes. The stroma contained heavy capsule and trabeculae which was composed of smooth muscle and reticular connective tissue. The vasculature included splenic arteries, trabeculae and white pulp arteries, arterioles, sheathed capillaries, sinuses and veins.

2.2. PHYSIOLOGY OF SPLEEN

Dellmann and Canthers (1996) and Nelson and Couto (2003) stated that major functions of the spleen were haematopoiesis, filtration and phagocytosis, remodelling of RBCs, removal of intraerythrocytic inclusions, reservoir of RBCs, metabolizing iron and haemopoiesis. White pulp was the body's source of the humoral antibodies.

2.3. INCIDENCE

Hyperplastic nodules and splenic haematomas were the most often encountered disease conditions of the canine spleen. Splenic haemangiosarcoma was second in overall prevalence (Spangler and Culbertson, 1992).

Wood *et al.* (1998) stated that out of 104 cases of haemangiosarcoma eight cases were in first stage (confined to spleen, no metastases) and 15 cases were in second stage (probably tumors which might or might not have regional lymph node metastasis).

Day *et al*. (1995) reported that among the 87 canine splenic biopsies, the commonly diagnosed condition was splenic neoplasia (n=38). The most frequently recognized neoplasm of the spleen was haemangiosarcoma (17 / 38). Other conditions included benign splenic enlargement secondary to nodular hyperplasia (n=6), haematoma (n=16) and non-specific changes including congestion, haemorrhages, extramedullary haematopoiesis and haemosiderin deposition (n=14), splenic infarction (n=3) with or without (n=7) torsion (n=10), abscessation (n=2) and focal mast cell proliferation (n=1).

Day *et al*. (1995) and Withrow and MacEwen (2001) reported that haemangiosarcoma was the most common neoplasm of the spleen in dogs which accounted for 51 to 66 per cent of all cases.

2.4. AGE

Day *et al.* (1995) reported that age of the affected dogs ranged from six months to 11 years and among them middle aged to older dogs were more prone to abnormalities of spleen.

According to Wilkerson *et al,* (2005), three to 14 years old dogs with an average of 8.5 years were prone to lymphoma.

The mean age of dogs with haemangioma was 9.3 years (four to 15 years) (Brown *et al.*,1985) and Sabattini and Bettini (2009).

Kimura *et al.* (2011) reported that the incidence of canine lymphoma was more in the age group of five to nine years (45 per cent), followed by ten to 13 years (34 per cent), below four years (17 per cent) and above 13 years (5 per cent). The mean age was 8.7 years with a range of five months to 15 years.

2.5. SEX

Spangler and Culbertson (1992) recorded that myeloid metaplasia or hyperplasia occurred five times more in females than males.

Day *et al.* (1995) recorded that sex predilection was more towards female dogs in splenic affections.

Wood *et al.* (1998) diagnosed splenic haemangiosarcoma (HSA) in 19 females (18 spayed and one intact) and 13 males (five neutered and eight intact).

The ratio of male to female dogs affected with diseases of spleen was 2.1:1. Male dogs were more prone to haemangiosarcoma (Brown *et al.,* 1985; Sabattini and Bettini, 2009).

Kimura *et al.* (2011) stated that out of 65 cases of lymphomas, 30 were recorded in females and 35 in males.

2.6. BREED

German Shepherd (27.6 per cent) were more prone to splenic diseases (Brown *et al*. 1985; Sabattini and Bettini, 2009) followed by Golden retrievers and Labrador retrievers (Spangler and Culbertson, 1992).

Day *et al.* (1995) reported that among the various breeds of dogs, the German Shepherd was most susceptible to splenic diseases.

Neath *et al.* (1997) stated that large and deep chested breeds like Great Dane and German Shepherd were at an increased risk for the isolated cases of splenic torsion.

According to Wilkerson *et al.* (2005), out of the 59 cases of lymphoma, Labrador retrievers (n=12) and Golden Retrievers (n=10) were found to be the most affected breeds.

Simeonova *et al.* (2007) reported that the primary torsion of spleen as well as gastric dilatation-volvulus occurred mostly in large, deep chested breeds such as St. Bernard, Labrador, Great Dane and German shepherds.

2.7. WEIGHT OF SPLEEN

Mallinckrodt and Gottfried (2011) reported that dogs with benign splenic masses had a significantly higher mean mass to splenic volume ratio and higher mean splenic weight than old dogs with haemangiosarcoma. The authors further suggested that splenic mass to volume ratio and spleen as a percentage of body weight might be useful in differentiating between haemangiosarcoma and benign splenic masses.

2.8. CLINICAL SIGNS

Signs of haemolysis (icterus, haemoglobinaemia, haemoglobinuria and bilirubinuria) and signs of increased erythropoiesis (anisocytosis, poikilocytosis, reticulocytosis, polychromatophilia, basophilic stippling and the presence of nucleated erythrocytes in peripheral blood), anaemia and leucocytosis might be present in most of the cases of splenic torsion. Death probably occurred within 24 hours following torsion (Maxie *et al.*, 1970).

Hardie *et al.* (1995) reported that the common presenting complaints of splenic diseases were anorexia, intermittent vomiting, lethargy, diarrhoea, polyuria, polydipsia, and red or dark coloured urine. The duration of disease varied from less than one day to one month, with most dogs noticeably ill for one to two weeks.

Simeonova *et al.* (2007) reported that the symptoms of splenic torsion depended on the degree of torsion which could vary from 90^0 to 180^0. Pain was a constant sign. Acute twisting of the splenic vessels led bleeding into abdomen resulting in infarction, melaena, haematemesis, anaemia, thrombocytopenia, weakness and collapse.

2.9. PHYSICAL FINDINGS

Maxie *et al.* (1970) observed gross distention of spleen in a case of splenic torsion. The reason for distention was attributed to thickening of arteries which helped in the permeated arterial blood flow into the spleen but obstructed venous out flow.

2.10. DIAGNOSIS

2.10.1. Haematogical findings

According to Ng and Mills (1985), the haematological findings in nine dogs with splenic or hepatic haemangiosarcoma were characterized by mild to moderate normochromic anaemia, neutrophilia, thrombocytopaenia, poikilocytosis and increased target cells.

Couto (1990) observed regenerative anaemia, thrombocytopaenia and neutrophilia in haemangiosarcoma and rarely in dogs with splenic haematoma or haemangioma.

Waner *et al*. (1997) and Unver *et al.* (2009) reported mild leukopenia and anaemia (usually normocytic, normochromic, non-regenerative) in acute stage of *Ehrlichia canis* (*E. canis*) infection. Chronic stages of canine monocytic ehrlichiosis (CME) were characterized by severe thrombocytopenia, leucopenia and anaemia.

2.10.2. Serum biochemical findings

Couto (1990) reported that serum biochemical abnormalities associated with splenomegaly were rare in dogs.

Hardie *et al.* (1995) reported biochemical abnormalities like hypoalbuminaemia, increased alkaline phosphatase activity, hyperbilirubinaemia, increased blood urea nitrogen (BUN) and creatinine concentration in dogs affected with splenic infarction.

Waner *et al*. (1997) observed hypoalbuminaemia and hypergammaglobulinaemia in canine monocytic ehrlichiosis (CME) affected dogs.

The author also observed mild transient increase in alanine aminotransferase and alkaline phosphatase activities in those cases.

2.11. ULTRASONOGRAPHY

2.11.1. Principle

Lamb (1990) and Carteen *et al.* (1993) explained the principle of ultrasound production and transmission through tissues.

Park *et al.* (1981) and Mahoney (2011) explained various modes of display and principles of image interpretation.

While viewing ultrasound images, the scanning surface or near field should be uppermost. Longitudinal, sagittal or parasagittal scan should be oriented with the cranial aspect of the animal to the viewer's left and caudal aspect to the viewer's right. While viewing transverse scans, the left and right sides should be oriented as though the viewer was observing the animal from the caudal aspect (Park *et al.,* 1981). The author also suggested that while evaluating a possible lesion ultrasonographically, the internal echo patterns, borders and adjacent echo patterns should be observed. Internal echo patterns were anechoic, hypoechoic, echoic and complex. Margins of lesions might be well defined or irregular or ill defined.

2.11.2. Indications

Indications of ultrasonography of spleen were splenomegaly, palpable splenic mass, cranial abdominal organomegaly, lethargy, collapse, anaemia, abnormal red blood cells and deep-chested dogs with vague clinical signs of abdominal discomfort (Nyland and Matton, 1995; Lamb, 1990 and Mahoney, 2011).

Indications of ultrasonography as a primary diagnostic technique included discrimination of cystic and solid mass, exploration of fluid filled body cavities, discrimination of texture of solid mass and biopsy guidance (Carteen *et al.,* 1993)

2.11.3. Procedure

The spleen was systematically scanned in sagittal and transverse planes from the ventral abdominal wall. A 7.5 MHz or 10 MHz transducer might be used to provide high resolution because of the superficial location of spleen. A standoff pad was helpful to position the spleen further from the transducer and more fully within the focal zone of the transducer if the ventral (parietal) surface was incompletely visualized in near field (Mahoney, 2011; Nyland and Matton, 1995).

According to Bread (2010), spleen was best viewed when patient was placed on right lateral recumbency and scanning procedure initiated from the splenic head in the left cranial abdomen, examined systematically in the sagittal and transverse planes to the tail of the spleen. The position of the tail of the spleen was variable and extended along the left body wall or across the midline ventrally. It was important to aim the transducer cranially under the border of the rib cage to visualize the entire splenic head.

Benter *et al.* (2011) suggested that spleen should be examined with the patient in a supine or lateral recumbent position. Imaging should be performed while the patient exhales because the upper pole of the spleen was otherwise covered by lung tissue. The 10^{th} and 11^{th} intercostal spaces constituted the ideal acoustic window for ultrasound transmission. The spleen was ultrasonographically examined from the diaphragmatic end to the lower pole.

2.11.4. Normal Ultrasonographic Pattern of Spleen.

The normal appearance of the spleen was a homogeneous, fine echo texture with medium to high level echoes (less coarse and more echogenic than the liver). It had a smooth echogenic capsule. Splenic veins appeared as anechoic tubular structures converging into several large veins at the hilus to form the splenic vein deep in the splenic parenchyma. Arteries were generally not visible except with colour doppler. Hyperechoic non-shadowing areas adjacent to superficial splenic vessels represented splenic capsular invagination and fat surrounding vessels at the splenic hilus was considered normal (Bread, 2010).

Benter *et al.* (2011) observed that the normal length of spleen in adult dogs was about 10 to 12 cm.

Mahoney (2011) opined that normal spleen was more echogenic than liver and renal cortex.

Lamb (1990) reported that normal splenic parenchyma had uniform to slightly coarse echo texture and was more echogenic than liver whereas it was hypoechoic or isoechoic compared to right kidney.

2.12. ABDOMINAL RADIOGRAPHY

Stickle (1989) reported that the abdominal radiographs revealed an enlarged spleen which usually caused pressure on other abdominal organs. The radiographic features of splenic torsion included suboptimal abdominal details, displacement of other abdominal organs and loss of visualization of the body of the spleen in its normal position on the ventrodorsal view with intrasplenic gas. The spleen appeared enlarged and folded into a 'C' shape in the central portion of the abdomen on the lateral radiographic view.

Wood *et al.* (1998) confirmed a case of haemangiosarcoma by abdominal radiography. Presence of abdominal fluid might obscure the diagnosis of splenic masses.

2.13. CYTOLOGY – FINE NEEDLE ASPIRATION BIOPSY

O'Keefe and Couto (1987) stated that transabdominal fine needle aspiration of tissue from the spleen was a safe and reliable method for evaluation of patients with splenomegaly. This procedure was safe even in patients with coagulopathies or neoplasia and provided fast and reliable results.

Christopher (2003) reported that the normal cellular components of the splenic aspirates were blood, stroma (red pulp) and lymphoid tissue (white pulp). The smear was dense with erythrocytes and might contain fibrin clots and platelet clumps.

Singh *et al.* (2010) reported that ultrasound guided fine needle aspiration biopsy was found to be accurate in diagnosing extramedullary haematopoiesis (EMH), suppurative splenitis and lymphoma.

2.14. SPLENIC DISEASES

On the basis of etiology splenic diseases were classified as primary and secondary.

2.14.1. Splenomegaly

Couto (1990) classified splenomegaly in dogs into localized and diffuse type. The localized splenic masses were classified as either neoplastic or non-neoplastic according to their histopathological features. Common non-neoplastic mass was haematoma and neoplastic masses were haemangioma and haemangiosarcoma. The diffuse splenomegalies were categorized as inflammatory lymphoreticular hyperplasia, congestive and infiltration splenomegaly, based on their pathogenesis.

Day *et al.* (1995) reported that the most commonly observed splenic changes were gross increase in size (splenomegaly). Nyland and Matton (1995) classified parenchymal changes in splenomegaly as diffuse, focal or multifocal. Bread (2010) also reported similar findings.

Buergelt (2002) reported that splenomegaly could be diagnosed by abdominal palpation and radiographic or ultrasonographic examination.

2.14.2. Diffuse Splenomegaly

Nyland and Matton (1995) documented that an enlarged spleen with low echogenicity was often found with malignant diseases such as lymphoma, plasmacytic neoplasia or acute congestion. Splenomegaly caused by chronic congestion, chronic inflammatory process or chronic myeloproliferative disorders produced a higher echogenicity to spleen than normal.

2.14.3. Inflammatory Splenomegaly

2.14.3.1. Etiology

Mahoney (2011) stated that inflammatory splenomegaly might be associated with extramedullary haemopoiesis, lymphoid hyperplasia, chronic inflammatory diseases, immune-mediated diseases, bacterial, fungal, or rickettsial diseases, and blunt abdominal trauma.

2.14.3.2. Pathogenesis

Couto (1990) reported that the inflammatory changes within the spleen (splenitis) usually resulted in localized or diffuse enlargement of the organ. Most disorders associated with splenitis were infectious or granulomatous in natures which were further classified the splenitis into suppurative, necrotizing, eosinophilic, lymphocytic, plasmacytic, granulomatous and pyogranulomatous type.

2.14.3.3. Ultrasonography

Nyland and Matton (1995) recorded a markedly hypoechoic, lacy parenchymal pattern in severe, acute inflammation. Splenitis associated with granulomatous diseases caused a diffusely hypoechoic spleen (Carteen *et al*., 1993).

2.14.4. Torsion of spleen

2.14.4.1. Etiology

Simeonova *et al.* (2007) stated that blunt abdominal trauma or surgery, splenomegaly or pregnancy might lead to tearing or abnormal relaxation of splenic suspensory ligaments causing splenic torsion.

Splenic torsion could be associated with gastric dilatation and volvulus, but occasionally occurred as a primary problem, typically in large or giant breed dogs, particularly German Shepherds and Great Danes (Mahoney, 2011).

2.14.4.2. Clinical Signs

Szatmari *et al.* (2000) recorded that the most common presenting complaints were anorexia, lethargy and vomiting. The clinical signs in splenic torsion were acute onset of abdominal pain, depression, abdominal distension and large firm mass palpable in the mid- abdomen.

Simeonova *et al.* (2007) reported that the symptoms of splenic torsion varied depending on the degree of torsion from 90^0 to 180^0. Pain was a constant sign and acute twisting of the splenic vessels resulted in bleeding into abdomen, melaena, haematemesis, anaemia, thrombocytopenia, weakness and collapse.

2.14.4.3. Physical findings

Maxie *et al.* (1970) studied the physical findings of splenic torsion as splenic venous obstruction which allowed arterial blood flow because of its thicker wall and subsequent gross distention which was easily palpable by abdominal palpation.

2.14.4.4. Diagnosis

2.14.4.4.1. Ultrasonography

Konde *et al*. (1989) recorded that the presence of a severely enlarged spleen with diffuse anechoic areas separated by small linear echoes appeared unique to splenic torsion.

A thorough abdominal ultrasonography detected severe splenomegaly. The spleen appeared to be normally positioned; however, the splenic parenchyma was diffusely heterogeneous with multiple parallel echogenic lines in a coarse, lacy, hypoechoic pattern. The splenic vessels were identified and appeared to be within normal limits, the blood flow was not detected with color flow Doppler. The spleen appeared hyperechoic, consistent with inflammation and/or peritonitis (Schnier, 2010).

2.14.4.4.2. Radiography

According to Stickle (1989), radiographic examination revealed reduced serosal details and local accumulation of gas within the spleen.

Splenomegaly or a large mid-abdominal mass was a common radiographic finding in dogs with isolated splenic torsion and ultrasonography was always necessary to confirm the diagnosis (Goldsmid *et al.*,1994).

2.14.4.4.3. Haematology

Stevenson *et al*. (1981) studied the haematological abnormalities which usually included regenerative anaemia, target cells, leucocytosis with a regenerative left shift and leucoerythroblastosis.

2.14.4.5. Pathology

2.14.4.5.1. Gross

The twisted splenic pedicle might distort the stomach and interfere either with eructation or the abnormal passage of ingesta and gas and finally lead to gastric tympany. The development of GDV syndrome with or without volvulus might play a role in the pathogenesis of splenic torsion (Neath *et al*., 1997).

2.14.4.5.2. Histopathology

Saunders *et al.* (1998) stated that the histopathological findings were splenic congestion, hemorrhages, thrombosis in splenic veins, infarction and necrosis.

2.14.5. Splenic Infarction

2.14.5.1. Etiology

Hardie *et al.* (1995) recorded that splenic infarction occurred in dogs with hypercoagulable conditions associated with liver diseases, renal diseases and hyper-adrenocorticism or as a consequence of uniform splenomegaly, neoplasia or thrombosis associated with cardiovascular diseases.

Mahoney (2011) stated that splenic infarction occurred secondary to hyperadrenocorticism, hypothyroidism, immune-mediated diseases, protein losing enteropathy, protein losing nephropathy, bacterial endocarditis and neoplasia.

2.14.5.2. Clinical signs

Day *et al*. (1995) observed lethargy, weakness with pyrexia or vomiting and acute collapse in splenic infarction.

2.14.5.3. Diagnosis

2.14.5.3.1. Ultrasonography

Wood *et al.* (1998) stated that the ultrasonography of the splenic infarction showed a focal hypoechoic or isoechoic region with a more diffuse, coarse mixed pattern of echogenicity.

According to Hardie *et al.* (1995), ultrasonogram of splenic infarction showed hypoechoic and lacy spleen.

Saunders *et al.* (1998) reported that the anechoic venous lumen with echogenicity indicated the occluding thrombus in splenic vessels with splenic infarction and necrosis due to splenic torsion.

2.14.5.4. Pathology

2.14.5.4.1. Gross

Hardie *et al.* (1995) described splenic infarction as solitary or multiple, well demarcated nodular swelling that ranged from 0.5 to 10 cm in dimension.

2.14.5.4.2 Histopathology

Day *et al*. (1995) observed that the microscopic appearance of splenic infarction was of vascular thrombus and vasculitis with fibrinoid necrosis of vessel wall.

Splenic infarction showed swollen splenic extremities and extensive coagulative necrosis that extended from the capsule inward and was distinct from the surrounding parenchyma (Hardie *et al.*, 1995).

2.14.6. Rupture of spleen

2.14.6.1. Etiology

Day *et al.* (1995) observed solitary masses in 10 dogs affected with haemangiosarcoma and its rupture leading to hemorrhage in six cases.

Valli (2007) reported that trauma to the spleen mostly occurred due to crushing injuries, punctured wounds as a result of falls, automobile accidents and gunshot wounds.

2.14.6.2. Ultrasonography

An acute rupture of the spleen was often difficult to detect with ultrasonography. Fissures might be hyperechoic or hypoechoic compared to splenic parenchyma (Benter *et al.*, 2011).

2.14.7. Haematoma

2.14.7.1. Etiology

Splenic haematoma was caused due to abdominal trauma or clotting disorders and usually associated with splenic neoplasia, such as haemangiosarcoma or lymphosarcoma (Nyland and Matton, 1995).

2.14.7.2. Diagnosis

O'keefe and Couto (1987) performed cytological examination of splenic aspirate and confirmed a case as haematoma.

2.14.7.2.1 Ultrasonography

Nyland and Matton (1995) found ultrasonographic appearances of splenic haematoma as hyperechoic with large collections of unclotted blood.

2.14.7.3. Pathology

2.14.7.3.1. Gross

Spangler and Kass (1998) recorded a case of haematoma of the spleen. The appearance of the sectioned surface depended on the duration of formation, amount of collagen, lymphoid elements and necrosis.

2.14.7.3.2. Histopathology

Day *et al.* (1995) observed fibroplasia and adjacent prominent lymphoid hyperplasia in splenic haematoma.

2.15. NEOPLASIA

2.15.1. Primary

2.15.1.1. Haemangioma

2.15.1.1.1. Clinical signs

Hardie *et al.* (1995) reported that the clinical signs in dogs which had splenic haemangioma were abdominal distension, weakness, weight loss, inappetance and cardiac arrhythmias.

2.15.1.1.2. Ultrasonography

Capillary haemangioma appeared as hyperechoic nodules whereas cavernous haemangioma was seen as heterogeneous hypoechoic mass, sometimes with calcification or multiple cystic areas (Giovagnoni *et al*., 2005).

2.15.1.1.3. Pathology

2.15.1.1.3.1. Gross

The size of the nodules ranged from seven to 20 cm (11 cm) in diameter (Day *et al.* 1995).

Benter *et al.* (2011) reported that haemangioma was the most common benign tumor of the spleen and typically did not exceed two cm in size.

2.15.1.1.3.2. Histopathology

Sabattini and Bettini (2009) studied the microscopic feature of haemangioma and most of the samples revealed cavernous subtype showing large, regular and well defined vascular space filled with erythrocytes, completely enclosed by a single layer of endothelial cells aligned on thin collagenous septa.

2.15.1.2. Haemangiosarcoma

2.15.1.2.1. Clinical signs

Brown *et al.* (1985) recorded that the clinical signs and physical findings in dogs with splenic haemangiosarcomas were anorexia, weight loss, abdominal distension, weakness, pallor, vomiting and splenic enlargement of spleen.

2.15.1.2.2. Diagnosis

2.15.1.2.2.1. Ultrasonography

Hammer and Couto (1992) observed that a mixed or non-homogenous echo pattern was commonly seen in dogs with splenic haemangiosarcoma.

2.15.1.2.2.2. Haematology

Ng and Mills (1985) noted that the haematological findings in haemangiosarcoma included anaemia, reticulocytosis, leucocytosis with neutrophilia, metarubricytosis, poikilocytosis, anisocytosis and increased target cells.

2.15.1.2.2.3. Cytology

Haemangiosarcomas could be diagnosed cytologically using samples obtained through fine needle aspiration biopsy or impression smears. The neoplastic cells appeared as spindle shaped and had large nucleus, one or more nucleoli, and a bluish, vacuolated cytoplasm (Hammer and Couto, 1992).

Cytologically, splenic haemangiosarcoma contained large amount of blood within the spleen. The neoplastic cells were variable in size and fusiform to squamous shaped. The nuclei were oval with coarsely clumped chromatin and contained prominent nucleoli (Hristov *et al.*, 2007).

2.15.1.2.3. Pathology

2.15.1.2.3.1. Gross

Couto (1990) stated that haemangiosarcomas were distributed commonly in the spleen, right atrium, subcutaneous tissue and liver.

Gulbahar (1998) reported that haemangiosarcoma, appeared as a mass of about 10 cm in size, in the ventral surface of spleen. The tumour was reddish brown with haemorrhages and necrosis on the cut surfaces.

2.15.1.2.3.2. Histopathology

Day *et al.* (1995) reported that haemangiosarcoma, consisted of irregular vascular spaces lined by elongated anaplastic endothelial cells. The nuclei were large and hyperchromatic and mitotic figures were often seen.

Hristov *et al.* (2007) described that neoplastic cells varied both in shapes and size with irregular mitotic figures. The cell's nuclei were pleomorphic and the cytoplasm had indistinct margins.

Microscopically, the haemangiosarcoma (HAS) cells had higher heterogeneous morphology that ranged from spindle shaped to polygonal to ovoid, with vasoformative to solid growth patterns (Sabattini and Bettini, 2009).

2.15.1.3. Lymphoma

2.15.1.3.1. Clinical signs

Matus *et al.* (1983) recorded clinical signs in dogs which had lymphoma as lethargy, anorexia, massive palpable splenomegaly, haemoperitoneum and slightly large lymph nodes.

2.15.1.3.2. Diagnosis

2.15.1.3.2.1. Ultrasonography

Mahoney (2011) observed that splenic neoplasia whether primary or secondary might cause multiple focal areas of both increased and decreased echogenicity. Diffuse splenic lymphosarcoma were seen associated with a general decrease in echogenicity of splenic parenchyma.

Nyland and Matton (1995) reported that lymphoma produced poorly marginated, hypoechoic to hyperechoic lesion.

2.15.1.3.2.2. Haematology

Oni *et al.* (1992) reported that normochromic normocytic anaemia, leucocytosis and thrombocytopenia were the characteristic features of lymphoma.

2.15.1.3.2.3. Cytology

Cienava *et al.* (2004) reported that splenic aspirate was highly cellular with a mild increase in the number of medium and large lymphocytes and plasma cells in cases of lymphoma.

Raskin and Meyer (2010) reported that lymphocytes varied in size ranging from small to large with round, indented or convoluted nuclei. Nucleoli were usually indistinct but prominent. Cytoplasm was scant to moderate and lightly basophilic. Uniformity of the lymphoid population without significant inflammation or plasma cell infiltration was noticed.

2.15.1.3.3. Pathology

2.15.1.3.3.1. Gross

Kahn (2006) stated that lymphoma was the most common malignant tumour in dogs. The tumour was classified into low and high grade types. Classification based on the location were multicentric, mediastinal, gastrointestinal and extranodal.

Fry and McGavin (2012) reported that in all canine lymphomas, multicentric lymphomas constituted 80 to 85 per cent of all canine lymphomas and the majority of these were intermediate to high grade tumours (80 per cent). Necropsy findings included enlarged lymph nodes that bulged on cut-section and were gray white in colour.

2.15.1.3.3.2. Histopathology

Valli (2007) described that the neoplastic proliferation started from periarterial lymphatic sheaths (PALS) in lymphoma.

2.15.1.4. Plasma cell tumour

2.15.1.4.1. Etiology

Jacobs *et al*. (2002) reported that etiology of the plasma cell tumor in domestic animals was not fully elucidated. The genetic predisposition, viral infections, chronic antigenic stimulation and exposure to environmental carcinogens were associated with this tumor.

2.15.1.4.2. Clinical signs

Silva *et al.* (2008) recorded that the clinical signs in a dog which had plasma cell tumour were lethargy, congested mucous membranes, polyuria, polydypsia, motor incoordination and normal body temperature without signs of apparent organomegaly but with sensitivity to caudal abdominal palpation.

2.15.1.4.3 Diagnosis

2.15.1.4.3.1 Haematology and Serum Biochemistry

Fry and McGavin (2012) observed hyperglobulinemia, hypercalcemia, leucopenia, anaemia and thrombocytopenia in plasma cell tumour.

2.15.1.4.3.2 Cytology

Christopher (2003) stated that the plasma cell tumours revealed large number of immature to well differentiated plasma cells, flaming plasma cells and abnormal mott cells that characterized some clones of neoplastic plasma cells.

2.15.1.4.4 Pathology

2.15.1.4.4.1 Histopathology

Jubb *et al.* (2007) stated that histologically, plasma tumour cells resembled normal plasma cell with marked anisocytosis, scattered nuclei and very large hyperchromatic nuclei with abundant cytoplasm.

2.15.1.5. Liposarcoma

2.15.1.5.1. Clinical signs

According to Weinstein *et al*. (1989), the clinical signs associated with splenic liposarcoma included anorexia or decreased appetite, abdominal distention, polydipsia, lethargy, vomiting, weight loss and weakness.

2.15.1.5.2. Diagnosis

2.15.1.5.2.1. Ultrasonography

Ultrasonography revealed that the tumor mass had mixed echogenicity, situated in the side of left abdomen, caudal to the liver and in front of urinary bladder (Chang and Liao, 2008).

2.15.1.5.2.2. Haematology and serum biochemistry

Haematological examination revealed a moderate normocytic, normochromic anaemia and leucocytosis consisting of mature neutrophils (Chang and Liao, 2008).

The serum chemistry profile showed mild hypoproteinemia and increased ratio of albumin/globulin, highly elevated of aspartate aminotransferase (AST), alanine amino transferase, lactate dehydrogenase and alkaline phosphatase (Chang and Liao, 2008).

2.15.1.5.3. Pathology

2.15.1.5.3.1. Histopathology

Day *et al*. (1995) reported that histopathologically liposarcomas composed of pleomorphic adipocytes in a fibrovascular matrix with scattered mitosis.

2.15.2. Secondary / Metastatic neoplasia

2.15.2.1. Adenocarcinoma

2.15.2.1.1. Clinical Signs

Rosa *et al.* (2012) reported that the clinical signs were characterized by haematochezia and anorexia, as well as weight loss and episodes of sporadic blood flowing out of the anus.

2.15.2.1.2. Haematology

Rosa *et al*. (2012) observed normochromic-normocytic anemia and elevated tumor markers, carcinoembryonic antigen (CEA) and carbohydrate antigen 19-9 (CA 19-9), without other alterations.

2.15.2.1.3. Pathology

2.15.2.1.3.2. Histopathology

According to Day *et al.* (1995), adenocarcinoma of spleen was of epithelial cell origin with cuboidal cells in acinar arrangement without infiltration below the capsule.

2.16. DEGENERATIVE DISORDERS OF SPLEEN

2.16.1.1. Atropy / ageing changes

Fry and McGavin (2012) stated that reduction in T and B lymphocytes in the secondary lymphoid organs was a sequlae of ageing. This resulted in secondary lymphoid atrophy and a small spleen with wrinkled capsule.

2.16.1.2. Siderotic Nodules

2.16.1.2.1. Pathology

Valli (2007) and Fry and McGavin (2012) reported that siderotic plaques of Gamma Gandy bodies were seen as encrustations or nodules, gray white to yellowish or black to brown in colour, present mostly on the splenic capsules or elsewhere in dogs.

2.16.1.2.2. Ultrasonography

On ultrasonographic evaluation, the diagnosis was considered as positive for siderotic nodules in the presence of dispersed, hyperechogenic, parenchymal foci non-attributable to thickened vessels walls in the spleen (Gonzalaz *et al*., 2008).

2.16.2. Haemopoietic alterations

2.16.2.1. Extramedullary haematopoiesis (EMH)

2.16.2.1.1. Etiopathogenesis

Couto (1990) stated that extra medullary haematopoiesis were more common in dogs and was attributed to a variety of stimuli such as red blood cell destruction, severe splenic or extrasplenic inflammation and neoplastic infiltration of the spleen, bone marrow hypoplasia, splenic congestion, pyometra, immune mediated thrombocytopenia, several infectious diseases and malignant neoplasms.

Johns and Christopher (2012) reported that EMH was often observed in animals without obvious haematologic abnormalities and suggested that local tissue injury, inflammation and repair were the major underlying pathogenesis of EMH.

2.16.2.1.2. Diagnosis

2.16.2.1.2.1 Ultrasonography

Ultrasonography revealed extremely enlarged and congested, hypoechoic spleen, almost half of the abdomen (Singh *et al.*, 2010).

Mahoney (2011) reported the ultrasonographic appearance of extramedullary haematopoiesis (EMH) as small hypoechoic nodules or mixed echogenic splenic mass.

2.16.2.1.2.2 Radiology

Radiographically splenic neoplastic masses showed lumpy margins with multiple lobulations (Hammer and Couto, 1992).

2.16.2.1.2.3 Cytology

In a clinical study on ultrasound guided fine needle aspiration biopsy of splenic affections in 10 dogs, the cytology revealed different stages of haematopoiesis like metamyelocytosis and myelocytes indicating extramedullary haematopoiesis (Singh *et al.*, 2010).

2.17. SECONDARY DISORDERS OF SPLEEN

2.17.1. Babesiosis

Buergelt (2002) studied the pathogenesis of canine diffuse splenomegaly. Splenomegaly could result from parasitized erythrocytes being trapped in the splenic sinusoids, the removal of damaged erythrocytes by splenic macrophages and chronic antigenic stimulation. In addition, both extravascular and intravascular haemolysis could contribute to splenomegaly. Blood parasites such as *Babesia* species promoted extravascular haemolysis induced splenomegaly.

According to Meinkoth *et al*. (2002), the haemogram of *Babesia* affected dogs typically had macrocytic, hypochromic and regenerative anaemia with moderate to marked thrombocytopenia

Farker, (2004) observed splenomegaly with localization of organisms and packed RBC's within the spleen in dogs experimentally infected with *Babesia*.

Jefferies *et al*. (2007) reported that the clinical manifestations of acute canine babesiosis included fever, haemolytic anaemia, thrombocytopenia, splenomegaly, and sometimes death.

2.17.2. Hepatozoonosis

Ivanov and Tsachev (2008) reported that the principal gross pathological findings in dogs, infected with *Hepatozooncanis* were cachexia, anaemia, icterus, slightly enlarged spleen and liver. Histologically, schizonts were observed in the skeletal muscle, lymph nodes, spleen, liver and kidney.

2.17.3. Ehrlichiosis

2.17.3.1. Etiology

Waner *et al*, (1997) reported that the etiologic agent of canine monocytic Ehrlichiosis (CME), *Ehrlichia canis* was a small pleomorphic gram-negative coccoid organism that parasitized circulating monocytes intracytoplasmically in clustures called morulae. It was also known as canine rickettsiosis, canine haemorrhagic fever , canine tick typhus and tropical canine pancytopenia.

2.17.3.2. Clinical manifestations

Neer (1996) stated that physical examination of these patients revealed lymphadenomegaly and splenomegaly in 20 per cent and 25 per cent of the patients respectively.

Harrus *et al.* (1997) speculated that the mechanism of thrombocytopenia in canine monocytic ehrlichiosis (CME) might be platelet consumption, increased splenic sequestration and decreased platelet lifespan.

2.17.3.2. Pathology

On cytology, ehrlichia organisms stained dark blue to purple with romanowsky stain. The morulae were well-defined, round to oval, eosinophilic to basophilic bodies found in the host membrane lined vacuoles within the cytoplasm of mononuclear cells (Ettinger and Feldman, 2000).

Castro *et al*. (2004) stated that the infected dogs revealed paleness of mucous membrane, subcutaneous tissues, liver and kidneys. Generalized lymphadenopathy and splenomegaly with marked hyperplasia of the white pulp were also observed in all infected dogs.

Pale mucous membranes, lymphadenopathy, splenomegaly, ascites and congestion, petechial and echymotic haemorrhages in the liver, lungs, spleen,

heart, lymph nodes and kidneys were observed in dogs affected with Ehrlichiosis (Unver *et al.*, 2009).

MATERIALS AND METHODS

The study was conducted in the Department of Clinical Veterinary Medicine, College of Veterinary and Animal Sciences, Mannuthy during the period from July 2013 to May 2014.

Dogs brought to the University Veterinary Hospital, Kokkalai and Teaching Veterinary Clinical Complex, Mannuthy with clinical signs of anorexia, weight loss, abdominal distension, weaknesss, pallor and vomiting, suggestive of splenic diseases were selected and ultilized for the present study.

3.1. OUTLINE OF STUDY

3.1.1. Clinical examination

Detailed clinical examination of patients was conducted as per Houston (2000) and the findings were recorded.

3.1.2. Ultrasound scanning

Normal size and echogenicity of spleen were studied in six apparently healthy dogs. All the clinical cases suspected of splenic diseases were subjected to ultrasound scanning.

3.1.2.1. Equipment

Selected dogs were subjected to ultrasound scanning using Mindray DC6 Vet machine with 3.0, 5.0 and 7.5 MHz transducer.

3.1.2.2. Ultrasound scanning procedure

Animals were placed on right lateral recumbency. Hairs on the abdominal area were removed and acoustic coupling gel was liberally applied over the skin. Imaging of spleen was done by placing the tranducer head directly under the border of the rib cage to visualize the entire splenic head. The position of the tail of the spleen was variable and extended along the left body wall or across the midline ventrally. Spleen was also scanned in transverse and sagittal planes (Nyland and Matton, 1995).

The ultrasonograms were reviewed for alterations in the homogeneous fine echotexture with medium to high level echoes (less coarse and more echogenic than the liver) and changes if any in smooth echogenic capsule.

3.1.2.3. Ultrasound Guided Biopsy

Ultrasound guided biopsy of spleen was performed using Tru-cut biopsy needle in cases where it was found necessary with the consent of the owner. Haemostatic profiles including whole blood clotting time by capillary tube method and platelet count by direct method using ReeEcker solution were evaluated (Benjamin, 1985) before the procedure to rule out coagulopathies.

For splenic biopsy, the scan head was placed on mid-abdomen, just under the rib cage and abdomen was scanned sagitally. The area to be biopsied was identified avoiding splenic vessels. The biopsy site was prepared under aseptic conditions. Local analgesia was attained with two per cent lignocaine solution through ring block. Patient was placed on right lateral recumbency. A small skin incision was made with No. 11 BP blade. The needle was introduced at an angle of 15^0 or 30^0 to the transducer (Plate 1). Biopsy was made when the needle tip and the target organ / lesion could be seen clearly (Mahoney, 2011). Tissue was immediately transferred to ten per cent formalin solution and labelled.

3.1.2.4. Splenic fine needle aspiration

Animal was placed on right lateral recumbency. Transabdominal ultrasound guided fine needle aspiration was performed under local anesthesia. A hypodermic needle (22 G) was used and suction applied with a syringe. The fluid collected was evaluated for cytological changes. The tissue collected in the lumen of the needle was expressed on a slide which was then subjected to cytological evaluation and identification of microorganisms, if any (Couto, 1990).

3.1.3. Radiography

The dog was positioned on right lateral recumbency for lateral view. A consistent set of technical specifications and a formal chart was used to establish the kilovolt (kVp) and milliampere-second (mAs) settings for all exposures. The spleen was observed for their normal size and changes in density (Burk and Ackerman, 1996).

3.2. PARAMETERS UNDER STUDY

The following parameters were studied in apparently healthy animals and clinical cases selected for the study.

1. Signalment, history and clinical signs
2. Haematology
3. Haemogram:

    Haemoglobin (Hb) [g/dl]
    Volume of packed red cells (VPRC) [%]
    Total Erythrocyte count (TEC) [10^6/mm^3]

4. Leukogram:

    Total leukocyte count (TLC) [10^3/mm^3]
    Differential leukocyte count (DLC) [%]

5. Platelet Count [10^5 /mm^3]4) Blood smear examination
6. Buffy coat smear examination
7. Serum Biochemistry:

    Alanine amino transferase (ALT) [U/L]
    Total protein [g/dl]
    Albumin[g/dl]
    Globulin [g/dl
    A:G ratio
    Calcium [mg/dl]

8. Ultrasonography
9. Radiography

3.3. CLINICAL PATHOLOGY

3.3.1. Collection of clinical materials

Relevant clinical materials were collected on the day of presentation. Five milliliters of whole blood was collected from saphenous or cephalic vein in dry glass vials with EDTA at the rate of 1-2 mg per ml as anticoagulant (Benjamin, 1985).

Ten ml of blood was collected in another vial on the day of presentation to seperate serum for biochemical examination. Sera thus seperated were stored at (-20^0C) till further analysis.

3.3.2. Haematology

Total erythrocyte count, haemoglobin, volume of packed red cells, total leukocyte count and platelet count were estimated as per the method described by Schalm *et al.* (1975).

3.3.3. Serum biochemistry

Total serum protein, albumin, globulin, A:G ratio, serum calcium and Alanine aminotransferase (ALT) were estimated as per standard procedures.

All biochemical estimations were done by spectrophotometry in Merck 200 spectrophotometer using commercially available kits.

Serum total protein was estimated by direct biuret method using standard kits supplied by Agappe diagnostics (Slot, 1965).

Serum albumin was estimated using bromocresol green methodology with standard kits supplied by Agappe diagnostics (Doumas, 1971).

Serum calcium was estimated using modified ortho-cresolphthalein complex methodology with standard kits supplied by Agappe diagnostics.

Alanine aminotransferase (ALT) was measured as per the reference method of International Federation of Clinical Chemistry (IFCC).

3.4. HISTOPATHOLOGY

In the event of mortality during the course of clinical investigation and treatment, spleen tissue was collected at necropsy.

Tissue collected was processed for histologic examination embedded in paraffin, four micrometer sections were made, stained with haematoxylin and eosin and subjected to microscopical examination (Bancroft and Gamble, 2008).

RESULT

Dogs presented to the Teaching Veterinary Clinical Complex, Mannuthy and University Veterinary Hospital, Kokkalai with clinical signs suggestive of spleen involvement on abdominal palpation were subjected to detailed clinical examination and 13 animals were identified. These animals were subjected to ultrasound scanning and those cases tentatively diagnosed as splenic disorders were selected for detailed laboratory investigation including haematobiochemical analysis, cytology of fine needle aspirate, ultrasonography, histopathology and tentative diagnosis were confirmed.

4.1. OCCURRENCE

Among the 13 cases, the primary disease conditions observed were splenitis (46.15 per cent), lymphoma (23.07 per cent) and splenic torsion (7.69 per cent). Secondary diseases recorded in the study were babesiosis (15.38 per cent) and ehrlichiosis (7.69 per cent).

Diseases identified	No of dogs affected
Splenitis	6
Lymphoma	3
Torsion of spleen	1
Babesiosis	2
Ehrlichiosis	1

4.1.1. Age

Splenic disorders were more common in the age group of three to six years (46.15 per cent), followed by six to nine years (23.07 per cent), less than three years (23.07 per cent) and more than nine years (7.69 per cent).

Table no. 1 Age-wise occurrence of splenic disorders in dogs.

S. No.	Age (Years)	No. of Cases	Occurrence (%)
1	< 3	3	23.07
2	3 - < 6	6	46.15
3	6 - < 9	3	23.07
4	> 9	1	7.69

4.1.2. Breed

The highest occurrence was encountered in German Shepherd (30.79 per cent) followed by Labrador retriever (23.07 per cent), Rottweiler (15.38 per cent), Dachshund (15.38 per cent), Great Dane (7.69 per cent), and Spitz (7.69 per cent).

4.1.3. Sex

Out of the 13 cases of splenic disorders eight were females (61.54 percent) and rest were males (38.46 per cent).

Table. 2. Sex- wise occurrence of splenic disorders in canines.

S. No.	Sex	No. of Cases	Occurrence (%)
1	Male	5	38.46
2	Female	8	61.54

4.1.4. Weight of spleen

The weight of spleen of dogs affected with various diseases conditions in which splenectomy or *post-mortem* was done are presented in table 3.

Table 3. Weight of spleen in various disease conditions of spleen in which splenectomy or necropsy was conducted.

Dog. No.	Breeds	Disease condition	Weight of Spleen(gm)
1	Great Dane	GDV / torsion	3400
2	Dachshund	Splenic tumor	2700
6	Labrador	Splenitis	210

4.1.5. Clinical findings

Clinical signs were non-specific and vague. The common clinical signs exhibited by animals were anorexia, vomiting, abdominal distension, weakness and limb edema. Physical examination revealed palpable lymph nodes, weight loss, pale mucous membrane, pyrexia and splenomegaly.

Table. 4. Clinical findings in splenic disorders

Clinical signs	No. of animals affected	Percentage (%)
Splenomegaly	12	92.31
Anorexia	9	69.23
Lymphadenopathy	8	61.53
Pyrexia	8	61.53
Vomiting	6	46.15
Weakness	5	38.46
Weight loss	4	30.79
Abdominal distension	3	23.07
Diarrhoea	1	7.69

4.2. PRIMARY SPLENIC DISEASES

4.2.1 Torsion of spleen

One case was diagnosed as splenic torsion. (Dog No. 1)

The case was reported in a five year old male Great Dane. The animal had anorexia, unproductive retching, hypersalivation and abdominal distension. Clinical examination revealed subnormal temperature (99.5 ^{0}F) and thready pulse.

4.2.1.1. Ultrasonography

The splenic parenchyma showed mixed echogenic appearance with splenomegaly (Plate 2). The stomach was distended with mixed echogenic content.

4.2.1.2. Radiography

Abdominal radiograph showed irregular splenic mass and this appeared as a radio-lucent area located in the middle of abdominal cavity (Plate 3).

4.2.1.3. Haemogram

Erythrocyte count was 3.5 x 10^6/mm^3. Haemoglobin and PCV were 7.9 gm/dl and 24 per cent respectively. Total leucocyte count was 25.2x 10^3/$mm^3$with neutrophils 80 per cent and lymphocytes 20 per cent. Platelet count was 2.1 x 10^5 /mm^3.

4.2.1.4. Serum biochemistry

Serum total protein was 5.4 g/dl. Serum albumin and globulin were 2.5 and 2.9 g/dl respectively with A:G ratio 0.86. Serum ALP was 46 U/L. Serum calcium was 12.7 g per cent.

4.2.1.5. Treatment

The animal was treated with parenteral fluids and inj. metaclopromide 0.2 mg/ kg BW IV; orogastric intubation was done. The animal did not respond to the treatment and was referred to the surgery department and splenectomy was performed.

4.2.1.6. Pathology

4.2.1.6.1. Gross

Spleen was blue black coloured, congested and weighed 3400 g (Plate 4).

4.2.1.6.2. Histopathology

Haemorrhage into the splenic parenchyma was observed. No lymphoid components were detected and only bits of trabeculae could be seen (Plate 5).

4.2.2. Neoplasia

4.2.2.1. Primary

4.2.2.1.1. Lymphoma

Three cases were diagnosed ultrasonographically as splenic lymphoma and were numbered as Dogs 2, 3 and 4.

Two cases were reported in twelve and fourteen year old female Dachshunds and one case was reported in a six month old male Rottweiler pup.

Dogs were presented with the history of anorexia, lethargy, depression and distension of abdomen. Abdominal palpation revealed a mass in the mid-abdomen in dog no 2 and 3.

4.2.2.1.1.1. Ultrasonography

Dog no 2 and 3: Ultrasonographic examinations revealed focal and mixed echogenic mass attached to the body of the spleen. The splenic parenchyma was hypoechoic (Plate 6 A and B).

Dog no 4: Splenic parenchyma showed multifocal or hypoechoic pattern with nodular areas on body of the spleen (Plate 6 C).

4.2.2.1.1.2. Radiography

The abdominal radiograph showed irregular splenic mass which appeared as radio-dense area in the middle of abdominal cavity in dog no 2 and 3 (Plate 7). Radiography was not performed in dog no 4.

4.2.2.1.1.3. Haemogram

Haemogram of the 12 year old dachshund (dog no 2) revealed an erythrocyte count of 3.5 x 10^6/mm^3. Haemoglobin and PCV were 7.9 g per cent and 24 per cent respectively. Platelet count was 1.68 x 10^5 /mm^3. Corresponding values of the other (dog no 3) were 2.84 x 10^6/mm^3, 6.82g per cent and 20 per cent respectively. Total leucocyte count of (dog no 2) was 10.4x 10^3/mm^3 with neutrophils 77 per cent and lymphocytes 23 per cent. Total leucocyte count (dog no 3) was 32.2 x 10^3/mm^3 with neutrophils 69 per cent and lymphocytes 31 per cent. Platelet count was 2.48x 10^5 /mm^3.

Dog no.4: Erythrocyte count was 4.9x 10^6/mm^3. Haemoglobin and PCV were 9.9 gm per cent and 30.2 per cent respectively. Total leucocyte count was 20.1 x 10^3/mm^3 with neutrophils 54 per cent, monocytes one per cent, basophils one per cent and lymphocytes 44 per cent. Platelet count was 4.10x 10^5 /mm^3.

4.2.2.1.1.4. Serum biochemistry

Dog no. 2: Serum total protein was 4.7 g/dl. Albumin and globulin were 2.1 and 2.6 g/dl respectively with A:G ratio 0.81. Serum ALP was 40 U/L. Serum calcium was 7.8 mg per cent.

Dog no. 3: Serum total protein was 6.1g/dl. Albumin and globulin were 4.9 and 1.2g/dl respectively with A:G ratio 4.08. Serum ALP was 20 U/L.

Dog no.4: Serum total protein was 6.7 g/dl. Albumin and globulin were 0.8 and 5.9 g/dl respectively with A:G ratio of 0.1. Serum ALP was 28 U/L. Serum calcium was 11.06 mg per cent.

4.2.2.1.1.5. Cytology

Dog no 4: The cytology of splenic aspirate revealed immature lymphocytes with meshed chromatin pattern (blast), numerous large lymphocytes and few neutrophils (Plate 10).

4.2.2.1.1.6. Treatment

Dog no. 2 was referred to the surgery department for removal of splenic mass. In surgery department laparotomy was performed and mass was removed. The animal died during surgery.

Dog no. 3 did not turn up after the first day.

Dog no. 4 was given symptomatic therapy with parenteral fluids, inj. Amoxycillin and cloxacillin @ 20 mg/ kg BW IV daily for five days. The animal didn't turn up for further treatment. It was reported that the dog died after one month.

4.2.2.1.1.7. Pathology

4.2.2.1.1.7.1. Gross

Gross pathological examination of the spleen of dog no. 2 revealed enlarged and whitish spleen with irregular borders and weighed 2700 g (Plate 8).

4.2.2.1.1.7.2. Histopathology

Dog no.2: Splenic histopathology revealed diffuse and multifocal congestion, periarteriolar lymphoid hyperplasia, and interlacing bundles of fibrous tissues (Plate 9).

4.2.3. Splenitis

Six cases were diagnosed as splenitis and were numbered from 5 to 10.

Average age of affected animals was 5.5 years with a range of 3 to 9 years. Three were German Shepherds, two were Labrador retrievers and one was Spitz. Five were females and only one was male.

These animals were presented with the history of anorexia, lethargy, vomiting, weight loss and distension of abdomen. Body temperatures ranged from 103.6 – 104.8^{0}F with a mean of 104.2^0 F. Popliteal lymph nodes were enlarged in all cases. Splenomegaly was the consistent finding on abdominal palpation.

4.2.3.1. Ultrasonography

Five cases showed diffuse splenic parenchyma with hypoechoic areas. The spleen had irregular borders (Plate 11).

In dog no 10, spleen was diffusely hypoechoic with splenomegaly, hepatomegaly and gall bladder sludge was also noticed (Plate 12).

4.2.3.2. Radiography

Moderate splenomegaly was observed in all animals (Plate 13).

4.2.3.3. Haemogram

Erythrocyte count in animals ranged from 3.2 to 6.7 x 10^6/mm^{3}with mean value of 4.81 x 10^6/mm^3. Haemoglobin values ranged from 9.1 to 15.9 g/dl with a mean of 11.4 g/dl. PCV ranged from 26.3 to 45.6 per cent with mean value of 34.58 per cent. Leucocyte count ranged from 11.4 to 21.0 x 10^3/mm^3 with a mean value of 15.7x 10^3/mm^3. Mean neutrophils, lymphocytic, monocyte and eosinophilie values were 79 per cent, 18 per cent, one per cent and two per cent respectively.

4.2.3.4. Serum biochemistry

Mean serum total protein, albumin, globulin and A:G ratio were 6.73, 2.93, 3.62 and 0.78 respectively. Serum ALT and calcium were 43.81 U/L and 9.26 mg per cent respectively.

4.2.3.5. Cytology

Dog no.5: The cytological examination revealed neutrophils with presence of toxic neutrophils (Plate 14).

Dog no.10: The cytological examination revealed neutrophils (Plate 15).

4.2.3.6. Treatment

Dog no. 7, 9 and 10 were treated with parenteral fluids, inj. Amoxycillin and cloxacillin @ 20 mg/ kg BW IV, inj. Pantoprazole @ 0.7 mg/ kg BW IV for 3

days and supportive treatment with Vitamin B complex. Animals showed marked improvement.

Dog no. 5 and 8 were treated with parenteral fluids, inj. Metronidazole @ 25 mg / kg BW IV, inj. Clindamycin @ 11 mg / kg BW IV and Tab Doxycycline @ 10 mg / kg BW orally for 10 days. Animals showed marked improvement.

Dog no. 6 was treated with parenteral fluids, inj. Pantoprazole @ 0.7 mg / kg BW IV, Inj. Ondansetron @ 0.2mg / kg BW IV for 2 days. The animal didn't respond to the treatment and died on the second day of treatment.

4.2.3.7. Pathology

4.2.3.7.1. Gross

On post mortem examination of dog no 6, spleen was found to be congested and weighed around 210g.

4.2.3.7.2. Histopathology

Dog no. 6: Histopathological examination revealed severe haemorrhage with lymphoid depletion. Accumulation of lymphocytes was seen within the sinus (Plate 16). Trabecular proliferation was also observed.

4.3. SECONDARY SPLENIC DISORDERS

4.3.1. Babesiosis

Two cases were diagnosed as babesiosis. (Dog no 11 and 12)

Peripherial blood smear positive for *Babesia gibsoni* in both cases (Plate 17).

The cases were reported in a four year old male Rottweiler (dog no.11) and seven year old female German shepherd (dog no.12).

These animals were presented with the history of anorexia, lethargy, weight loss, vomiting and distension of abdomen. Body temperature was 103.6 and 105^0F respectively. Popliteal lymph nodes were enlarged in both the cases. Haemoglobinuria was reported in dog no 11. Splenomegaly was observed on abdominal palpation. Both the cases had the history of *Rhipicephalus* infestation.

4.3.1.1. Ultrasonography

Spleen was diffusely heterogeneous with severe splenomegaly in both dogs (Plate 18)

4.3.1.2. Radiography

Moderate splenomegaly was observed in dog no.11 and mild splenomegaly was noticed in dog no 12 (Plate 19).

4.3.1.3. Haemogram

Dog no 11: Erythrocyte count was 1.69 x 10^6/mm^3. Haemoglobin and PCV were 2.9 g per cent and 10.3 per cent respectively. Total leucocyte count was 18.9 x 10^3/mm^3 with neutrophils 78 per cent, lymphocytes 18 per cent, monocytes one per cent and eosinophils three per cent. Platelet count was 1.37 x 10^5 /mm^3.

Serum total protein was 7.0 g/dl. Albumin and globulin were 2.8 and 4.2 g/dl respectively with A:G ratio of 0.66. Serum ALP was 16 U/L. Serum calcium was 9.1 mg per cent.

Dog no 12: Erythrocyte count was 1.79 x 10^6/mm^3. Haemoglobin and PCV were 4.9 g per cent and 14.7 per cent respectively. Total leucocyte count was 19.2 x 10^3/mm^3 with neutrophils 72 per cent, lymphocytes 24 per cent, monocytes three per cent and eosinophils one per cent. Platelet count was 0.75 x 10^5 /mm^3.

Serum total protein was 6.7 g/dl. Albumin and globulin were 2.6 and 4.1 g/dl respectively with A:G ratio of 0.63. Serum ALP was 12 U/L. Serum calcium was 8.2 mg per cent.

4.3.1.4. Cytology

Dog no 11. cytological examination revealed numerous lymphocytes with meshed chromatin and it contained more than one nucleoli (Plate 20).

Dog no 12. cytological examination revealed large numbers of immature lymphoid cells (Plate 21).

4.3.1.5. Treatment

The animals were treated with parenteral fluids, inj. Metronidazole @ 25 mg / kg BW IV, inj. Clindamycin @ 11 mg / kg BW IV and Tab Doxycycline @ 10 mg / kg BW orally for 10 days. Both animals showed marked improvement and were discharged.

4.3.2. Ehrlichiosis.

One case was diagnosed as Ehrlichiosis. (Dog no. 13)

The case was reported in a four year old male Labrador retriever. The animal exhibited anorexia, nasal discharge, cough and vomiting. Clinical examination revealed pyrexia. Popliteal lymph nodes were enlarged. Splenomegaly was detected on abdominal palpation. Ehrlichia morulae could be detected in the buffy coat smear (Plate 22).

4.3.2.1. Ultrasonography

The splenic parenchyma was diffuse hyperechoic in appearance and spleen was large in size (Plate 23).

4.3.2.2. Radiography

Moderate enlargement of spleen was observed on abdominal radiography (Plate 24).

4.3.2.3. Haemogram

Erythrocyte count was 3.8 x 10^6/mm^3. Haemoglobin and PCV were 13.3 g per cent and 37.3 per cent respectively. Total leucocyte count was 12.0 x 10^3/mm^3 with neutrophils 80 per cent, lymphocytes 15 per cent, monocytes three per cent and eosinophils two per cent. Platelet count was 0.56 x 10^5 /mm^3.

4.3.2.4. Serum biochemistry

Serum total protein was 6.7 g/dl. Albumin and globulin were 3.5 and 3.2 g/dl respectively with A:G ratio of 1.09. Serum ALP was 45 U/L. Serum calcium was 8.5 mg per cent.

4.3.2.5. Cytology

The cytological examination revealed presence of small, medium sized lymphocytes (Plate 25).

4.3.2.6. Treatment

The animal was treated with parenteral fluids, inj. Oxytetracycline @ 10 mg / kg BW IV SID for 14 days and inj. Prednisolone @ 1mg / kg BW IM SID and supportive treatment with Vitamin B complex. The animal responded to the treatment.

DISCUSSION

5.1. OCCURRENCE

5.1.1. Age

Maximum occurrence of splenic disorders (46.15 per cent) was in the age group of three to six years whereas the occurrence was 23.07 per cent each in dogs below 3 years of age and in the age group of six to nine years. The least occurrence of splenic disorders was in dogs above nine years (7.69 per cent).

Day *et al.* (1995) reported that dogs aged from six months to 11 years (6.75± 3.2) were found to be more affected with splenic disorders. Wilkerson *et al.* (2005) recorded lymphoma cases in the age group of three to 14 years with an average age of 8.5 years while Kimura *et al.* (2011) reported that the incidence of canine lymphoma was higher among dogs aged five to nine years (45 per cent), followed by 10 to 13 years (34 per cent) and four to five years (17 per cent).

5.1.2. Breed

In the present study, the highest occurrence was noticed in German Shepherd, followed by Labrador retriever, Rottweiler, Dachshund, Great Dane, Spitz and Boxer.

The findings were in accordance with that of Brown *et al.* (1985) and Sabattini and Bettini (2009) in which German Shepherds were ranked first (27.60 per cent), followed by Labrador retrievers.

Primary torsion of spleen as well as gastric dilatation volvulus was found to be more common in large, deep chested breeds such as St. Bernand, Labrador, Great Dane and German shepherd (Neath *et al.*, 1997; Simeonova *et al.*, 2007).

5.1.3. Sex

Splenic disorders were reported to be more common in females dogs (Day *et al.*, 1995). In the present work also, out of 14 cases of splenic disorders, eight were females and rest were males.

Similar observations were also made by Spangler and Culbertson (1992) who reported that splenic hyperplasia was five times more in females than in males.

On the contrary, the ratio of affected male to female dogs was 2.1:1 (Brown *et al.,* 1985; Sabattini and Bettini, 2009). Kimura *et al.* (2011) stated that among the 65 cases of lymphoma, 35 cases were recorded in males and 30 were in females.

5.1.4. Weight of spleen

In dogs affected with torsion of spleen, tumours and splenitis, the weight of spleen were definitely increased. Mallinckrodt and Gottfried (2011) reported that the weight of spleen was more in benign splenic *tumours.*

5.1.5. Clinical findings

The clinical findings recorded in this study were nonspecific. Most of the animals had anorexia, splenomegaly, weakness, weight loss, vomiting, anaemia, abdominal distension and limb oedema. Similar findings were recorded by Ng and Mills (1985) and Couto (1990). Pallor of mucous membrane and anaemia were reported by Hardie *et al.* (1995).

5.2. PRIMARY SPLENIC DISEASES

Focal or multifocal parenchymal changes were observed in 23.07 per cent (3/13) cases and diffuse changes were seen in others.

5.2.1. Torsion of spleen

In the present study, splenic torsion was diagnosed in a five year old male Great Dane.

Mahoney (2011) observed that Great Dane and German Shepherd dog were prone to gastric dilatation and volvulus (GDV) associated splenic torsion.

The increased incidence of torsion of spleen in Great Dane dogs might be due to blunt abdominal trauma or surgery. Splenomegaly and pregnancy might

also lead to tearing or abnormal relaxation of the splenic suspensory ligaments (Simeonova *et al.,* 2007).

Animal was presented with anorexia, unproductive retching, hyper-salivation, abdominal distension, subnormal temperature (99.5^0F) and thready pulse. Similar signs were also reported by Szatmari *et al.* (2000) in a Saint *Bernard* dog.

Simeonova *et al.* (2007) observed that associated abdominal pain was one of the major clinical signs. In torsion acute twisting of the splenic vessels might result in bleeding into abdomen, melaena, haematemesis, anaemia, weakness and collapse.

Diffuse parenchyma with mixed echogenicity and splenomegaly observed in the present case agreed with the observations made by Schnier (2010). Enlargement of spleen with reticular pattern, characterized by lacy appearance with homogenous echogenic splenic parenchyma was reported by Saunders *et al.* (1998).

Enlargement of spleen, in *toto,* with diffuse anechoic areas separated by small linear echoes appeared unique in cases of splenic torsion (Konde *et al.*, 1989).

Splenomegaly or a mid-abdominal mass was found on the radiograph of the present case. Konde *et al.* (1989) also observed similar finding in the radiograph of a dog affected with splenic torsion. Radiographic evidence of splenomegaly with loss of serosal details and gastrointestinal displacement might be suggestive of the presence of splenic torsion (King and Pack, 2002).

The blood picture revealed anaemia, neutrophilia, lymphopaenia and leukocytosis. It might be due to the slow flow of blood through the red pulp, exposure of cells to lower glucose, cholesterol, and pH than in the central circulation. Collectively, these biochemical changes could contribute to premature ageing of erythrocytes and platelets resulting in accelerated destruction (Weiss and Wardrop, 2007). In the present study, the serum values of ALT and calcium were slightly elevated. The haemato-biochemical changes observed in

the present study agreed with the observations made by Maxie *et al.,* (1970) and Schnier (2010).

Torsion of spleen generally was seen to respond poorly to medical treatment and thus warranted surgical intervention (Schnier, 2010). Hence splenectomy was done and spleen was sent for histopathology.

Grossly, the spleen was blue-black colored with enlargement. Fry and McGavin (2012) also reported similar finding.

Histopathology revealed haemorrhages. However, absence of any necrotic areas indicated that the condition had not worsened. The surgery was followed by a brief uneventful period but by second week the condition of the animal got worsened and resulted in death.

5.2.2. Neoplasia

5.2.2.1. Primary

5.2.2.1.2. Lymphoma

Ultrasonographically three cases were diagnosed as splenic lymphomas in the present study.

The clinical signs were anorexia, depression and distension of abdomen. Similar findings were also reported by Matus *et al.* (1983). There was marked abdominal distension in two cases (Dog no 2 and 3). Palpation of mid-abdomen of dogs (no. 2 and 3) immediately behind the last thoracic rib revealed a hard mass suggestive of splenomegaly and/ tumors in the spleen. Distended abdomen and palpable splenic mass were detected in splenic lymphoma (Oni *et al.*, 1992).

Ultrasonography in dogs 2 and 3 revealed presence of a focal or mixed echogenic mass attached to the body of spleen. In certain cases of lymphoma Mahoney (2011) observed that the splenic parenchyma could also be hypoechoic.

Nyland and Matton (1995) observed splenic lymphoma with non-uniform appearance in ultrasonography, ranging from hypoechoic to hyperechoic lesion without distal acoustic enhancement.

In the present study, multifocal or hypoechoic nodular splenic parenchyma was detected in one case (Dog no. 4). Mahoney (2011) also made similar finding. Multifocal splenic parenchyma could be due to neoplasia or other disease processes which affect spleen (Mannion, 2006).

The erythrocyte counts were 3.5, 2.84 and 4.9 x 10^6/mm^3 respectively in dogs 2, 3 and 4. Anaemia was a common haematological finding as reported by Oni *et al.* (1992) in splenic lymphoma. Total leucocyte counts were 10.4, 32.2 and 20.1 x 10^3/mm^3 respectively in dogs 2, 3 and 4. Leucocytosis might be interpreted as an inflammatory response or a stress response.

Globulin level was low in all dogs. Hypoglobulinemia could be due to immunodeficiency disorder such as benign lymphoid hyperplasia (Wright, 2012). Thangapandiyan *et al.* (2013) reported that hypoglobulinemia and hypercalcemia were associated with canine lymphoma.

Grossly, the neoplastic spleen was enlarged, whitish in colour and had irregular borders. Kansal *et al.* (2003) also had similar finding in humans.

Histopathology of the spleen showed diffuse and multifocal congestion, periarteriolar lymphoid hyperplasia and interlacing bundles of fibrous tissue which confirmed the case (Dog no.2) as lymphoma. Similar findings were also observed by Kansal *et al.* (2003).

The cytological examination of splenic aspirate revealed immature lymphocytes with mesh chromatin pattern (blast), numerous large lymphocytes and few neutrophils. These observations were in accordance with those made by Raskin and Meyer (2010).

5.2.3. Splenitis

Splenitis was diagnosed in six cases and the age of the affected animals ranged from three to nine years. Day *et al.* (1995) reported that the incidence of splenitis was higher in the age of 6.75 ± 3.2 years. Buergelt (2002) reported a case of bacterial splenitis in a 12 years old Boxer whereas, Neer (1996) reported splenitis in a 18 months old dog.

Most common clinical signs of splenitis in the present study were anorexia, vomiting, weight loss, pyrexia, lymphadenopathy and abdominal distension.

Couto (1990) and Neath *et al.* (1997) also observed varying degrees of anorexia, vomiting and weight loss.

Distended abdomen and splenomegaly were detected in splenitis by Ginel *et al.* (2001). Splenomegaly might be due to vascular distension secondary to relaxation of smooth muscle cells, portal hypertension and vascular outflow obstruction (Tillson, 2003).

The ultrasonographic appearance of spleen showed diffused hypoechoic parenchyma in all cases in both sagittal and transverse scan. Nyland and Matton (1995) also observed hypoechoic splenic parenchyma in acute inflammation of spleen. Carteen *et al*. (1993) also observed mixed echogenic splenic parenchyma and irregular borders of spleen, which might be due to granulomatous diseases.

In dog no. 11 ultrasonography revealed diffused hypoechoic splenomegaly, hepatomegaly and gall bladder sludge, which might be due to portal hypertension (Tillson, 2003).

The radiographic appearance of the spleen showed moderate enlargement in all animals. Burk and Ackerman (1996) also observed moderate enlargement of spleen in cases of splenitis.

Mild anaemia and leucocytosis observed in the present study was also observed by Neer (1996). Couto (1990) reported that *haemato-biochemical* changes were rare in splenitis.

Fine needle splenic aspirate cytology of dog no. 6 and 11 revealed large number of neutrophils and dog no. 6 revealed toxic neutrophils also. The morphology of the inflammatory cells were dependent on the type of organism involved, toxin production, amount of necrosis and length of time the inflammation had persisted (Christopher, 2003).

Five animals responded well to the treatment adopted and dog no. 7 succumbed to death.

Postmortem examination of dead animal revealed congested spleen.

Histopathology of the spleen showed severe haemorrhages with lymphoid depletion. Accumulation of lymphocytes seen within sinus were confirmative of lymphadenitis which was also observed by Fry and McGavin (2012).

5.3. SECONDARY SPLENIC DISORDERS

5.3.1. Babesiosis

Chaudhuri e*t al.* (2008) reported that the seroprevalence of Babesiosis was higher in adult dogs than in dogs younger than one year. In the present study affected animals were four (dog no 12) and seven (dog no 13) years old.

Nonspecific clinical signs observed in this study were anorexia, lethargy, weight loss, vomiting and enlarged popliteal lymph nodes which were similar to the findings of Gopegui *et al,* (2007) and Konto *et al,* (2014). Splenomegaly was detected in both the cases. Splenomegaly was observed in cases of babesiosis by Nalubamba *et al.* (2011).

Haemoglobinuria was reported in one case. Haemoglobinuria might be due to intravascular lysis of erythrocytes and depletion of plasma haptoglobin (Giger, 1992).

Thrombocytopenia observed in present study with babesiosis was mainly due to the sequestration of platelets in the spleen (Takeda, 1995).

In the present study, the erythrocyte counts were lower, as erythrocytes get destroyed during the parasitized phase (Murase *et al.,* 1996).

Ultrasonography revealed diffused heterogenecity with splenomegaly which could be supportive in the diagnosis of *Babesia* infection (Fraga *et al.,* 2011).

Splenomegaly was found on the abdominal radiographs of the dogs diagnosed with babesiosis. Similar findings were also reported by Maele *et al.* (2008).

Svante (2012) observed that the lymphoid cells seen in tissue fragments, trapped in a meshwork of endothelial cells as a non-specific finding in splenomegaly. In Dog no.12 cytological examination revealed numerous, lymphocytes with meshed chromatin.

The cytological evaluation of splenic aspirate in dog no. 13 revealed large numbers of immature lymphoid cells, few neutrophils and plasma cells.

Christopher *et al.* (2003) opined that immature lymphocytes, neutrophils and plasma cells, could be found in splenic inflammation.

The animals responded well to the treatment regime adopted and was in agreement with Greene (1998).

5.3.2. Ehrlichiosis

In the present study, Ehrlichiosis was diagnosed in a four year old Labrador dog by buffy coat smear examination. Faria *et al.* (2010) also diagnosed Ehrlichiosis by detecting *Ehrlichia* morulae in monocytes during buffy coat examination.

Dogs affected with ehrlichiosis showed diffused hyperechoic splenic parenchyma and splenomegaly by ultrasonography as reported by Varshney *et al.* (2011).

Abdominal radiograph revealed moderate enlargement of spleen.

Anaemia and thrombocytopenia observed in this study resembled those reported by Tresamol *et al*, (1995) and Neer (1996). Thrombocytopenia was attributed to increased consumption, immune mediated destruction of platelets and splenic sequestration (Brunker and Hoover, 2007).

Splenic aspirate revealed small, medium sized lymphocytes. Couto (1990) reported that lymphoplasmacytic splenitis commonly occurred in sub-acute or chronic infections such as infectious canine hepatitis, canine ehrlichiosis, pyometra, brucellosis and haemobartonellosis.

The dog responded well to the treatment. Greene (1998) also reported successful treatment of canine ehrlichiosis using inj. Prednisolone and inj. Oxytetracycline.

SUMMARY

A study was undertaken to find out the occurrence of splenic disorders in dogs and to evaluate the clinic-pathological changes associated with splenomegaly.

Based on abdominal palpation, clinical signs, ultrasonographic changes, cytology or histopathology, haematology and serum biochemistry 13 cases of splenic disorders were selected. Splenic diseases were classified into primary and secondary. Parenchymal changes were focal or multifocal and diffuse.

Clinical signs were usually non-specific and vague. The common clinical signs exhibited by animals were anorexia, vomiting, abdominal distention, weakness and limb oedema. Physical examination revealed palpable lymph nodes, weight loss, pale mucous membrane, pyrexia and splenomegaly.

Transverse and sagittal scan of the abdomen of the animal was performed in right lateral recumbancy. Changes in the echogenicity of splenic parenchyma and capsule were noted.

Primary splenic diseases that produced focal or multifocal changes were splenic neoplasia. Splenitis produced diffuse parenchymal changes. Babesiosis and Ehrlichiosis caused diffuse splenomegaly.

Age of the affected animals ranged from 6 months to 12 years. Among dog breeds, highest occurrences was found in German Shepherd dogs (30.79 percent), followed by Labrador Retriever (23.07 percent). Females (61.54 percent) were more affected than the male dogs.

The haematological findings in the present study revealed regenerative normocytic anaemia in all cases.

All the clinical cases in this study revealed palpable splenomegaly and anaemia. The spleen enlarged as macrophages accumulated and blood flow through red pulp got impeded.

The serum biochemical values were within the normal range.

The most common finding in the radiography of the spleen was generalized enlargement, as the spleen is a very dynamic organ.

Ultrasonographic examination showed homogenous enlargement of splenic parenchyma. Two tumour cases revealed mixed echogenicity. Splenic parenchyma showed numerous small hypoechoic nodules in lymphoma. Splenic torsion showed enlargement with reticular pattern, characterized by 'lacy' appearance with homogenous echogenic splenic parenchyma. Ultrasound guided FNAB helped to diagnosis lymphoma, splenitis and protozoan diseases.

Out of 13 cases 3 were neoplasia of spleen. Lymphoma revealed enlarged and whitish spleen with irregular borders which weighed 2700g. Histopathologically, lymphoma showed diffuse and multifocal congestion, periarteriolar lymphoid hyperplasia, and interlacing bundles of fibrous tissues. Cytologically, lymphoma showed immature lymphocytes with meshed chromatin pattern (blast), numerous large lymphocytes and few neutrophils.

Out of the 13 cases six were splenitis. Histopathologically, splenitis showed severe haemorrhages with lymphoid depletion. Accumulation of lymphocytes was seen within the sinus. Cytologically, splenitis showed neutrophils with presence of toxic neutrophils (neutrophils contained basophilic granules).

Two cases of babesiosis had splenomegaly. It could be attributed to parasitized erythrocytes being trapped in the splenic sinusoids, the removal of damaged erythrocytes by splenic macrophages and chronic antigenic stimulation. In addition extravascular and intravascular haemolysis might have contributed to splenomegaly.

The case of ehrlichioisis also had splenomegaly. The clinical signs observed were anorexia, dullness, vomiting, pyrexia and splenomegaly. Cytological examination revealed small and medium sized lymphocytes.

Based on above finding following conclusions were made.

Conclusions:-

1. Palpable splenomegaly was the major clinical sign to recognise splenic disease.
2. Occurrence of splenic disorder was more in German Shepherds and Labrador retrievers.
3. There were no significant changes in the serum biochemistry values in splenic disorders.
4. Abdominal radiography and ultrasonography helped in identifying splenomegaly, torsion and tumour cases.
5. Ultrasound was an additional diagnostic aid to differentiate diffuse, focal or multifocal masses in the spleen.
6. Ultrasound guided FNAB was helpful in the specific diagnosis of lymphoma, splenitis.

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CLINICO-PATHOLOGICAL STUDIES ON SPLENOMEGALY IN DOGS

SHIVASHANKARANAND GAJANAN RAUT

(12-MVM-03)

Abstract of the thesis submitted in partial fulfillment of the requirement for the degree of

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2014

Faculty of Veterinary and Animal Sciences
Kerala Veterinary and Animal Sciences University

Department of Clinical Veterinary Medicine
COLLEGE OF VETERINARY AND ANIMAL SCIENCES
MANNUTHY, THRISSUR – 680651
KERALA, INDIA

ABSTRACT

An investigation was undertaken to study the occurrence of splenic disorders in dogs and clinico-pathological changes associated with splenomegaly. The cytological / histopathological findings and ultrasonographic changes were also studied.

Based on clinical signs, physical examination, radiography, cytology and ultrasonographic studies splenic disease were diagnosed in 13 cases which included neoplasm (23.07 per cent), splenic torsion (7.69 per cent), splenitis (46.15 per cent) and haemoprotozoan diseases (23.07 per cent).

Age of affected animals ranged from 6 months to 12 years. Among the breeds, highest occurrence was in German Shepherd dogs (30.79 per cent), followed by Labrador Retriever (23.07 per cent). Females (61.54 per cent) were more affected.

The common clinical signs exhibited by affected animals were anorexia, vomiting, abdominal distention, weakness and limb oedema. Physical examination revealed palpable lymph nodes, weight loss, pale mucous membrane, pyrexia and splenomegaly.

Haematological studies revealed regenerative normocytic normochromic anaemia and thrombocytopenia. There were no significant changes in the serum biochemistry values.

Primary splenic diseases that produced ultrasound changes were splenic neoplasia and splenic torsion. Splenitis produced diffuse parenchymal changes. Diffuse splenomegaly could be observed in *Babesiosis* and *Ehrlichiosis.*

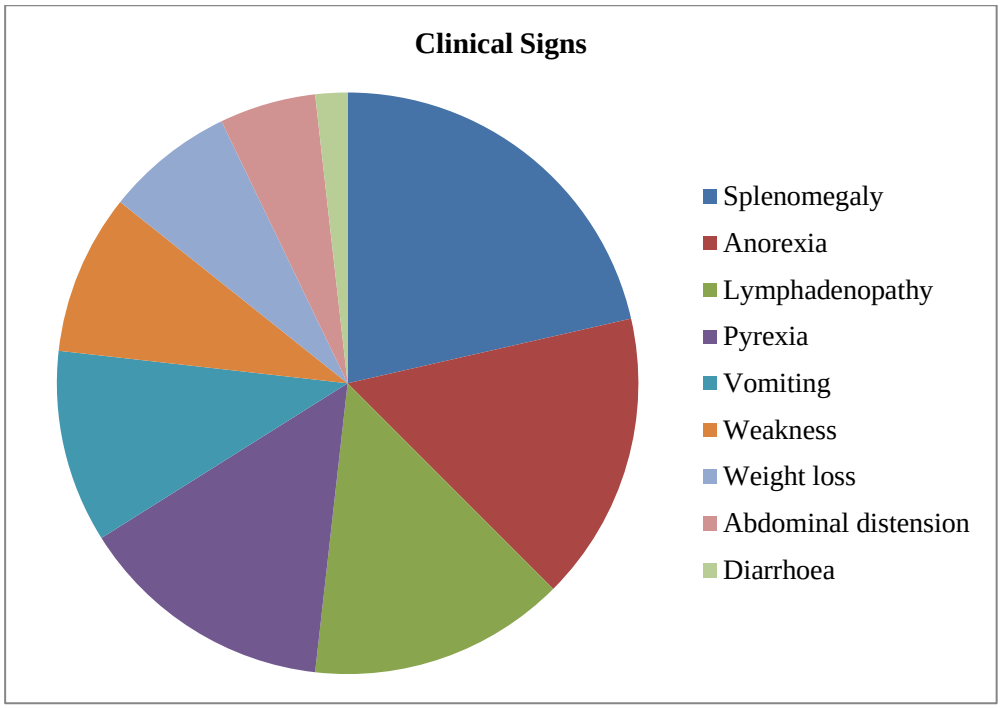
Abdominal ultrasonography was more useful than abdominal radiography in diagnosing splenomegaly, torsion and tumour. Ultrasound guided FNAB helped in specific diagnosis of lymphoma and splenitis.

Radiographically, lymphoma was characterized by radio-dense area in the middle of abdominal cavity.

Cytologically, presence of numerous small, medium, round lymphocytes was typical of lymphoma. Splenitis was characterized by presence of large number of toxic neutrophils in splenic aspirate.

Histopathologically, torsion was characterized by haemorrhage into the parenchyma with lymphoid depletion, Lymphoma was characterized by diffuse and multifocal congestion, periarteriolar lymphoid hyperplasia and interlacing bundles of fibrous tissues.

Fig.1 Common clinical signs of splenic diseases



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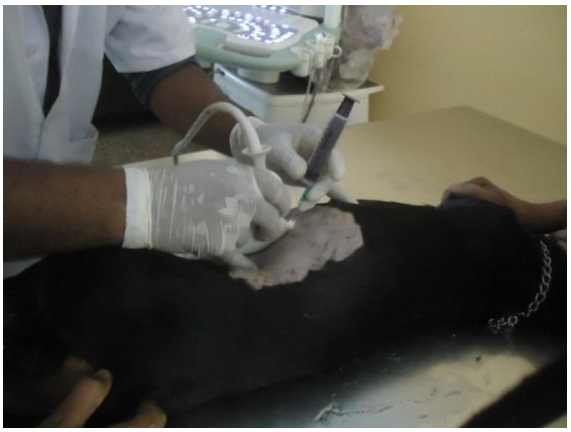

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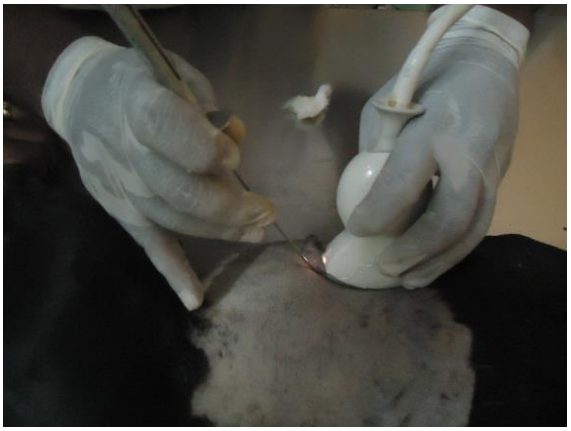


C

Plate 1. Ultrasound Guided Spleen Biopsy Technique

A- Positioning dogs for ultrasound examination of the spleen.

B- Position for ultrasound guided biopsy – free hand approach.

C- Position for ultrasound guided fine needle aspiration.

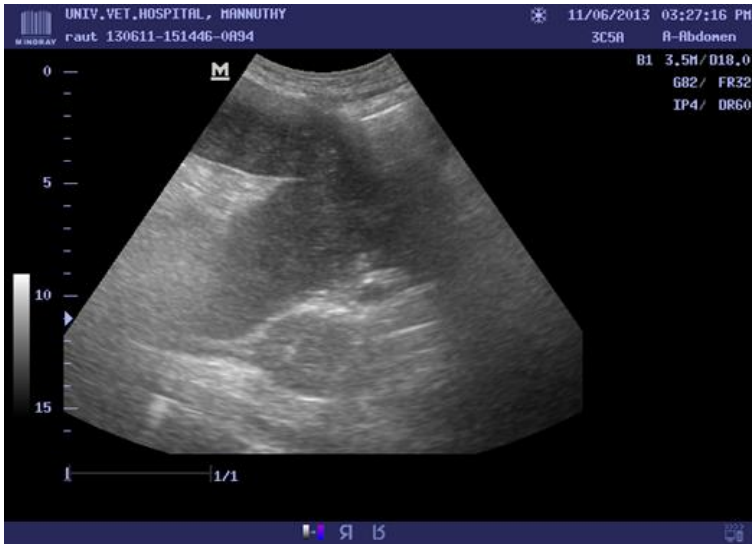


Plate 2. Ultrasonography : Torsion of spleen - Splenomegaly with mixed echogenic appearance

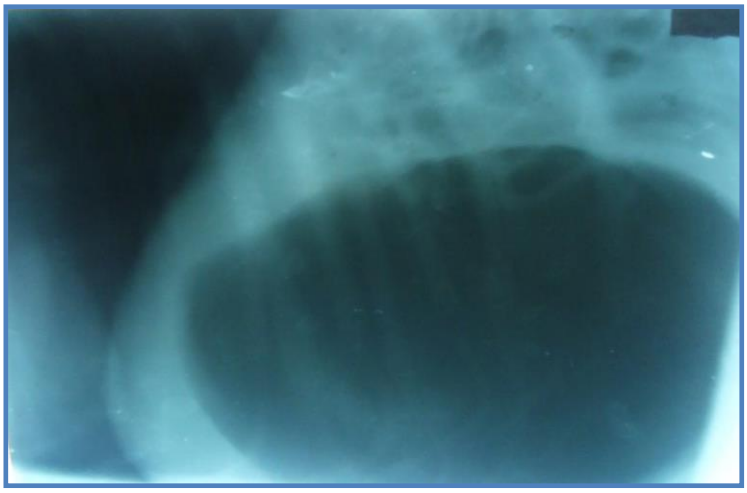


Plate 3. Radiography :- Irregular mass that appeared as radio-lucent area located in the middle of abdominal cavity

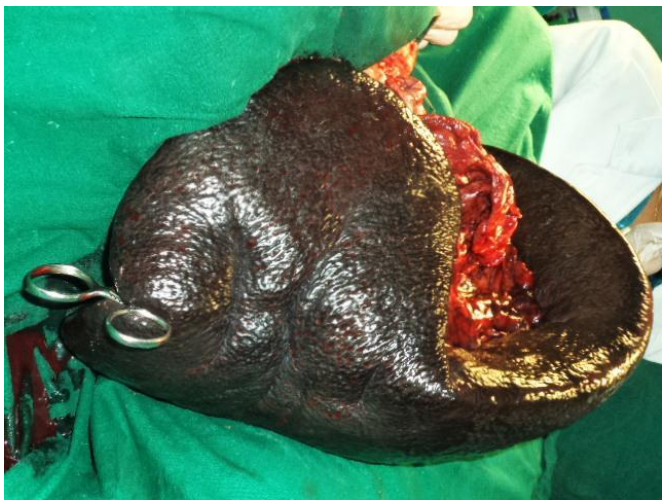


Plate 4. Gross lesion : Torsion of spleen - bluish black colour weight 3.4Kg

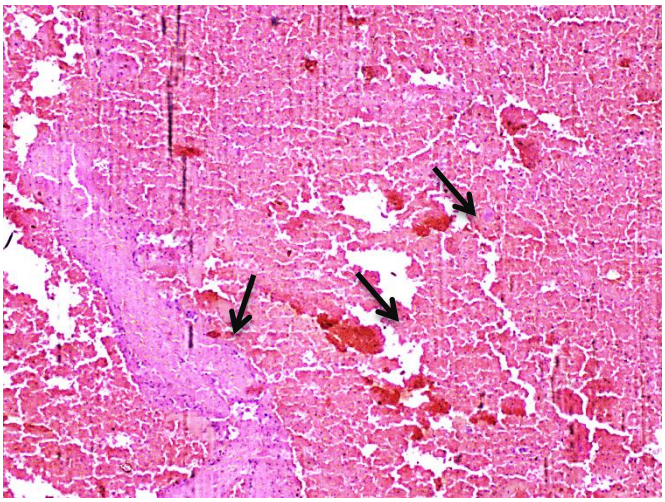
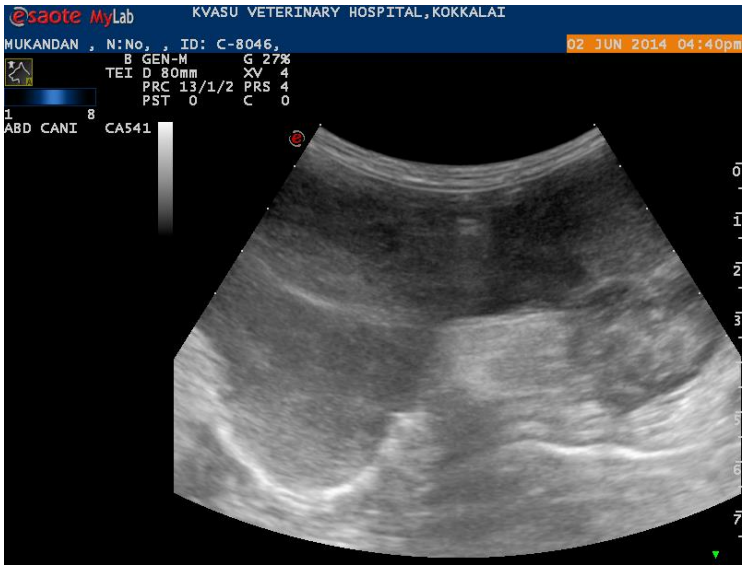


Plate 5. Histopathology : Torsion of spleen - haemorrhage into the splenic parenchyma and bits of trabeculae

6 A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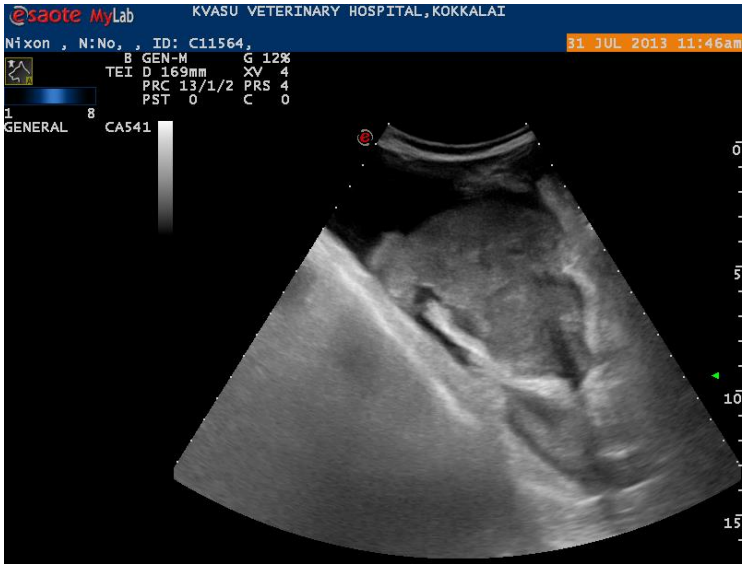
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Plate 6 A and B. Ultrasonography : Lymphoma - focal areas with mixed echogenic mass, splenic parenchyma hypoechoic

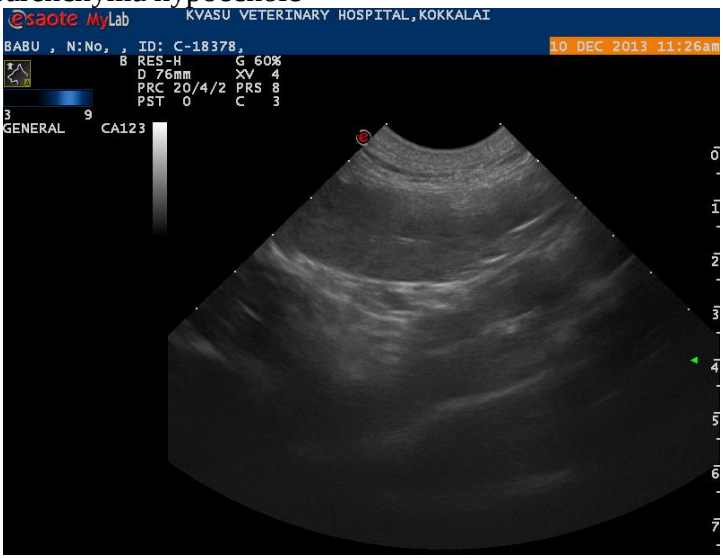


Plate 6 C. Ultrasonography :- Parenchyma with numerous small hypoechoic nodules

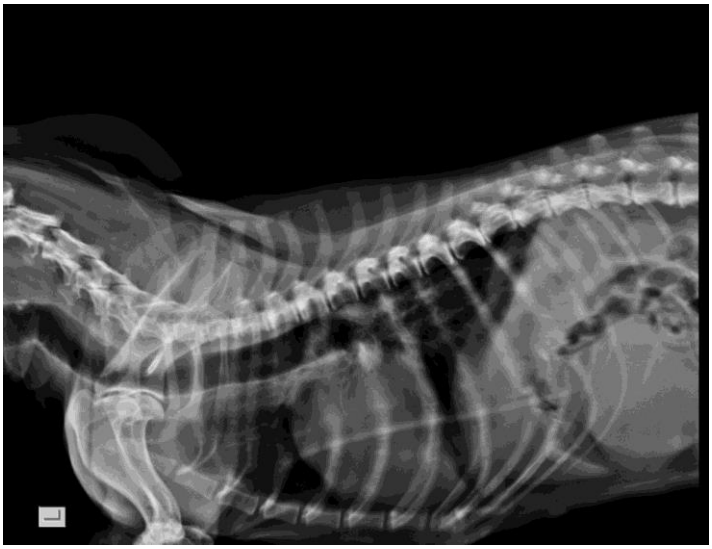


Plate 7. Radiography : Lymphoma - Irregular splenic mass which appeared as radio-dense area in the middle of abdominal cavity

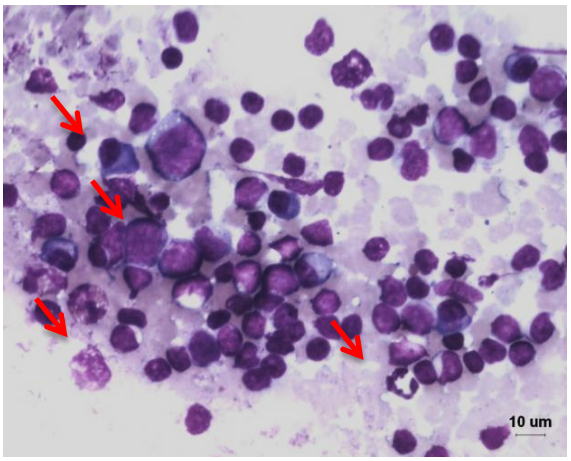


Plate 8. Cytology :- Immature lymphocytes with meshed chromatin pattern (blast), lymphocytosis, and few neutrophils in lymphoma

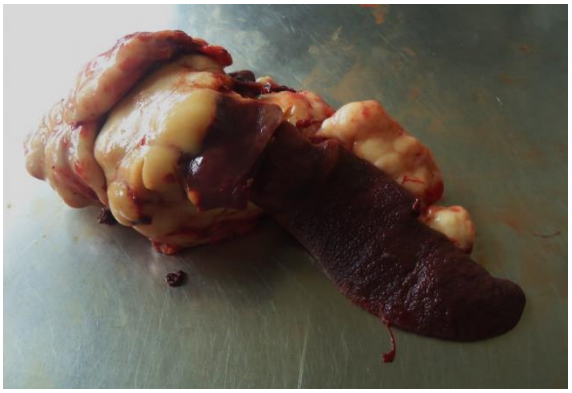
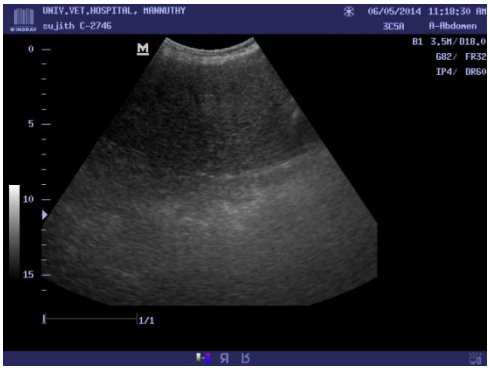


Plate 9 Gross lesion : Lymphoma - whitish colour, weight 2.7 Kg

Plate 10. Histopathology : Lymphoma - diffuse and multifocal congestion with interlacing bundles of fibrous tissues



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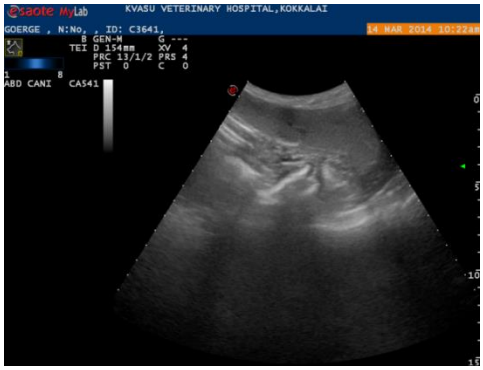

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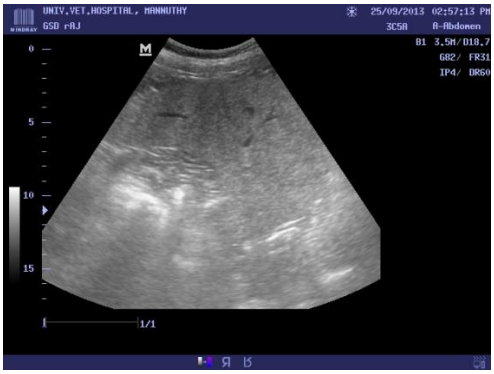

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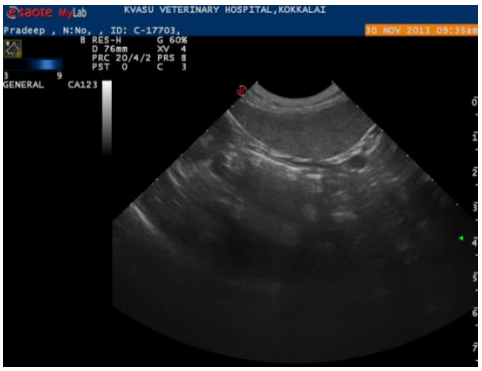

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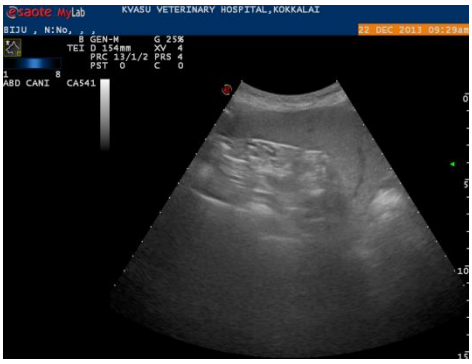

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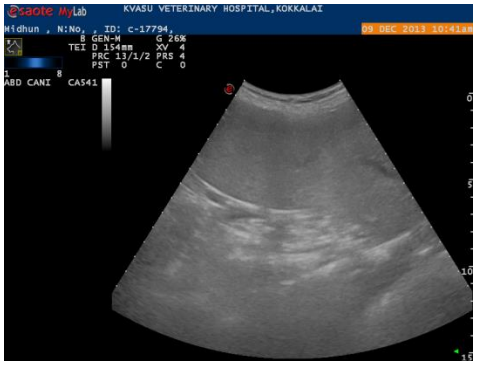


F

Plate 11 A, B, C, D, E and F. Ultrasonography :- Splenic parenchyma with hypoechoic areas and splenomegaly in splenitis

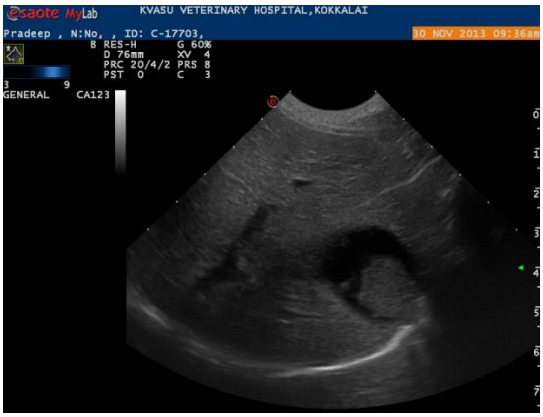
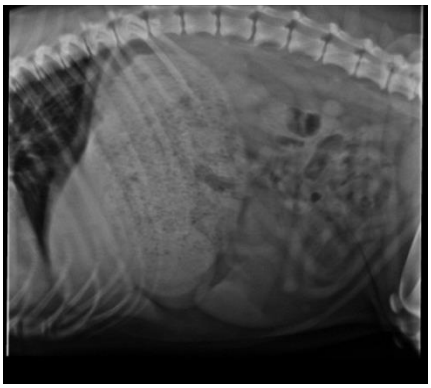
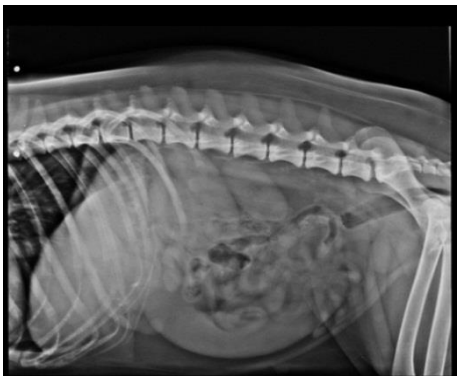


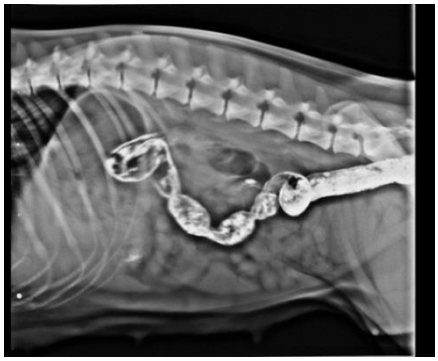
Plate 12. Ultrasonography:- Hepatomegaly and gall bladder sludge



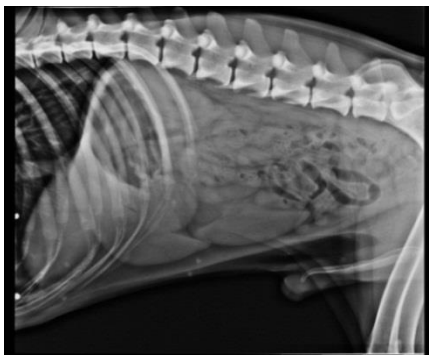
A



B



C



D

Plate 13 A, B, C and D. Radiography :- Moderate splenomegaly in splenitis

Plate 14. Cytology :- Numerous neutrophils / toxic neutrophils in splenitis

Plate 15. Cytology :- Numerous neutrophils in splenitis

Plate 16. Histopathology :- Haemorrhage with lymphoid depletion and lymphocytes within the sinus.

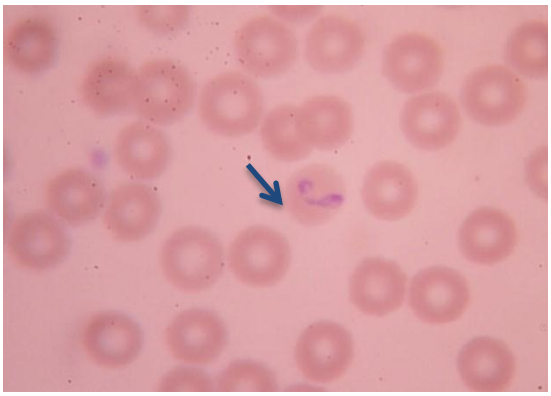


Plate 17. Peripherial Blood smear:- Blood smear positive for *Babesia gibsoni*

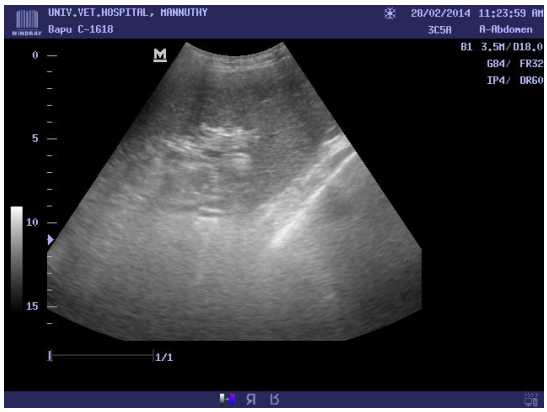


Plate 18. Ultrasonography :- Diffusly hypoechoic spleen with splenomegaly

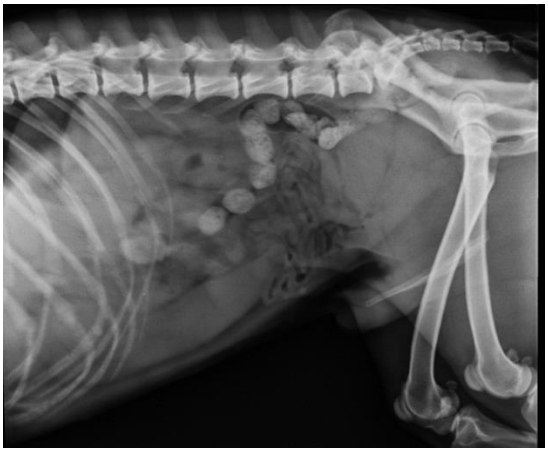
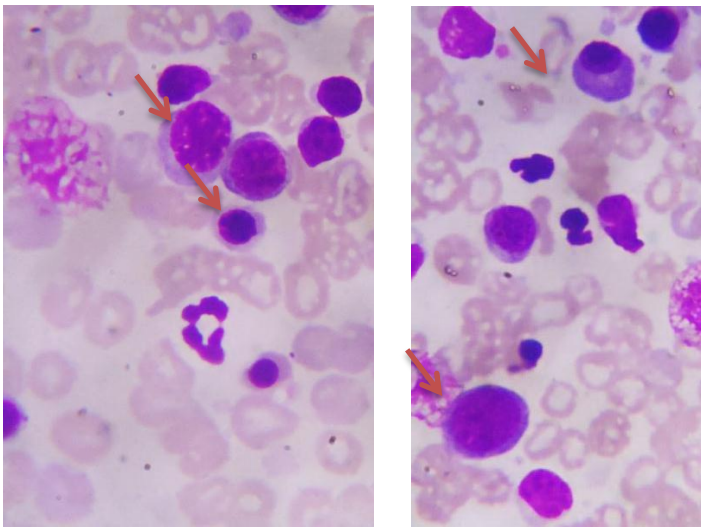


Plate 19. Radiography :- Moderate splenomegaly in Babesiosis



A B

Plate 20 A and B. Cytology :- Numerous lymphocytes with meshed chromatin in Babesiosis

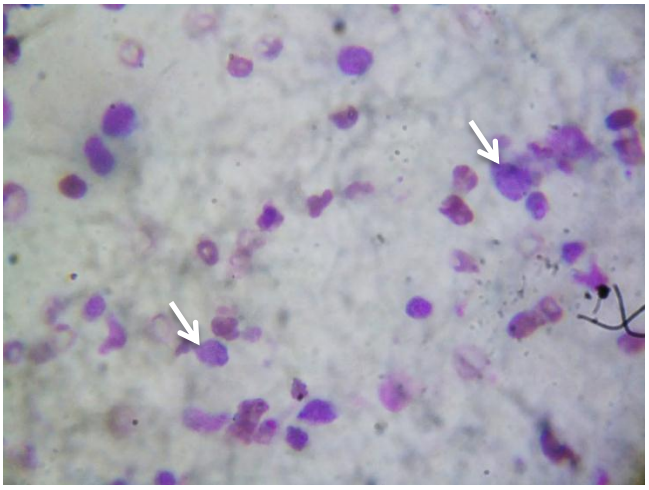


Plate 21. Cytology :- Large numbers of immature lymphoid cells

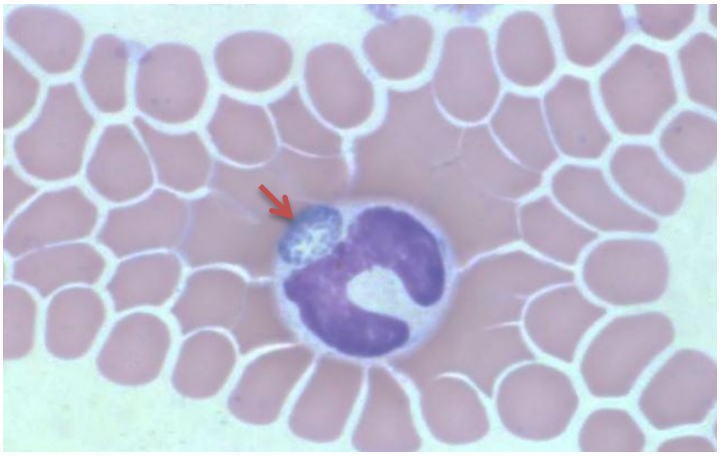


Plate 22. Buffy coat smear :- *Ehrlichia* morulae in monocytes, (buffy coat examination).

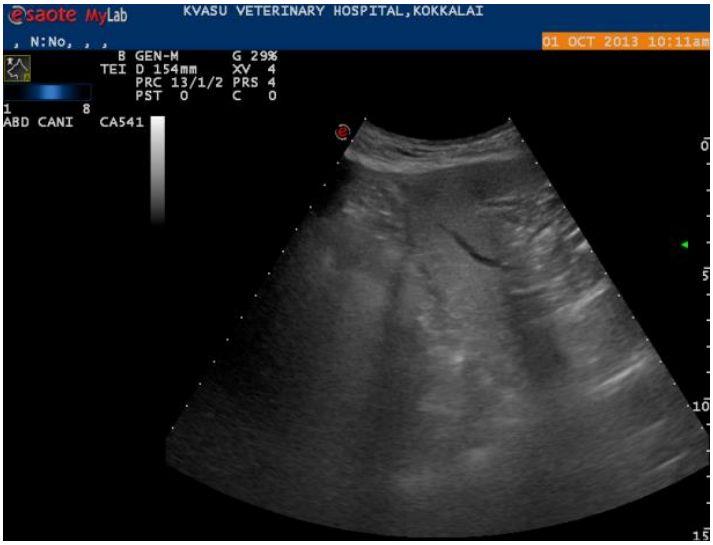


Plate 23. Ultrasonography :- Diffuse hyperechoic appearance and splenomegaly in Ehrlichiosis

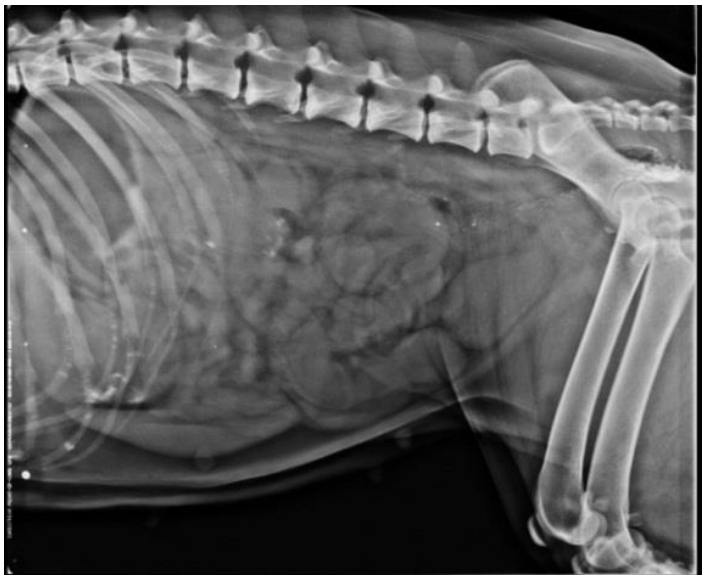


Plate 24. Radiography :- Moderate enlargement of spleen in Ehrlichiosis

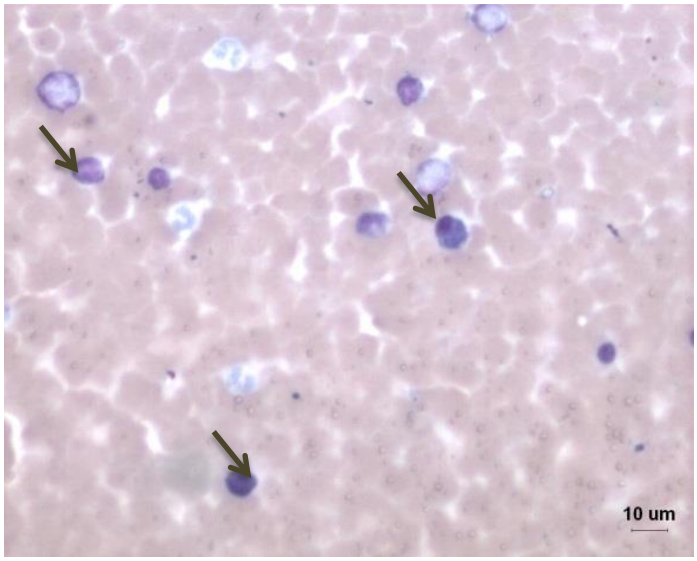


Plate 25. Cytology :- Large number of small, medium sized lymphocytes in splenic aspirate smear in Ehrlichiosis.